

POLYNUCLEOTIDES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This PCT application claims the priority benefit of U.S. Provisional Application No. 63/228,892, filed August 3, 2021, which is incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY VIA EFS WEB

[0002] The content of the electronically submitted sequence listing (4597_008PC01_Seqlisting_st26.xml; Size: 96,785 bytes; and Date of Creation: August 3, 2022) submitted in this application is incorporated herein by reference in its entirety.

FIELD OF THE DISCLOSURE

[0003] The present disclosure provides isolated polynucleotides (*e.g.*, replicons) or a set of polynucleotides that comprise a first nucleotide sequence encoding innate immune inhibitor, *e.g.*, an influenza non-structural (NS1) protein, and a second nucleotide sequence encoding a heterologous target mRNA. Such polynucleotides are capable of driving enhanced and persistent expression of the heterologous target mRNA in a cell.

BACKGROUND OF THE DISCLOSURE

[0004] Nucleic acid therapeutics have emerged as a promising and rapidly developing treatment for a wide variety of diseases. These therapies rely on cells, *in vitro*, *ex vivo*, or *in vivo*, to produce biologically active molecules, such as functional RNAs and/or therapeutic polypeptides, in a way that retains native conformations and post-translational modifications, which are often difficult to achieve with recombinant proteins. Synthetic mRNA has proven to be a valuable tool, with an improved safety profile relative to viral or DNA-based modalities. However, the human immune system naturally degrades RNA, limiting the potency and persistence of administered synthetic RNAs (*e.g.*, circular RNAs). As such, there remains a need in the art for RNA therapies that provide potent and durable effects *in vitro* and *in vivo*.

BRIEF SUMMARY OF THE DISCLOSURE

[0005] Some aspects of the present disclosure are directed to a polynucleotide or a set of polynucleotides comprising a first nucleic acid molecule encoding an influenza non-structural (NS1) protein and a second nucleic acid molecule encoding a heterologous target mRNA.

[0006] In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are present in a first vector. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein is present in a first vector, and wherein the second nucleic acid molecule encoding the target mRNA is present in a second vector.

[0007] In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein is expressed under the control of a first promoter. In some aspects, the second nucleic acid molecule encoding the target mRNA is expressed under the control of a second promoter. In some aspects, the first promoter and the second promoter are the same. In some aspects, the first promoter and the second promoter are the different.

[0008] In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are expressed under the control of a first promoter, wherein the first promoter drives expression of both the influenza NS1 protein and the target mRNA. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are linked by an IRES sequence. In some aspects, the first vector, the second vector, or both comprise one or more regulatory elements.

[0009] In some aspects, the expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, or at least about 300% relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the increase in the expression of the target mRNA persists for at least about 6 hours, at least about 12 hours, at least about 18 hours, at least

about 24 hours, at least about 30 hours, at least about 36 hours, at least about 42 hours, or at least about 48 hours.

[0010] In some aspects, the target mRNA encodes a biologically active polypeptide. In some aspects, the biologically active polypeptide comprises a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof. In some aspects, the cytokine is IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, IFN- α , IFN- β , IFN- γ , CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β , TGF- β 1, TGF- β 2, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, MSP, a fragment thereof, a variant thereof, or any combination thereof.

[0011] In some aspects, the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof. In some aspects, the target mRNA encodes a p35 subunit of IL-12 and a p40 subunit of IL-12. In some aspects, the p35 subunit and the p40 subunit are expressed from a single promoter. In some aspects, the p35 subunit and the p40 subunit are expressed as a single contiguous polypeptide. In some aspects, the p35 subunit and the p40 subunit are linked by one or more covalent bonds. In some aspects, the p35 subunit and the p40 subunit are linked by one or more peptide bonds. In some aspects, a portion of the mRNA that encodes the p35 subunit is separated from a portion of the mRNA that encodes the p40 subunit by an IRES.

[0012] In some aspects, the target mRNA encodes a miRNA, siRNA, shRNA, a dsRNA, antisense oligonucleotide, a guide RNA, or any combination thereof.

[0013] In some aspects, the first promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter. In some aspects, the second promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.

[0014] In some aspects, the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof. In some aspects, the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, or any combination thereof. In some aspects, the influenza NS1 is H5N1 NS1. In some aspects, the influenza NS1 is H1N1 NS1. In some aspects, the H1N1 NS1 is the H1N1 TX91 variant NS1.

[0015] In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set

forth in SEQ ID NO: 1 or 2. In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2. In some aspects, the first nucleic acid molecule comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein. In some aspects, the first nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein. In some aspects, (i) the first nucleic acid molecule, (ii) the second nucleic acid molecule, or (iii) both (i) and (ii) are circular RNA

[0016] In some aspects, the polynucleotide or the set of polynucleotides comprises one or more modified nucleic acid molecule.

[0017] Some aspects of the present disclosure are directed to a polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA, wherein the self-replicating target mRNA comprises one or more modified nucleic acid molecule. In some aspects, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, or less than about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, the one or more modified nucleic acid molecule is a modified rNTP. In some aspects, the one or more modified nucleic acid molecule comprises N1-methylpseudo uracil, 5-methyl cytosine, N6-methyladenosine or combinations thereof.

[0018] Some aspects of the present disclosure are directed to a vector or a set of vectors comprising a polynucleotide or a set of polynucleotides disclosed herein. In some aspects, the vector is a replicon. In some aspects, the vector is a Venezuelan equine encephalitis (VEE) replicon or a derivative or portion thereof. In some aspects, the vector is a Venezuelan equine encephalitis (VEE) replicon comprising a nucleotide sequence encoding a lysine at residue 739, according to the wild-type amino acid sequence VEE.

[0019] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof.

[0020] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; and (vi) a VEE 3'UTR or a derivative thereof.

[0021] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; (v) an E1 sequence; and (vi) a VEE 3'UTR or a derivative thereof.

[0022] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.

[0023] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0024] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.

[0025] T In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof.

[0026] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.

[0027] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target

mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0028] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.

[0029] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0030] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0031] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0032] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.

[0033] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a

second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

[0034] In some aspects, the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE. In some aspects, the one or more nsP comprises a VEE nsP or a derivative thereof. In some aspects, the VEE nsP is selected from nsP2, nsP3, nsP4, and any combination thereof.

[0035] Some aspects of the present disclosure are directed to a cell comprising a polynucleotide or a set of polynucleotides disclosed here or a vector or a set of vectors disclosed herein. In some aspects, the cell is a mammalian cell. In some aspects, the cell is a human cell. In some aspects, the cell is an immune cell.

[0036] Some aspects of the present disclosure are directed to a pharmaceutical composition comprising a polynucleotide or a set of polynucleotides disclosed herein, a vector or a set of vectors disclosed herein, or a cell disclosed herein and a pharmaceutically acceptable carrier.

[0037] Some aspects of the present disclosure are directed to a method of expressing a target mRNA in a cell, comprising transfecting the cell with a polynucleotide or a set of polynucleotides disclosed herein or a vector or a set of vectors disclosed herein. In some aspects, the cell is a human cell. In some aspects, the cell is an ex vivo human cell. In some aspects, the cell is a human immune cell.

[0038] Some aspects of the present disclosure are directed to a method of treating a subject in need thereof, comprising administering to the subject a polynucleotide or a set of polynucleotides disclosed herein, a vector or a set of vectors disclosed herein, a cell disclosed herein, or a pharmaceutical composition disclosed herein.

[0039] Some aspects of the present disclosure are directed to a method of expressing a target mRNA in a subject in need thereof, comprising administering to the subject a polynucleotide or a set of polynucleotides disclosed herein, a vector or a set of vectors disclosed herein, a cell disclosed herein, or a pharmaceutical composition disclosed herein.

[0040] In some aspects, the subject is afflicted with a cancer. In some aspects, the cancer is selected from the group consisting of melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma,

cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, and various types of head and neck cancer, including squamous cell head and neck cancer. In some aspects, the cancer can be melanoma, lung cancer, colorectal cancer, renal-cell cancer, urothelial carcinoma, Hodgkin's lymphoma, and any combination thereof.

[0041] Some aspects of the present disclosure are directed to a method of expressing a target mRNA in a cell, comprising co-expressing the target mRNA and an influenza NS1 protein in the cell, wherein the target mRNA is not an influenza mRNA. In some aspects, the influenza NS1 protein is encoded by a first nucleic acid molecule and the target mRNA is encoded by a second nucleic acid molecule.

[0042] In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are present in a first vector. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein is present in a first vector, and wherein the second nucleic acid molecule encoding the target mRNA is present in a second vector.

[0043] In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein is expressed under the control of a first promoter. In some aspects, the second nucleic acid molecule encoding the target mRNA is expressed under the control of a second promoter. In some aspects, the first promoter and the second promoter are the same. In some aspects, the first promoter and the second promoter are the different. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are expressed under the control of a first promoter, wherein the first promoter drives expression of both the influenza NS1 protein and the target mRNA. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are linked by an IRES sequence. In some aspects, the first vector, the second vector, or both comprise one or more regulatory elements. In some aspects, expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, (i) the first nucleic acid molecule, (ii) the second nucleic acid molecule, or (iii) both (i) and (ii) are circular RNA.

[0044] Some aspects, of the present disclosure are directed to a method of expressing a target mRNA in a cell, comprising transfecting the cell with a polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA comprising one or more modified

nucleic acid molecules. In some aspects, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, or less than about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, the one or more modified nucleic acid molecules is a modified rNTP. In some aspects, the one or more modified nucleic acid molecules comprises N1-methylpseudo uracil, 5-methyl cytosine, N6-methyladenosine, or combinations thereof.

[0045] In some aspects, expression of the target mRNA is increased relative to the expression of the target mRNA from a self-replicating target mRNA not comprising one or more modified nucleic acid molecules. In some aspects, the expression of the target mRNA is increased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, or at least about 300%.

[0046] In some aspects, the increase in the expression of the target mRNA persists for at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 24 hours, at least about 30 hours, at least about 36 hours, at least about 42 hours, or at least about 48 hours.

[0047] In some aspects, the target mRNA encodes a biologically active polypeptide. In some aspects, the biologically active polypeptide comprises a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof. In some aspects, the cytokine is IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, IFN- α , IFN- β , IFN- γ , CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β , TGF- β 1, TGF- β 2, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, MSP, a fragment thereof, a variant thereof, or any combination thereof.

[0048] In some aspects, the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof. In some aspects, the target mRNA encodes a p35 subunit of IL-12 and a p40 subunit of IL-12. In some aspects, the p35 subunit and the p40 subunit are expressed from a single promoter. In some aspects, the p35 subunit and the p40 subunit are expressed as a single contiguous polypeptide. In some aspects, the p35 subunit and the p40 subunit are linked by one or more covalent bonds. In some aspects, the p35 subunit and the p40 subunit are linked by one or more

peptide bonds. In some aspects, a portion of the mRNA that encodes the p35 subunit is separated from a portion of the mRNA that encodes the p40 subunit by an IRES.

[0049] In some aspects, the target mRNA comprises a miRNA, a siRNA, a shRNA, a dsRNA, an antisense oligonucleotide, a guide RNA, a circular RNA, or any combination thereof.

[0050] In some aspects, the first promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter. In some aspects, the second promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.

[0051] In some aspects, the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof. In some aspects, the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, or a combination thereof. In some aspects, the influenza NS1 is H5N1 NS1. In some aspects, the influenza NS1 is H1N1 NS1. In some aspects, the H1N1 NS1 is the H1N1 TX91 variant NS1.

[0052] In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2. In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2. In some aspects, the first nucleic acid molecule comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein. In some aspects, the first nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0053] FIGs. 1A-1B are illustrations of a sample non-cytopathic-EGFP vector (FIG. 1A) and a non-cytopathic-NS1-EGFP vector (FIG. 1B).

[0054] FIGs. 2A-2D are images of 4T1 cells 24-hour post-transfection with Strand-mCherry (FIG. 2A), mCherry modRNA (FIG. 2B), and Strand-mCherry plus NS1 modRNA mRNAs (FIGs. 2C-2D) using Lipofectamine MessengerMax.

[0055] FIG. 2E summarizes the mean fluorescence intensity observed for each mRNA transfection tested in FIGs. 2A-2D.

[0056] FIGs. 3A-3D are images of 4T1 cells 24-hour post-transfection with Strand-mCherry (FIG. 3A), mCherry modRNA (FIG. 3B), and Strand-mCherry plus NS1 modRNA mRNAs (FIGs. 3C-3D) using TT3 lipid nanoparticle.

[0057] FIGs. 3E-3F summarize the mean fluorescence intensity observed for each mRNA transfected by tandem NS1 expression (FIG. 3E) or NS1 cotransfection (FIG. 3F).

[0058] FIGs. 4A-4H are graphical representations of flow cytometry data illustrating the number of cells expressing mCherry at 24 hours (FIGs. 4A-4D) and 48 hours (FIGs. 4E-4H) after transfection of Hcc38 tumor cells with NS1 modRNA (FIGs. 4B and 4F), NS1 repRNA (FIGs. 4C and 4G), or NS1-P2A-mCherry mRNA (FIGs. 4D and 4H) using Lipofectamine MessengerMax.

[0059] FIGs. 4I-4K show median fluorescence intensity (MFI) of Scc9 HNSCC cells (FIG. 4I) and FaDu HNSCC cells (FIGs. 4J-4K).

[0060] FIGs. 5A-5D are graphical representations of flow cytometry data illustrating the number of cells expressing EGFP at 24 hours following transfection of BT20 cancer using TT3 LNP comprising an EGFP-encoding replicon vector containing either the original (Strand) backbone (FIGs. 5A-5B) or one containing a Q739L mutation (non-cytopathic; FIGs. 5C-5D).

[0061] FIGs. 5E-5F are graphical representations of median fluorescence intensity (MFI; FIG. 5E) and transfection efficiency as measured by the percent of GFP-positive cells (FIG. 5F).

[0062] FIGs. 6A-6F are graphical representations of flow cytometry data illustrating the number of B16.F10 cells (FIGs. 6A-6C) and 4T1 cells (FIGs. 6D-6F) expressing mCherry following electroporation with replicon made either using unmodified rNTPs (Unmodified Rep; FIGs. 6A and 6D) or using a ratio of 1:1 (50%; FIGs. 6B and 6E) or 1:3 (25%; FIGs. 6C and 6F) UTP to N1-methyl-pseudoUTP (denoted by psi).

[0063] FIG. 6G is a graphical representation of median fluorescence intensity (MFI) of cells described in FIGs. 6A-6G.

[0064] FIG. 7 is a bar graph illustrating the relative luminescence of 4T1 cells transfected with firefly luciferase (Fluc)-encoded replicons made either using unmodified rNTPs (unmod) or using a 1:3 ratio of certain NTPs- U: Ψ (M1) or U: Ψ ; 1:3 C:5me-C (M2), where C refers to Cytidine and 5me-C refers to 5-methyl-cytidine. Luminescence was measured at 24 as 48hrs post-transfection via electroporation.

[0065] FIGs. 8A-8B are bar graphs illustrating the relative luminescence (FIG. 8A) and type I IFN activity as measured using the SEAP reporter assay using colorimetry (FIG. 8B) of B16-ISG cells, an interferon inducible cell line, transfected with firefly luciferase (Fluc)-encoded replicons made either using unmodified rNTPs (unmod) or using a 1:3 ratio of certain NTPs- U: Ψ (M1) or U: Ψ; 1:3 C:5me-C (M2), where C refers to Cytidine and 5me-C refers to 5-methylcytidine.

[0066] FIGs. 9A-9C are graphical representations of payload expression (FIG. 9A), signal intensity (FIG. 9B), and Type I IFN activity (FIG. 9C) of B16-ISG cells transfected with a Q739L replicon expressing NS1-EGFP (P2A linker) made either using unmodified or singly (M1) or doubly modified rNTPs.

[0067] FIGs. 10A-10F are images of GFP expression of T cells activated for 2 days with Anti-CD3/CD28/CD2 cocktail with high dose IL-2 with (FIGs. 10D-10F) or without (FIGs. 10A-10C) addition of a recombinant protein (Enhancer) to the media. T cells were transfected with lipid 1 (FIGs. 10A and 10D), lipid 2-cholesterol (FIGs. 10B and 10E), or lipid 2-β-sitosterol (FIGs. 10C and 10F).

[0068] FIGs. 11A-11C are graphical representations of transfection efficiency (percent GFP positive cells; FIG. 11A), median fluorescence intensity (FIG. 11B), and IFN-gamma levels (FIG. 11C) in primary human T cells activated using IL-2 and anti-CD3/CD28/CD2 for 2 days post thaw and transfected using the Q739L replicon driving NS1-EGFP, as used above, and made using unmodified or singly (M1) or doubly modified (M2) mRNAs.

[0069] FIGs. 12A-12B are graphical representations of transfection efficiency (percent GFP positive cells; FIG. 12A) and IFN-alpha activation (FIG. 12B) in human PBMCs isolated from three healthy donors and either grown with low dose IL-2 (Resting) or with high dose of IL-2 in presence of Anti-CD3/CD28/CD2 cocktail (Activated) for 2 days followed by mRNA:lipid delivery with M2 modified Q739L replicons driving NS1-EGFP or EGFP or with conventional EGFP mRNA (EGFP-mod). Measurements were taken 24 hours post transfection.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0070] Some aspects of the present disclosure are directed to a polynucleotide or a set of polynucleotides comprising a first nucleic acid molecule encoding an influenza non-structural (NS1) protein and a second nucleic acid molecule encoding a heterologous target mRNA. The present disclosure provides that expression of a target mRNA encoded by a polynucleotide, *e.g.*, a replicon, can be enhanced in the presence of influenza NS1. In particular, coexpression of influenza

NS1 and a target mRNA increases the expression level of the target mRNA and the persistence of the expression of the target mRNA in a cell. As such, some aspects of the present disclosure are directed to methods of expressing a target mRNA in a cell, *e.g.*, a human cell, comprising transfecting the cell with a polynucleotide (*e.g.*, circular RNA) or a set of polynucleotides (*e.g.*, set of circular RNAs) comprising a first nucleic acid molecule encoding an influenza non-structural (NS1) protein and a second nucleic acid molecule encoding a heterologous target mRNA. In some aspects, the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof.

[0071] Additional aspects of the present disclosure are provided throughout the present application.

I. Definitions

[0072] In order that the present disclosure can be more readily understood, certain terms are first defined. As used in this application, except as otherwise expressly provided herein, each of the following terms shall have the meaning set forth below. Additional definitions are set forth throughout the application.

[0073] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a nucleotide sequence," is understood to represent one or more nucleotide sequences. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein. It is further noted that the claims can be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a negative limitation.

[0074] The term "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0075] It is understood that wherever aspects are described herein with the language "comprising," otherwise analogous aspects described in terms of "consisting of" and/or "consisting essentially of" are also provided.

[0076] As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value and within a range of

values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value). When the term "approximately" or "about" is applied herein to a particular value, the value without the term "approximately" or "about" is also disclosed herein.

[0077] As described herein, any concentration range, percentage range, ratio range, or integer range is to be understood to include the value of any integer within the recited range and, when appropriate, fractions thereof (such as one tenth and one hundredth of an integer), unless otherwise indicated.

[0078] As used herein, the terms "ug" and "uM" are used interchangeably with "µg" and "µM," respectively.

[0079] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0080] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Where a range of values is recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed, along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither, or both limits are included is also encompassed within the disclosure. Thus, ranges recited herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 10 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

[0081] Where a value is explicitly recited, it is to be understood that values that are about the same quantity or amount as the recited value are also within the scope of the disclosure. Where a combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the disclosure. Conversely, where different

elements or groups of elements are individually disclosed, combinations thereof are also disclosed. Where any element of a disclosure is disclosed as having a plurality of alternatives, examples of that disclosure in which each alternative is excluded singly or in any combination with the other alternatives are also hereby disclosed; more than one element of a disclosure can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

[0082] Nucleotides are referred to by their commonly accepted single-letter codes. Unless otherwise indicated, nucleotide sequences are written left to right in 5' to 3' orientation. Nucleotides are referred to herein by their commonly known one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Accordingly, 'a' represents adenine, 'c' represents cytosine, 'g' represents guanine, 't' represents thymine, and 'u' represents uracil.

[0083] Amino acid sequences are written left to right in amino to carboxy orientation. Amino acids are referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission.

[0084] The terms "administration," "administering," and grammatical variants thereof refer to introducing a composition (*e.g.*, such as an isolated polynucleotide described herein) into a subject via a pharmaceutically acceptable route. The introduction of a composition into a subject can be done by any suitable route, including intratumorally, orally, pulmonarily, intranasally, parenterally (intravenously, intra-arterially, intramuscularly, intraperitoneally, or subcutaneously), rectally, intralymphatically, intrathecally, periorcularly or topically. Administration includes self-administration and the administration by another. A suitable route of administration allows the composition to perform its intended function. For example, if a suitable route is intravenous, the composition can be administered by introducing the composition into a vein of the subject.

[0085] As used herein, the term "cancer" refers to a broad group of various diseases characterized by the uncontrolled growth of abnormal cells in the body. Unregulated cell division and growth divide and grow results in the formation of malignant tumors that invade neighboring tissues and can also metastasize to distant parts of the body through the lymphatic system or bloodstream. The compositions disclosed herein can be used in the treatment of any cancer, including but not limited to melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, and various types

of head and neck cancer, including squamous cell head and neck cancer. In some aspects, the cancer can be melanoma, lung cancer, colorectal cancer, renal-cell cancer, urothelial carcinoma, Hodgkin's lymphoma, and any combination thereof.

[0086] The term "coding sequence" or sequence "encoding" is used herein to mean a DNA or RNA region (the transcribed region) which "encodes" a particular protein, *e.g.*, an influenza NS1 protein or a target heterologous protein. A coding sequence is transcribed (DNA) and translated (RNA) into a polypeptide, *in vitro* or *in vivo*, when placed under the control of an appropriate regulatory region, such as a promoter. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotes or eukaryotes, genomic DNA from prokaryotes or eukaryotes, and synthetic DNA sequences. A transcription termination sequence can be located 3' to the coding sequence.

[0087] The term "downstream" refers to a nucleotide sequence that is located 3' to a reference nucleotide sequence. In some aspects, downstream nucleotide sequences relate to sequences that follow the starting point of transcription. For example, the translation initiation codon of a gene is located downstream of the start site of transcription.

[0088] The terms "excipient" and "carrier" are used interchangeably and refer to an inert substance added to a pharmaceutical composition to further facilitate administration of a compound.

[0089] The term "expression," as used herein, refers to a process by which a polynucleotide produces a gene product, *e.g.*, RNA or a polypeptide (*e.g.*, therapeutic protein, *e.g.*, influenza NS1 nonstructural protein). It includes, without limitation, transcription of the polynucleotide into micro RNA binding site, small hairpin RNA (shRNA), small interfering RNA (siRNA), or any other RNA product. It includes, without limitation, transcription of the polynucleotide into messenger RNA (mRNA), and as well as the translation of mRNA into a polypeptide. Expression produces a "gene product." As used herein, a gene product can be, *e.g.*, a nucleic acid, such as an RNA produced by transcription of a gene. As used herein, a gene product can be either a nucleic acid, RNA (*e.g.*, circular RNA) or miRNA produced by the transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation or splicing, or polypeptides with post translational modifications, *e.g.*, phosphorylation, methylation, glycosylation, the addition of lipids, association with other protein subunits, or proteolytic cleavage.

[0090] As used herein, the term "heterologous target mRNA" refers to any mRNA (linear or circular) that is not naturally present in a target cell that can be expressed in the target cell using the polynucleotides described herein. Unless indicated otherwise, a heterologous target mRNA can encode a polypeptide or RNA molecules that have regulatory function (such as miRNA, dsDNA, lncRNA, siRNA, antisense oligonucleotide, a phosphorodiamidate morpholino oligomer (PMO), a peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO), or combinations thereof). Accordingly, as used herein, the term "encode" refers to the production of a moiety of interest (*e.g.*, polypeptide or a RNA molecule, such as circular RNA) from a nucleic acid molecule (*e.g.*, heterologous target mRNA). In some aspects, the heterologous target mRNA encodes a biologically active polypeptide, including but not limited to a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof. In some aspects, the heterologous target mRNA is referred to herein as a "payload." Unless indicated otherwise, the term "target mRNA" and "heterologous target mRNA" are used interchangeably.

[0091] In some aspects, two or more sequences are said to be "identical" if they are 100% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are at least about 70% identical, at least about 80% identical, at least about 90% identical, or at least about 95% identical to one another. In some aspects, two or more sequences are said to be "highly conserved" if they are about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. In some aspects, two or more sequences are said to be "conserved" if they are at least about 30% identical, at least about 40% identical, at least about 50% identical, at least about 60% identical, at least about 70% identical, at least about 80% identical, at least about 90% identical, or at least about 95% identical to one another. In some aspects, two or more sequences are said to be "conserved" if they are about 30% identical, about 40% identical, about 50% identical, about 60% identical, about 70% identical, about 80% identical, about 90% identical, about 95% identical, about 98% identical, or about 99% identical to one another. Conservation of sequence can apply to the entire length of a polynucleotide or polypeptide or can apply to a portion, region or feature thereof.

[0092] As used herein, the term "identity" refers to the overall monomer conservation between polymeric molecules, *e.g.*, between polypeptide molecules or polynucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules). The term "identical" without any additional qualifiers, *e.g.*, protein A is identical to protein B, implies the sequences are 100% identical (100% sequence identity). Describing two sequences as, *e.g.*, "70% identical," is equivalent to describing them as having, *e.g.*, "70% sequence identity."

[0093] Calculation of the percent identity of two polypeptide or polynucleotide sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second polypeptide or polynucleotide sequences for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In some aspects, the length of a sequence aligned for comparison purposes is at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, or about 100% of the length of the reference sequence. The amino acids at corresponding amino acid positions, or bases in the case of polynucleotides, are then compared.

[0094] When a position in the first sequence is occupied by the same amino acid as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm.

[0095] Suitable software programs are available from various sources, and for alignment of both protein and nucleotide sequences. One suitable program to determine percent sequence identity is *bl2seq*, part of the BLAST suite of program available from the U.S. government's National Center for Biotechnology Information BLAST web site (blast.ncbi.nlm.nih.gov). *Bl2seq* performs a comparison between two sequences using either the BLASTN or BLASTP algorithm. BLASTN is used to compare nucleic acid sequences, while BLASTP is used to compare amino acid sequences. Other suitable programs are, *e.g.*, Needle, Stretcher, Water, or Matcher, part of the EMBOSS suite of bioinformatics programs and also available from the European Bioinformatics Institute (EBI) at worldwideweb.ebi.ac.uk/Tools/psa.

[0096] Sequence alignments can be conducted using methods known in the art such as MAFFT, Clustal (ClustalW, Clustal X or Clustal Omega), MUSCLE, etc.

[0097] Different regions within a single polynucleotide or polypeptide target sequence that aligns with a polynucleotide or polypeptide reference sequence can each have their own percent sequence identity. It is noted that the percent sequence identity value is rounded to the nearest tenth. For example, 80.11, 80.12, 80.13, and 80.14 are rounded down to 80.1, while 80.15, 80.16, 80.17, 80.18, and 80.19 are rounded up to 80.2. It also is noted that the length value will always be an integer.

[0098] In some aspects, the percentage identity (%ID) of a first amino acid sequence (or nucleic acid sequence) to a second amino acid sequence (or nucleic acid sequence) is calculated as $\%ID = 100 \times (Y/Z)$, where Y is the number of amino acid residues (or nucleobases) scored as identical matches in the alignment of the first and second sequences (as aligned by visual inspection or a particular sequence alignment program) and Z is the total number of residues in the second sequence. If the length of a first sequence is longer than the second sequence, the percent identity of the first sequence to the second sequence will be higher than the percent identity of the second sequence to the first sequence.

[0099] One skilled in the art will appreciate that the generation of a sequence alignment for the calculation of a percent sequence identity is not limited to binary sequence-sequence comparisons exclusively driven by primary sequence data. It will also be appreciated that sequence alignments can be generated by integrating sequence data with data from heterogeneous sources such as structural data (*e.g.*, crystallographic protein structures), functional data (*e.g.*, location of mutations), or phylogenetic data. A suitable program that integrates heterogeneous data to generate a multiple sequence alignment is T-Coffee, available at www.tcoffee.org, and alternatively available, *e.g.*, from the EBI. It will also be appreciated that the final alignment used to calculate percent sequence identity can be curated either automatically or manually.

[0100] As used herein, the terms "isolated" and "purified," and grammatical variants thereof, are used interchangeably and refer to the state of a preparation of desired composition of the present disclosure that has undergone one or more processes of purification. In some aspects, isolating or purifying as used herein is the process of removing, partially removing (*e.g.*, a fraction) of a composition of the present disclosure from a sample containing contaminants. In some aspects, an isolated composition has no detectable undesired activity or, alternatively, the level or amount of the undesired activity is at or below an acceptable level or amount. In some aspects, an isolated composition has an amount and/or concentration of desired composition of the present disclosure, at or above an acceptable amount and/or concentration and/or activity. In some aspects, the isolated composition is enriched as compared to the starting material from which the composition is obtained. This enrichment can be by at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, at least about 99.99%, at least about 99.999%, at least about 99.9999%, or greater than 99.9999% as compared to the starting material. In some aspects, isolated preparations are substantially free of residual biological products. In some aspects, the isolated

preparations are 100% free, at least about 99% free, at least about 98% free, at least about 97% free, at least about 96% free, at least about 95% free, at least about 94% free, at least about 93% free, at least about 92% free, at least about 91% free, or at least about 90% free of any contaminating biological matter. Residual biological products can include abiotic materials (including chemicals) or unwanted nucleic acids, proteins, lipids, or metabolites.

[0101] The term "linked" as used herein refers to a first amino acid sequence or polynucleotide sequence covalently or non-covalently joined to a second amino acid sequence or polynucleotide sequence, respectively. The first amino acid or polynucleotide sequence can be directly joined or juxtaposed to the second amino acid or polynucleotide sequence or alternatively an intervening sequence can covalently join the first sequence to the second sequence. The term "linked" means not only a fusion of a first polynucleotide sequence to a second polynucleotide sequence at the 5'-end or the 3'-end, but also includes insertion of the whole first polynucleotide sequence (or the second polynucleotide sequence) into any two nucleotides in the second polynucleotide sequence (or the first polynucleotide sequence, respectively). The first polynucleotide sequence can be linked to a second polynucleotide sequence by a phosphodiester bond or a linker. The linker can be, *e.g.*, a polynucleotide.

[0102] As used herein, the terms "modulate," "modify," and grammatical variants thereof, generally refer when applied to a specific concentration, level, expression, function or behavior, to the ability to alter, by increasing or decreasing, *e.g.*, directly or indirectly promoting/stimulating/up-regulating or interfering with/inhibiting/down-regulating the specific concentration, level, expression, function or behavior, such as, *e.g.*, to act as an antagonist or agonist. In some instances, a modulator can increase and/or decrease a certain concentration, level, activity or function relative to a control, or relative to the average level of activity that would generally be expected or relative to a control level of activity.

[0103] As used herein, the term "nonstructural protein" refers to a protein encoded by a virus but that is not part of the viral particle. More specifically, the nonstructural proteins described herein comprise the influenza NS1 protein, including but not limited to a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, an H1N1 NS1, an H1N2 NS1, an H2N2 NS1, an H3N2 NS1, an H5N1 NS1, an H7N9 NS1, an H7N7 NS1, an H9N2 NS1, an H7N2 NS1, an H7N3 NS1, an H5N2 NS1, an H10N7 NS1, combinations thereof, or variants thereof. Additional disclosures relating to such nonstructural proteins are provided elsewhere in the present disclosure.

[0104] "Nucleic acid," "nucleic acid molecule," "nucleotide sequence," and grammatical variants thereof, are used interchangeably and refer to the phosphate ester polymeric form of ribonucleosides (adenosine, guanosine, uridine or cytidine; "RNA molecules") or deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxythymidine, or deoxycytidine; "DNA molecules"), or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form or a double-stranded helix. Additionally, as is apparent from the present disclosure, in some aspects, a nucleic acid molecule can also be in a circular form (*e.g.*, circular RNA). Single stranded nucleic acid sequences refer to single-stranded DNA (ssDNA) or single-stranded RNA (ssRNA). Double stranded DNA-DNA, DNA-RNA and RNA-RNA helices are possible. The term nucleic acid molecule, and in particular DNA or RNA molecule, refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear or circular DNA molecules (*e.g.*, restriction fragments), plasmids, supercoiled DNA and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences can be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the non-transcribed strand of DNA (*i.e.*, the strand having a sequence homologous to the mRNA). A "recombinant DNA molecule" is a DNA molecule that has undergone a molecular biological manipulation. DNA includes, but is not limited to, cDNA, genomic DNA, plasmid DNA, synthetic DNA, and semi-synthetic DNA.

[0105] As used herein, the term "circular RNA" refers to a polyribonucleotide that forms a circular structure through covalent bonds. As is apparent from the present disclosure, any of the polynucleotides, sets of polynucleotides, first nucleic acid molecule, and second nucleic acid molecule can be circular in structure. For instance, in some aspects, a polynucleotide described herein (*e.g.*, comprising a first nucleic acid molecule encoding an influenza NS1 protein and a second nucleic acid encoding a heterologous target mRNA) comprises a circular RNA. In some aspects, a first nucleic acid molecule provided herein (*e.g.*, encoding an influenza NS1 protein) comprises a circular RNA. In some aspects, a second nucleic acid molecule provided herein (*e.g.*, encoding a heterologous target mRNA) comprises a circular RNA. In some aspects, a polynucleotide provided herein comprises a circular RNA, which comprises the first nucleic acid molecule and the second nucleic acid molecule.

[0106] The terms "pharmaceutically-acceptable carrier," "pharmaceutically-acceptable excipient," and grammatical variations thereof, encompass any of the agents approved by a regulatory agency of the U.S. Federal government or listed in the U.S. Pharmacopeia for use in

animals, including humans, as well as any carrier or diluent that does not cause the production of undesirable physiological effects to a degree that prohibits administration of the composition to a subject and does not abrogate the biological activity and properties of the administered compound. Included are excipients and carriers that are useful in preparing a pharmaceutical composition and are generally safe, non-toxic, and desirable.

[0107] As used herein, the term "pharmaceutical composition" refers to one or more of the polynucleotides described herein mixed, or intermingled with, or suspended in one or more other chemical components, such as pharmaceutically-acceptable carriers and excipients. In some aspects, a purpose of a pharmaceutical composition is to facilitate administration of preparations of polynucleotides to a subject.

[0108] The term "polynucleotide" as used herein refers to polymers of nucleotides of any length, including ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. In some aspects, a polynucleotide useful for the present disclosure can be linear. In some aspects, a polynucleotide is circular (*e.g.*, circular RNA). This term refers to the primary structure of the molecule. Thus, the term includes triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide.

[0109] More particularly, the term "polynucleotide" includes polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, hRNA, siRNA, and mRNA (including circular RNA), whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing normucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids "PNAs") and polymorpholino polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA.

[0110] The terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. The polymer can comprise modified amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids such as

homocysteine, ornithine, p-acetylphenylalanine, D-amino acids, and creatine), as well as other modifications known in the art. The term "polypeptide," as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. Polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the foregoing. A polypeptide can be a single polypeptide or can be a multi-molecular complex such as a dimer, trimer or tetramer. They can also comprise single chain or multi-chain polypeptides. Most commonly, disulfide linkages are found in multi-chain polypeptides. The term polypeptide can also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid. In some aspects, a "peptide" can be less than or equal to 50 amino acids long, *e.g.*, about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[0111] The terms "prevent," "preventing," and variants thereof, as used herein, refer partially or completely delaying onset of an disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular disease, disorder, and/or condition; partially or completely delaying progression from a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the disease, disorder, and/or condition. In some aspects, preventing an outcome is achieved through prophylactic treatment.

[0112] As used herein, the term "similarity" refers to the overall relatedness between polymeric molecules, *e.g.*, between polynucleotide molecules (*e.g.*, DNA molecules and/or RNA molecules) and/or between polypeptide molecules. Calculation of percent similarity of polymeric molecules to one another can be performed in the same manner as a calculation of percent identity, except that calculation of percent similarity takes into account conservative substitutions as is understood in the art. It is understood that percentage of similarity is contingent on the comparison scale used, *i.e.*, whether the amino acids are compared, *e.g.*, according to their evolutionary proximity, charge, volume, flexibility, polarity, hydrophobicity, aromaticity, isoelectric point, antigenicity, or combinations thereof.

[0113] The terms "subject," "patient," "individual," and "host," and variants thereof, are used interchangeably herein and refer to any mammalian subject, including without limitation, humans, domestic animals (*e.g.*, dogs, cats and the like), farm animals (*e.g.*, cows, sheep, pigs, horses and the like), and laboratory animals (*e.g.*, monkey, rats, mice, rabbits, guinea pigs and the

like) for whom diagnosis, treatment, or therapy is desired, particularly humans. The disclosures provided herein are applicable to both human therapy and veterinary applications.

[0114] The terms "treat," "treatment," or "treating," as used herein refers to, *e.g.*, the reduction in severity of a disease or condition; the reduction in the duration of a disease course; the amelioration or elimination of one or more symptoms associated with a disease or condition; the provision of beneficial effects to a subject with a disease or condition, without necessarily curing the disease or condition. The term also include prophylaxis or prevention of a disease or condition or its symptoms thereof. In some aspects, the term "treating" or "treatment" means inducing an immune response in a subject against an antigen (*e.g.*, heterologous payload disclosed herein).

[0115] The term "upstream" refers to a nucleotide sequence that is located 5' to a reference nucleotide sequence.

II. Compositions of the Disclosure

[0116] Some aspects of the present disclosure are directed to a polynucleotide or a set of polynucleotides comprising a first nucleic acid molecule encoding an innate immune inhibitor and a second nucleic acid molecule encoding a heterologous target mRNA. In some aspects, the first nucleic acid molecule is circular. In some aspects, the second nucleic acid molecule is circular. In some aspects, both the first nucleic acid molecule and the second nucleic acid molecule are circular. As described herein, in some aspects, the polynucleotide or set of polynucleotides comprise circular RNA. Accordingly, in some aspects, provided herein is a circular RNA comprising a first nucleic acid molecule encoding an innate immune inhibitor and a second nucleic acid molecule encoding a heterologous target mRNA. Also provided herein is a set of circular RNAs comprising a first nucleic acid molecule encoding an innate immune inhibitor and a second nucleic acid molecule encoding a heterologous target mRNA.

[0117] Any innate immune inhibitor can be used in the compositions and methods disclosed herein. Examples of innate immune inhibitors include, but are not limited to Influenza NS1, African swine fever virus (ASFV) g5R, Cocksackievirus B3 (CVB3) 2A protease, CVB3 3C protease, encephalomyocarditis virus (EMCV) 2A protein (*e.g.*, without NLS), EMCV 3C protease, feline calicivirus (FCV) 3C-like protease, foot-and mouth disease virus (FMDV) L protease, group A rotavirus (RVA) NSP3, hantavirus N, human adenovirus 5 (Ad5) 100K, human immunodeficiency virus 1 (HIV-1) protease, human rhinovirus (HRV) 2A protease, HRV 3C protease, human herpesvirus 1 (HSV) vhs, human herpesvirus 1 (HSV) vhs, human T-cell leukemia

virus (HTLV-1) protease, Influenza A virus (FluAv) Pol, human herpesvirus 8 (KSHV) SOX, MD145-12 3C-like protease, measles virus (MV) N, poliovirus (PV) 2A protease, PV 3C protease, moloney murine leukemia virus (MMLV) protease 3C, rabies virus (RV) M, SARS-CoV NSP1, SARS-CoV S, SARS-CoV spike, simian virus 40 (SV40) small T antigen, vaccinia virus (VV) D10, VV D9, mouse 4E-BP1 (*e.g.*, constitutive active), mouse 4E-BP2 (*e.g.*, constitutive active), mouse 4E-BP3 (*e.g.*, constitutive active), mouse 4EHP, mouse Ago1, mouse Ago2, mouse Ago3, mouse Ago4, mouse CPEB2, mouse DDX6, mouse eIF4E, mouse eIF4E (S209A), mouse eIF4E (S209D), mouse eIF4E (S209E), mouse eIF4g (N-term), mouse FMRP, mouse GW182, mouse p54, mouse p56 mouse p60, mouse PABP (eIF4G binding domain), mouse PDCD4, mouse RNase L (NA385: constitutive active), mouse Upf1 (*e.g.*, constitutive active), mouse (Me31B), EBFP2, any derivative thereof, and any combination thereof. In some aspects, the innate immune inhibitor is an innate immune inhibitor disclosed in US Publication No. 20180296702 A, which is incorporated by reference herein in its entirety. In some aspects, the innate immune inhibitor comprises a viral non-structural (NS) protein, *e.g.*, an influenza NS1 protein. In some aspects, the first nucleic acid molecule encodes a viral non-structural (NS) protein. In some aspects, the first nucleic acid molecule encodes an influenza NS1 protein or a derivative thereof.

[0118] In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the second nucleic acid molecule encoding the target mRNA are present in a first vector. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, is present in a first vector, and the second nucleic acid molecule encoding the target mRNA is present in a second vector.

[0119] In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the second nucleic acid molecule encoding the target mRNA are expressed under the control of a single promoter. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the second nucleic acid molecule encoding the target mRNA are transcribed as a polycistronic mRNA. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, is expressed under the control of a first promoter, and the second nucleic acid molecule encoding the target mRNA is expressed under the control of a second promoter. In some aspects, the first promoter and the second promoter are the same. In some aspects, the first promoter and the second promoter are different.

[0120] In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the second nucleic acid molecule encoding the target

mRNA are expressed under the control of a first promoter, wherein the first promoter drives expression of both the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the target mRNA. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, and the second nucleic acid molecule encoding the target mRNA are linked by an IRES sequence.

[0121] In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, the second nucleic acid molecule encoding the target mRNA, or both are expressed under the control of an inducible promoter. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, the second nucleic acid molecule encoding the target mRNA, or both are expressed under the control of tissue specific promoter. In some aspects, the first nucleic acid molecule encoding the innate immune inhibitor, *e.g.*, influenza NS1 protein, the second nucleic acid molecule encoding the target mRNA, or both are expressed under the control of a constitutively active promoter.

[0122] Some aspects of the present disclosure are directed to a polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA, wherein the self-replicating target mRNA comprises one or more modified nucleic acid molecule. In some aspects, less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, or less than about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, about 20%, about 21%, about 22%, about 23%, about 24%, about 25%, about 26%, about 27%, about 28%, about 29%, or about 30% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules. In some aspects, the one or more modified nucleic acid molecule is a modified rNTP.

[0123] In some aspects, the one or more modified nucleic acid molecules comprise N1-methylpseudo uracil. In some aspects, the one or more modified nucleic acid molecules comprise 5-methyl cytosine. In some aspects, the one or more modified nucleic acid molecules comprise N1-methylpseudo uracil and 5-methyl cytosine. Non-limiting examples of additional modified nucleic acid molecules that can be used with the present disclosure include: 6-aza-cytidine, 2-thio-cytidine, α -thio-cytidine, pseudo-iso-cytidine, 5-aminoallyl-uridine, 5-iodo-uridine, 5,6-dihydrouridine, α -thio-uridine, 4-thio-uridine, 6-aza-uridine, 5-hydroxy-uridine, deoxy-thymidine, pseudo-uridine, inosine, α -thio-guanosine, 8-oxo-guanosine, O6-methyl-guanosine, 7-deaza-guanosine, N1-methyl adenosine, 2-amino-6-chloro-purine, N6-methyl-2-amino-purine, 6-chloro-purine, N6-methyl-adenosine, α -thio-adenosine, 8-azido-adenosine, 7-deaza-adenosine, pyrrolo-cytidine, N4-acetyl-cytidine, 5-methyl-uridine, 5-iodo-cytidine, 1,6-Dimethyl-pseudo-uracil, 1-

(optionally substituted C1-C6 Alkyl)-6-(1-propynyl)-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-(2-propynyl)-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-allyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-ethynyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-homoallyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-vinyl-pseudo-uracil, 1-Methyl-6-(2,2,2-Trifluoroethyl)-pseudo-uracil, 1-Methyl-6-(4-morpholino)-pseudo-uracil, 1-Methyl-6-(4-thiomorpholino)-pseudo-uracil, 1-Methyl-6-(optionally substituted phenyl)-pseudo-uracil, 1-Methyl-6-amino-pseudo-uracil, 1-Methyl-6-azido-pseudo-uracil, 1-Methyl-6-bromo-pseudo-uracil, 1-Methyl-6-butyl-pseudo-uracil, 1-Methyl-6-chloro-pseudo-uracil, 1-Methyl-6-cyano-pseudo-uracil, 1-Methyl-6-dimethylamino-pseudo-uracil, 1-Methyl-6-ethoxy-pseudo-uracil, 1-Methyl-6-ethylcarboxylate-pseudo-uracil, 1-Methyl-6-ethyl-pseudo-uracil, 1-Methyl-6-fluoro-pseudo-uracil, 1-Methyl-6-formyl-pseudo-uracil, 1-Methyl-6-hydroxyamino-pseudo-uracil, 1-Methyl-6-hydroxy-pseudo-uracil, 1-Methyl-6-iodo-pseudo-uracil, 1-Methyl-6-iso-propyl-pseudo-uracil, 1-Methyl-6-methoxy-pseudo-uracil, 1-Methyl-6-methylamino-pseudo-uracil, 1-Methyl-6-phenyl-pseudo-uracil, 1-Methyl-6-propyl-pseudo-uracil, 1-Methyl-6-tert-butyl-pseudo-uracil, 1-Methyl-6-trifluoromethoxy-pseudo-uracil, 1-Methyl-6-trifluoromethyl-pseudo-uracil, 6-(2,2,2-Trifluoroethyl)-pseudo-uracil, 6-(4-Morpholino)-pseudo-uracil, 6-(4-Thiomorpholino)-pseudo-uracil, 6-(optionally substituted-Phenyl)-pseudo-uracil, 6-Amino-pseudo-uracil, 6-Azido-pseudo-uracil, 6-Bromo-pseudo-uracil, 6-Butyl-pseudo-uracil, 6-Chloro-pseudo-uracil, 6-Cyano-pseudo-uracil, 6-Dimethylamino-pseudo-uracil, 6-Ethoxy-pseudo-uracil, 6-Ethylcarboxylate-pseudo-uracil, 6-Ethyl-pseudo-uracil, 6-Fluoro-pseudo-uracil, 6-Formyl-pseudo-uracil, 6-Hydroxyamino-pseudo-uracil, 6-Hydroxy-pseudo-uracil, 6-Iodo-pseudo-uracil, 6-iso-Propyl-pseudo-uracil, 6-Methoxy-pseudo-uracil, 6-Methylamino-pseudo-uracil, 6-Methyl-pseudo-uracil, 6-Phenyl-pseudo-uracil, 6-Propyl-pseudo-uracil, 6-tert-Butyl-pseudo-uracil, 6-Trifluoromethoxy-pseudo-uracil, 6-Trifluoromethyl-pseudo-uracil, 1-(3-Amino-3-carboxypropyl)-pseudo-uracil, 1-(2,2,2-Trifluoroethyl)-pseudo-uracil, 1-(2,4,6-Trimethylbenzyl)-pseudo-uracil, 1-(2,4,6-Trimethyl-phenyl)-pseudo-uracil, 1-(2-Amino-2-carboxyethyl)-pseudo-uracil, 1-(2-Amino-ethyl)-pseudo-uracil, 1-(3-Amino-propyl)-pseudo-uracil, 1-(4-Amino-4-carboxybutyl)-pseudo-uracil, 1-(4-Amino-benzyl)-pseudo-uracil, 1-(4-Amino-butyl)-pseudo-uracil, 1-(4-Amino-phenyl)-pseudo-uracil, 1-(4-Methoxy-benzyl)-pseudo-uracil, 1-(4-Methoxy-phenyl)-pseudo-uracil, 1-(4-Methyl-benzyl)-pseudo-uracil, 1-(4-Nitro-benzyl)-pseudo-uracil, 1-(4-Nitro-phenyl)-pseudo-uracil, 1-(5-Amino-pentyl)-pseudo-uracil, 1-(6-Amino-hexyl)-pseudo-uracil, 1-Aminomethyl-pseudo-uracil, 1-Benzyl-pseudo-uracil, 1-Butyl-pseudo-uracil, 1-Cyclobutylmethyl-pseudo-uracil, 1-Cyclobutyl-pseudo-uracil, 1-Cycloheptylmethyl-

pseudo-uracil, 1-Cycloheptyl-pseudo-uracil, 1-Cyclohexylmethyl-pseudo-uracil, 1-Cyclohexyl-pseudo-uracil, 1-Cyclooctylmethyl-pseudo-uracil, 1-Cyclooctyl-pseudo-uracil, 1-Cyclopentylmethyl-pseudo-uracil, 1-Cyclopentyl-pseudo-uracil, 1-Cyclopropylmethyl-pseudo-uracil, 1-Cyclopropyl-pseudo-uracil, 1-Ethyl-pseudo-uracil, 1-Hexyl-pseudo-uracil, 1-iso-Propyl-pseudo-uracil, 1-Pentyl-pseudo-uracil, 1-Phenyl-pseudo-uracil, 1-Propyl-pseudo-uracil, 1-p-toluy-pseudo-uracil, 1-tert-Butyl-pseudo-uracil, 1-Trifluoromethyl-pseudo-uracil, 3-(optionally substituted C1-C6 Alkyl)-pseudo-uracil, Pseudo-uracil-N1-2-ethanoic acid, Pseudo-uracil-N1-3-propionic acid, Pseudo-uracil-N1-4-butanoic acid, Pseudo-uracil-N1-5-pentanoic acid, Pseudo-uracil-N1-6-hexanoic acid, Pseudo-uracil-N1-7-heptanoic acid, Pseudo-uracil-N1-methyl-p-benzoic acid, 6-phenyl-pseudo-uracil, 6-azido-pseudo-uracil, Pseudo-uracil-N1-p-benzoic acid, N3-Methyl-pseudo-uracil, 5-Methyl-amino-methyl-uracil, 5-Carboxy-methyl-amino-methyl-uracil, 5-(carboxyhydroxymethyl)uracil methyl ester 5-(carboxyhydroxymethyl)uracil, 2-anhydro-cytosine, 2-anhydro-uracil, 5-Methoxycarbonylmethyl-2-thio-uracil, 5-Methylaminomethyl-2-seleno-uracil, 5-(iso-Pentenylaminomethyl)-uracil, 5-(iso-Pentenylaminomethyl)-2-thio-uracil, 5-(iso-Pentenylaminomethyl)-uracil, 5-Trideuteromethyl-6-deutero-uracil, 5-(2-Chloro-phenyl)-2-thio-cytosine, 5-(4-Amino-phenyl)-2-thio-cytosine, 5-(2-Furanyl)-uracil, 8-Trifluoromethyl-adenosine, 2-Trifluoromethyl-adenosine, 3-Deaza-3-fluoro-adenosine, 3-Deaza-3-bromo-adenosine, 3-Deaza-3-iodo-adenosine, 1-Hydroxymethyl-pseudo-uracil, 1-(2-Hydroxyethyl)-pseudo-uracil, 1-Methoxymethyl-pseudo-uracil, 1-(2-Methoxyethyl)-pseudo-uracil, 1-(2,2-Diethoxyethyl)-pseudo-uracil, 1-(2-Hydroxypropyl)-pseudo-uracil, (2R)-1-(2-Hydroxypropyl)-pseudo-uracil, (2S)-1-(2-Hydroxypropyl)-pseudo-uracil, 1-Cyanomethyl-pseudo-uracil, 1-Morpholinomethyl-pseudo-uracil, 1-Thiomorpholinomethyl-pseudo-uracil, 1-Benzoyloxymethyl-pseudo-uracil, 1-(2,2,3,3,3-Pentafluoropropyl)-pseudo-uracil, 1-Thiomethoxymethyl-pseudo-uracil, 1-Methanesulfonylmethyl-pseudo-uracil, 1-Vinyl-pseudo-uracil, 1-Allyl-pseudo-uracil, 1-Homoallyl-pseudo-uracil, 1-Propargyl-pseudo-uracil, 1-(4-Fluorobenzyl)-pseudo-uracil, 1-(4-Chlorobenzyl)-pseudo-uracil, 1-(4-Bromobenzyl)-pseudo-uracil, 1-(4-Iodobenzyl)-pseudo-uracil, 1-(4-Methylbenzyl)-pseudo-uracil, 1-(4-Trifluoromethylbenzyl)-pseudo-uracil, 1-(4-Methoxybenzyl)-pseudo-uracil, 1-(4-Trifluoromethoxybenzyl)-pseudo-uracil, 1-(4-Thiomethoxybenzyl)-pseudo-uracil, 1-(4-Methanesulfonylbenzyl)-pseudo-uracil, Pseudo-uracil 1-(4-methylbenzoic acid), Pseudo-uracil 1-(4-methylbenzenesulfonic acid), 1-(2,4,6-Trimethylbenzyl)-pseudouracil, 1-(4-Nitrobenzyl)-pseudo-uracil, 1-(4-Azidobenzyl)-pseudo-uracil, 1-(3,4-Dimethoxybenzyl)-pseudo-uracil, 1-(3,4-Bis-trifluoromethoxybenzyl)-pseudo-uracil, 1-Acetyl-pseudo-uracil, 1-Trifluoroacetyl-pseudo-uracil, 1-Benzoyl-pseudo-uracil, 1-

Pivaloyl-pseudo-uracil, 1-(3-Cyclopropyl-prop-2-ynyl)-pseudouracil, Pseudo-uracil 1-methylphosphonic acid diethyl ester, Pseudo-uracil 1-methylphosphonic acid, Pseudo-uracil 1-[3-(2-ethoxy)]propionic acid, Pseudo-uracil 1-[3-{2-(2-ethoxy)-ethoxy}] propionic acid, Pseudo-uracil 1-[3-{2-(2-[2-ethoxy]-ethoxy)-ethoxy}]propionic acid, Pseudo-uracil 1-[3-{2-(2-[2-(2-ethoxy)-ethoxy]-ethoxy)-ethoxy}]propionic acid, Pseudo-uracil 1-[3-{2-(2-[2-{2(2-ethoxy)-ethoxy}-ethoxy]-ethoxy)-ethoxy}]propionic acid, 1-{3-[2-(2-Aminoethoxy)-ethoxy]-propionyl}pseudo-uracil, 1-[3-(2-{2-[2-(2-Aminoethoxy)-ethoxy]-ethoxy}-ethoxy)-propionyl]pseudo-uracil, 1-Biotinyl-pseudo-uracil, 1-Biotinyl-PEG2-pseudo-uracil, 5-(C3-8 cycloalkyl)-cytosine, 5-methyl-N6-acetyl--cytosine, 5-(carboxymethyl)-N6-trifluoroacetyl-cytosine trifluoromethyl ester, N6-propionyl-cytosine, 5-monofluoromethyl-cytosine, 5-trifluoromethoxy-cytosine, N6-(1,1,1-trifluoro-propionyl)-cytosine, 4-acetyl-pseudo-isocytosine, 1-ethyl-pseudo-isocytosine, 1-hydroxy-pseudo-isocytosine, or 1-(2,2,2-trifluoroethyl)-pseudo-uracil, 1,6-Dimethyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-(1-propynyl)-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-(2-propynyl)-pseudouracil, 1-(optionally substituted C1-C6 Alkyl)-6-allyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-ethynyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-homoallyl-pseudo-uracil, 1-(optionally substituted C1-C6 Alkyl)-6-vinyl-pseudo-uracil, 1-Methyl-6-(2,2,2-Trifluoroethyl)pseudo-uracil, 1-Methyl-6-(4-morpholino)-pseudo-uracil, 1-Methyl-6-(4-thiomorpholino)-pseudo-uracil, 1-Methyl-6-(optionally substituted phenyl)pseudo-uracil, 1-Methyl-6-amino-pseudo-uracil, 1-Methyl-6-azido-pseudo-uracil, 1-Methyl-6-bromo-pseudo-uracil, 1-Methyl-6-butyl-pseudo-uracil, 1-Methyl-6-chloro-pseudo-uracil, 1-Methyl-6-cyano-pseudo-uracil, 1-Methyl-6-dimethylamino-pseudo-uracil, 1-Methyl-6-ethoxy-pseudo-uracil, 1-Methyl-6-ethylcarboxylate-pseudo-uracil, 1-Methyl-6-ethyl-pseudo-uracil, 1-Methyl-6-fluoro-pseudo-uracil, 1-Methyl-6-formyl-pseudo-uracil, 1-Methyl-6-hydroxyamino-pseudo-uracil, 1-Methyl-6-hydroxy-pseudo-uracil, 1-Methyl-6-iodo-pseudo-uracil, 1-Methyl-6-iso-propyl-pseudo-uracil, 1-Methyl-6-methoxy-pseudo-uracil, 1-Methyl-6-methylamino-pseudo-uracil, 1-Methyl-6-phenyl-pseudo-uracil, 1-Methyl-6-propyl-pseudo-uracil, 1-Methyl-6-tert-butyl-pseudo-uracil, 1-Methyl-6-trifluoromethoxy-pseudo-uracil, 1-Methyl-6-trifluoromethyl-pseudo-uracil, 6-(2,2,2-Trifluoroethyl)-pseudo-uracil, 6-(4-Morpholino)-pseudo-uracil, 6-(4-Thiomorpholino)-pseudo-uracil, 6-(Substituted-Phenyl)-pseudo-uracil, 6-Amino-pseudo-uracil, 6-Azido-pseudo-uracil, 6-Bromo-pseudo-uracil, 6-Butyl-pseudo-uracil, 6-Chloro-pseudo-uracil, 6-Cyanopseudo-uracil, 6-Dimethylamino-pseudo-uracil, 6-Ethoxy-pseudo-uracil, 6-Ethylcarboxylate-pseudo-uracil, 6-Ethyl-pseudo-uracil, 6-Fluoro-pseudo-uracil, 6-Formyl-pseudo-uracil, 6-Hydroxyamino-pseudo-uracil, 6-Hydroxy-pseudo-

uracil, 6-Iodo-pseudo-uracil, 6-iso-Propyl-pseudo-uracil, 6-Methoxy-pseudo-uracil, 6-Methylamino-pseudo-uracil, 6-Methyl-pseudo-uracil, 6-Phenyl-pseudo-uracil, 6-Phenyl-pseudo-uracil, 6-Propyl-pseudo-uracil, 6-tert-Butyl-pseudo-uracil, 6-Trifluoromethoxy-pseudo-uracil, 6-Trifluoromethyl-pseudo-uracil, 1-(3-Amino-3-carboxypropyl)pseudo-uracil, 1-(2,2,2-Trifluoroethyl)-pseudo-uracil, 1-(2,4,6-Trimethyl-benzyl)pseudo-uracil, 1-(2,4,6-Trimethyl-phenyl)pseudo-uracil, 1-(2-Amino-2-carboxyethyl)pseudo-uracil, 1-(2-Amino-ethyl)pseudo-uracil, 1-(3-Amino-propyl)pseudo-uracil, 1-(4-Amino-4-carboxybutyl)pseudo-uracil, 1-(4-Amino-benzyl)pseudo-uracil, 1-(4-Amino-butyl)pseudo-uracil, 1-(4-Amino-phenyl)pseudo-uracil, 1-(4-Methoxy-benzyl)pseudo-uracil, 1-(4-Methoxy-phenyl)pseudo-uracil, 1-(4-Methyl-benzyl)pseudo-uracil, 1-(4-Nitro-benzyl)pseudo-uracil, 1-(4-Nitro-phenyl)pseudo-uracil, 1-(5-Amino-pentyl)pseudo-uracil, 1-(6-Amino-hexyl)pseudo-uracil, 1-Aminomethyl-pseudo-uracil, 1-Benzyl-pseudo-uracil, 1-Butyl-pseudo-uracil, 1-Cyclobutylmethyl-pseudo-uracil, 1-Cyclobutyl-pseudo-uracil, 1-Cycloheptylmethyl-pseudo-uracil, 1-Cycloheptyl-pseudo-uracil, 1-Cyclohexylmethyl-pseudo-uracil, 1-Cyclohexyl-pseudo-uracil, 1-Cyclooctylmethyl-pseudo-uracil, 1-Cyclooctyl-pseudo-uracil, 1-Cyclopentylmethyl-pseudo-uracil, 1-Cyclopentyl-pseudo-uracil, 1-Cyclopropylmethyl-pseudo-uracil, 1-Cyclopropyl-pseudo-uracil, 1-Ethyl-pseudo-uracil, 1-Hexyl-pseudo-uracil, 1-iso-Propyl-pseudo-uracil, 1-Pentyl-pseudo-uracil, 1-Phenyl-pseudo-uracil, 1-Propyl-pseudo-uracil, 1-p-tolyl-pseudo-uracil, 1-tert-Butyl-pseudo-uracil, 1-Trifluoromethyl-pseudo-uracil, 3-(optionally substituted C1-C6 Alkyl)-pseudo-uracil, Pseudo-uracil-N1-2-ethanoic acid, Pseudo-uracil-N1-3-propionic acid, Pseudo-uracil-N1-4-butanoic acid, Pseudo-uracil-N1-5-pentanoic acid, Pseudo-uracil-N1-6-hexanoic acid, Pseudo-uracil-N1-7-heptanoic acid, Pseudo-uracil-N1-methyl-p-benzoic acid, 6-phenyl-pseudo-uracil, 6-azido-pseudo-uracil, or Pseudo-uracil-N1-p-benzoic acid, N3-Methyl-pseudo-uracil, 5-Methyl-amino-methyl-uracil, 5-Carboxy-methyl-amino-methyl-uracil, 5-(carboxyhydroxymethyl)uracil methyl ester or 5-(carboxyhydroxymethyl)uracil, and combinations thereof.

[0124] In some aspects, the compositions described herein have increased heterologous target mRNA expression in a host cell. In some aspects, the expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased relative to the expression of the target mRNA expressed from a replicating RNA not comprising one or more modified nucleic acid molecule. In some aspects, the expression of the target mRNA is increased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least

about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, at least about 300%, at least about 350%, at least about 400%, at least about 450%, or at least about 500%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 100%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 200%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 300%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 400%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 500%, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.

[0125] In some aspects, the expression of the target mRNA is increased by at least about 2-fold, at least about 2.5-fold, at least about 3-fold, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, or at least about 6-fold, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 2-fold, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 3-fold, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 4-fold, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 5-fold, *e.g.*, relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein. In some aspects, the expression of the target mRNA is increased by at least about 6-fold, *e.g.*, relative to the expression

of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.

[0126] In some aspects, the compositions described herein have increased heterologous target mRNA expression and/or increased heterologous target mRNA persistence in a host cell. In some aspects, expression of the target mRNA, *e.g.*, increased expression relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein or relative to the expression of the target mRNA expressed from a replicating RNA not comprising one or more modified nucleic acid molecule, persists for at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 24 hours, at least about 30 hours, at least about 36 hours, at least about 42 hours, at least about 48 hours, at least about 60 hours, at least about 72 hours, at least about 84 hours, at least about 96 hours, at least about 108 hours, or at least about 120 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 48 hours. In some aspects, expression of the target mRNA persists for at least about 48 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 60 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 72 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 84 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 96 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 108 hours, following transfection of the cell. In some aspects, expression of the target mRNA persists for at least about 120 hours, following transfection of the cell.

II.A. Heterologous Target mRNA

[0127] The heterologous target mRNA of the present disclosure can encode any polypeptide or functional RNA of interest. In some aspects, the target mRNA encodes a biologically active polypeptide. In some aspects, the biologically active polypeptide comprises a cytokine, a chemokine, a growth factor, a clotting factor, a hormone, a receptor, a mitogen, an immunoglobulin (*e.g.*, an antibody), an enzyme, or any combination thereof.

[0128] In some aspects, the cytokine comprises a cytokine selected from IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, IFN- α , IFN- β , IFN- γ , CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β , TGF- β 1, TGF- β 2, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, MSP, a fragment

thereof, a variant thereof, or any combination thereof. In some aspects, the IL-12 is a single chain IL-12 (scIL-12), protease sensitive IL-12, destabilized IL-12, membrane bound IL-12, intercalated IL-12. In some aspects, the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof. In some aspects, the IL-12 is a human IL-12. In some aspects, the target mRNA encodes a p35 subunit of IL-12 and a p40 subunit of IL-12. In some aspects, the p35 subunit and the p40 subunit are expressed from a single promoter. In some aspects, the p35 subunit and the p40 subunit are expressed as a single contiguous polypeptide. In some aspects, the p35 subunit and the p40 subunit are linked by one or more covalent bonds. In some aspects, the p35 subunit and the p40 subunit are linked by one or more peptide bonds. In some aspects, the target mRNA encodes a human IL-12 polypeptide comprising a p35 subunit of IL-12 covalently linked to a p40 subunit of IL-12. In some aspects, the p35 subunit of IL-12 and the p40 subunit of IL-12 are linked by a linker comprising one or more amino acid.

[0129] In some aspects, the target mRNA comprises a first portion and a second portion, wherein the first portion of the target mRNA encodes a p35 subunit of IL-12 and the second portion of the target mRNA encodes a p40 subunit of IL-12, wherein the first portion and the second portion are separated by an IRES.

[0130] In some aspects, the target mRNA encodes a chemokine. Exemplary chemokines include, but are not limited to, CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5 (RANTES), CCL6, CCL7, CCL8, CCL9 (or CCL10), CCL11, CCL12, CCL13, CCL14, CCL15, CCL16, CCL17, CCL18, CCL19, CCL20, CCL21, CCL22, CCL23, CCL24, CCL25, CCL26, CCL27, CCL28, CXCL1, CXCL2, CXCL3, CXCL4, CXCL5, CXCL6, CXCL7, CXCL8, CXCL9, CXCL10, CXCL11, CXCL12, CXCL13, CXCL14, CXCL15, CXCL16, CXCL17, XCL1, XCL2, and CX3CL1.

[0131] In some aspects, the target mRNA encodes an interferon (IFN). Exemplary interferons include, but are not limited to, interferon type I (*e.g.*, IFN- α , IFN- β , IFN- ϵ , IFN- κ , and IFN- ω), interferon type II (*e.g.*, IFN- γ), and interferon type III. In some aspects, IFN- α is further classified into about 13 subtypes including IFNA1, IFNA2, IFNA4, IFNA5, IFNA6, IFNA7, IFNA8, IFNA10, IFNA13, IFNA14, IFNA16, IFNA17, and IFNA21.

[0132] In some aspects, the target mRNA encodes a growth factor. Exemplary growth factors include, but are not limited to, a bone morphogenetic protein (BMP), angiopoietin, CNTF, LIF, M-CSF, G-CSF, GM-CSF, epidermal growth factor (EGF), an ephrin, erythropoietin (EPO), a fibroblast growth factor (FGF), a glial cell line-derived neurotrophic factor (GDNF), growth differentiation factor-9 (GDF9), hepatocyte growth factor (HGF), hepatoma-derived growth factor

(HDGF), insulin, insulin-like growth factors Insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), keratinocyte growth factor (KGF), migration-stimulating factor (MSF), macrophage-stimulating protein (MSP; also known as hepatocyte growth factor-like protein, HGFLP), myostatin (GDF-8), a neuregulins (NRG), a neurotrophins (*e.g.*, brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4)), placental growth factor (PGF), platelet-derived growth factor (PDGF), renalase (RNLS), T-cell growth factor (TCGF), thrombopoietin (TPO), transforming growth factors Transforming growth factor alpha (TGF- α), transforming growth factor beta (TGF- β), tumor necrosis factor-alpha (TNF- α), vascular endothelial growth factor (VEGF), and members of the Wnt signaling pathway.

[0133] In some aspects, the target mRNA encodes an enzyme. In some aspects, the target mRNA encodes cas9. In some aspects, the target mRNA encodes a zinc finger endonuclease.

[0134] In some aspects, the target mRNA encodes a functional RNA. In some aspects, the target mRNA encodes a miRNA, siRNA, shRNA, a dsRNA, antisense oligonucleotide, a guide RNA, or any combination thereof. In some aspects, the target mRNA encodes a guide RNA, *e.g.*, for use in combination with cas9.

II.B. Influenza NS1

[0135] Any influenza NS1 can be used in the compositions and methods of the present disclosure. In some aspects, the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof. In some aspects, the influenza NS1 is a type A influenza virus NS1. In some aspects, the influenza NS1 is a type B influenza virus NS1. In some aspects, the influenza NS1 is a type C influenza virus NS1.

[0136] In some aspects, the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, a variant thereof, or a combination thereof. In some aspects, the influenza NS1 is an H1N2 NS1. In some aspects, the influenza NS1 is an H2N2 NS1. In some aspects, the influenza NS1 is an H3N2 NS1. In some aspects, the influenza NS1 is an H7N9 NS1. In some aspects, the influenza NS1 is an H7N7 NS1. In some aspects, the influenza NS1 is an H9N2 NS1. In some aspects, the influenza NS1 is an H7N2 NS1. In some aspects, the influenza NS1 is an H7N3 NS1. In some aspects, the influenza NS1 is an H5N2 NS1. In some aspects, the influenza NS1 is an H10N7 NS1.

[0137] In some aspects, the influenza NS1 is an H1N1 NS1. In some aspects, the influenza is the H1N1 TX91 variant NS1. In some aspects, the influenza NS1 encoded by the first nucleic

acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 (Table 1). In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1. In some aspects, the influenza NS1 is the H1N1 TX91 variant NS1 encoded by the nucleotide sequence set forth in SEQ ID NO: 1. In some aspects, the first nucleic acid molecule encoding the influenza NS1 comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1, wherein the nucleotide sequence encodes an influenza NS1 protein. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein comprises the nucleotide sequence set forth in SEQ ID NO: 1, wherein the nucleotide sequence encodes an influenza NS1 protein.

[0138] In some aspects, the influenza NS1 is an H5N1 NS1. In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 2 (Table 1). In some aspects, the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 2. In some aspects, the influenza NS1 is the H1N1 TX91 variant NS1 encoded by the nucleotide sequence set forth in SEQ ID NO: 2. In some aspects, the first nucleic acid molecule encoding the influenza NS1 comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 2, wherein the nucleotide sequence encodes an influenza NS1 protein. In some aspects, the first nucleic acid molecule encoding the influenza NS1 protein comprises the nucleotide sequence set forth in SEQ ID NO: 2, wherein the nucleotide sequence encodes an influenza NS1 protein.

Table 1. Influenza NS1 Sequences

Influenza H1N1 TX91 variant NS1 (SEQ ID NO: 1)

1	AGATTACAAA	GGCAACGAAA	TAATGACGGC	AGCTGCCTCT	CAAGGGCTGA	CCCGTAAAGG
61	TGTGTATGCC	GTTTCGGTACA	AGGTGAATGA	AAATCCTCTG	TACGCACCCA	CCTCAGAACA
121	TGTGAACGTC	CTACTGACCC	GCACGGAGGA	CCGCATCGTG	TGGAAAACAC	TAGCCGGCGA
181	CCCATGGATA	AAAACACTGA	CTGCCAAGTA	CCCTGGGAAT	TTCACTGCCA	CGATAGAGGA
241	GTGGCAAGCA	GAGCATGATG	CCATCATGAG	GCACATCTTG	GAGAGACCCG	ACCTTACCGA
301	CGTCTTCCAG	AATAAGGCAA	ACGTGTGTTG	GGCCAAGGCT	TTAGTGCCCG	TGCTGAAGAC
361	CGCTGGCATA	GACATGACCA	CTGAACAATG	GAACACTGTG	GATTATTTTG	AAACGGACAA
421	AGCTCACTCA	GCAGAGATAG	TATTGAACCA	ACTATGCGTG	AGGTTCTTTG	GACTCGATCT
481	GGACTCCGGT	CTATTTTCTG	CACCCACTGT	TCCGTTATCC	ATTAGGAATA	ATCACTGGGA
541	TAACTCCCCG	TCGCCTAACA	TGTACGGGCT	GAATAAAGAA	GTGGTCCGTC	AGCTCTCTCG
601	CAGGTACCCA	CAACTGCCTC	GGGCAGTTGC	CACTGGAAGA	GTCTATGACA	TGAACACTGG
661	TACTGCGC	AATTATGATC	CGCGCATAAA	CCTAGTACCT	GTAACACGAA	GACTCCCTCA
721	TGCTTTTAGTC	CTCCACCATA	ATGAACACCC	ACAGAGTGAC	TTTTCTTCAT	TCGTACGCAA
781	ATTGAAGGGC	AGAACTGTCC	TGGTGGTCGG	GGAAAAGTTG	TCCGTCCCAG	GCAAAATGGT
841	TGACTGGTTG	TCAGACCGGC	CTGAGGCTAC	CTTCAGAGCT	CGGCTGGATT	TAGGCATCCC
901	AGGTGATGTG	CCCAAATATG	ACATAATATT	TGTTAATGTG	AGGACCCCAT	ATAAATACCA
961	TCACTATCAG	CAGTGTGAAG	ACCATGCCAT	TAAGCTTAGC	ATGTTGACCA	AGAAAGCTTG
1021	TCTGCATCTG	AATCCCGGCG	GAACCTGTGT	CAGCATAGGT	TATGGTTACG	TATGACGGGC
1081	CAGCGAAAGC	ATCATTTGGTG	CTATAGCGCG	GCAGTTCAAG	TTTTCCCCGG	TATGCAAAAC
1141	GAAATCCTCA	CTTGAAGAGA	CGGAAGTTCT	GTTTGTATTC	ATTGGGTACG	ATCGCAAGGC
1201	CCGTACGCAC	AATCCTTACA	AGCTTTCATC	AACCTTGACC	AACATTTTATA	CAGGTTCCAG
1261	ACTCCACGAA	GCCGGATGTG	CACCCTCATA	TCATGTGGTG	CGAGGGGATA	TTGCCACGGC
1321	CACCGAAGGA	GTGATTATAA	ATGCTGCTAA	CAGCAAAGGA	CAACCTGGCG	GAGGGGTGTG
1381	CGGAGCGCTG	TATAAGAAAT	TCCCGGAAAG	CTTCGATTTA	CAGCCGATCG	AAGTAGGAAA
1441	AGCGCGACTG	GTCAAAGGTG	CAGCTAAACA	TATCATTCAT	GCCGTAGGAC	CAAACCTCAA
1501	CAAAGTTTCG	GAGGTTGAAG	GTGACAAACA	GTTGGCAGAG	GCTTATGAGT	CCATCGCTAA
1561	GATTGTCAAC	GATAACAATT	ACAAGTCAGT	AGCGATTCCA	CTGTTGTCCA	CCGGCATCTT
1621	TTCCGGGAAC	AAAGATCGAC	TAACCCAATC	ATTGAACCAT	TTGCTGACAG	CTTTAGACAC
1681	CACTGATGCA	GATGTAGCCA	TATACTGCAG	GGACAAGAAA	TGGGAAATGA	CTCTCAAGGA
1741	AGCAGTGGCT	AGGAGAGAAG	CAGTGGAGGA	GATATGCATA	TCCGACGACT	CTTCAGTGAC
1801	AGAACCTGAT	GCAGAGCTGG	TGAGGGTGCA	TCCGAAGAGT	TCTTTGGCTG	GAAGGAAGGG
1861	CTACAGCACA	AGCGATGGCA	AAACTTTCTC	ATATTTGGAA	GGGACCAAGT	TTCACCAGGC
1921	GGCCAAGGAT	ATAGCAGAAA	TTAATGCCAT	GTGGCCCCGT	GCAACGGAGG	CCAATGAGCA
1981	GGTATGCATG	TATATCCTCG	GAGAAAGCAT	GAGCAGTATT	AGGTCGAAAT	GCCCCGTCGA
2041	AGAGTCGGAA	GCCTCCACAC	CACCTAGCAC	GCTGCCTTGC	TTGTGCATCC	ATGCCATGAC
2101	TCCAGAAAGA	GTACAGCGCC	TAAAAGCCTC	ACGTCCAGAA	CAAATTACTG	TGTGCTCATC
2161	CTTTCCATTG	CCGAAGTATA	GAATCACTGG	TGTGCAGAAG	ATCCAATGCT	CCCAGCCTAT
2221	ATTGTTCTCA	CCGAAAGTGC	CTGCGTATAT	TCATCCAAGG	AAGTATCTCG	TGGAAACACC
2281	ACCGGTAGAC	GAGACTCCGG	AGCCATCGGC	AGAGAACCAA	TCCACAGAGG	GGACACCTGA
2341	ACAACCACCA	CTTATAACCG	AGGATGAGAC	CAGGACTAGA	ACGCCTGAGC	CGATCATCAT
2401	CGAAGAGGAA	GAAGAGGATA	GCATAAGTTT	GCTGTGAGAT	GGCCCCACCC	ACCAGGTGCT
2461	GCAAGTCGAG	GCAGACATTG	ACGGGCCGCC	CTCTGTATCT	AGCTCATCCT	GGTCCATTCC
2521	TCATGCATCC	GACTTTGATG	TGGACAGTTT	ATCCATACTT	GACACCCTGG	AGGGAGCTAG
2581	CGTGACCAGC	GGGGCAACGT	CAGCCGAGAC	TAACCTTTAC	TTCGCAAAGA	GTATGGAGTT
2641	TCTGGCGCGA	CCGGTGCCTG	CGCCTCGAAC	AGTATTTCAGG	AACCCTCCAC	ATCCCCTCC
2701	GCGCACAAAG	ACACCGTCAC	TTGCAACCCAG	CAGGGCCTGC	TCGAGAACCA	GCCTAGTTTC
2761	CACCCCGCCA	GGCGTGAATA	GGGTGATCAC	TAGAGAGGAG	CTCGAGGCGC	TTACCCCGTC
2821	ACGCACTCCT	AGCAGGTCGG	TCTCGAGAAC	CAGCCTGGTC	TCCAACCCGC	CAGGCGTAAA
2881	TAGGGTGATT	ACAAGAGAGG	AGTTTGAGGC	GTTCTGTAGCA	CAACAACAAT	GACGGTTTGA
2941	TGCGGGTGCA	TACATCTTTT	CCTCCGACAC	CGGTCAAGGG	CATTTACAAC	AAAAATCAGT
3001	AAGGCAAACG	GTGCTATCCG	AAGTGGTGTT	GGAGAGGACC	GAATTGGAGA	TTTCGTATGC
3061	CCCGCGCCTC	GACCAAGAAA	AAGAAGAATT	ACTACGCAAG	AAATTACAGT	TAAATCCCAC
3121	ACCTGCTAAC	AGAAGCAGAT	ACCAGTCCAG	GAAGGTGGAG	AACATGAAAG	CCATAACAGC
3181	TAGACGTATT	CTGCAAGGCC	TAGGGCATTG	TTTGAAGGCA	GAAGGAAAAG	TGGAGTGCTA
3241	CCGAACCCCTG	CATCCTGTTT	CTTTGTATTC	ATCTAGTGTG	AACCGTGCCT	TTTCAAGCCC
3301	CAAGGTCGCA	GTGGAAGCCT	GTAACGCCAT	GTTGAAAGAG	AACTTTCCGA	CTGTGGCTTC
3361	TTACTGTATT	ATTCCAGAGT	ACGATGCCTA	TTTGGACATG	GTTGACGGAG	CTTCATGCTG
3421	CTTAGACACT	GCCAGTTTTT	GCCCTGCAAA	GCTGCGCAGC	TTTCCAAGAA	AACACTCCTA
3481	TTTGGAACCC	ACAATACGAT	CGGCAGTGCC	TTTCAAGGATC	CAGAACACGC	TCCAGAACGT

3541	CCTGGCAGCT	GCCACAAAAA	GAAATTGCAA	TGTCACGCAA	ATGAGAGAAT	TGCCCCGTATT
3601	GGATTTCGGCG	GCCTTTAATG	TGGAATGCTT	CAAGAAATAT	GCGTGTAAATA	ATGAATATTG
3661	GGAAACGTTT	AAAGAAAACC	CCATCAGGCT	TACTGAAGAA	AACGTGGTAA	ATTACATTAC
3721	CAAATTAAAA	GGACCAAAAG	CTGCTGCTCT	TTTTCGGAAG	ACACATAATT	TGAATATGTT
3781	GCAGGACATA	CCAATGGACA	GGTTTGTAAT	GGACTTAAAG	AGAGACGTGA	AAGTGACTION
3841	AGGAACAAAA	CATACTGAAG	AACGGCCCCA	GGTACAGGTG	ATCCAGGCTG	CCGATCCGCT
3901	AGCAACAGCG	TATCTGTGCG	GAATCCACCG	AGAGCTGGTT	AGGAGATTAA	ATGCGGTCCCT
3961	GCTTCCGAAC	ATTTCATACAC	TGTTTGATAT	GTCGGCTGAA	GACTIONTGACG	CTATTATAGC
4021	CGAGCACTTC	CAGCCTGGGG	ATTGTGTTCT	GGAAACTGAC	ATCGCGTCGT	TTGATAAAAG
4081	TGAGGACGAC	GCCATGGCTC	TGACCGCGTT	AATGATTCTG	GAAGACTTAG	GTGTGGACGC
4141	AGAGCTGTTG	ACGCTGATTG	AGGCGGCTTT	CGGCGAAATT	TCATCAATAC	ATTTGCCCCAC
4201	TAAAACATAA	TTTAAATTCTG	GAGCCATGAT	GAAATCTGGA	ATGTTCCCTCA	CACCTGTTGT
4261	GAACACAGTC	ATTAACATTG	TAATCGCAAG	CAGAGTGTG	AGAGAACGCG	TAACCGGATC
4321	ACCATGTGCA	GCATTTCATTG	GAGATGACAA	TATCGTGAAA	GGAGTCAAAT	CGGACAAAATT
4381	AATGGCAGAC	AGGTGCGCCA	CCTGGTTGAA	TATGGAAGTC	AAGATTATAG	ATGCTGTGGT
4441	GGGCGAGAAA	GCGCCTTATT	TCTGTGGAGG	GTTTATTTTG	TGTGACTCCG	TGACCGGCAC
4501	AGCGTGCCGT	GTGGCAGACC	CCCTAAAAAG	GCTGTTTAAAG	CTTGGCAAAC	CTCTGGCAGC
4561	AGACGATGAA	CATGATGATG	ACAGGAGAAAG	GGCATTGCAT	GAAGAGTCAA	CACGCTGGAA
4621	CCGAGTGGGT	ATTCTTTCAG	AGCTGTGCAA	GGCAGTAGAA	TCAAGGTATG	AAACCGTAGG
4681	AACTTCCATC	ATAGTTATGG	CCATGACTAC	TCTAGCTAGC	AGTGTTAAAT	CATTACAGCTA
4741	CCTGAGAGGG	GCCCCATATA	CTCTCTACGG	CTAACCTGAA	TGGACTACGA	CATAGTCTAG
4801	TCCGCCAAGG	CCACCAatgga	cagcaacacg	gtgtcaagct	tccaggtcga	ctgcttctctg
4861	tggcacgtgc	gcaagcaggt	ggcggaccag	gagctgggcg	acgccccgtt	cctcgaccg
4921	ctgcgccg	accagaagtc	cctgaagggg	cggggcagca	cgctcggcct	gaacatcgag
4981	accgcgacgt	gcgtcggcaa	gcagatcggt	gagcggatcc	tcaaggaggga	gagcgacgag
5041	gccttccgca	tgaccatggc	gagcgccctg	gcgtcgcgct	acctcacgga	catgacgatc
5101	gaggagatga	gccgggactg	gttcatgctc	atgcccgaagc	agaaggtggc	ggggcccttc
5161	tgctgcgga	tggaccaggc	catcatggac	aagaacatca	tcctgaaggc	gaacttcagc
5221	gtgatcttcg	accgcctgga	gacgtcgacc	ctcctgcgcg	cattcaccca	ggagggggcg
5281	atcgctggcg	agatcagccc	gctgcggtcc	ctgcgggggc	acacgaacga	ggacgtcaag
5341	aacgccatcg	gcgtcctcat	cgggggcctc	gagtggaacg	acaacacgct	cgggtctctc
5401	gagacgctcc	agcgggttcgc	gtggcggagc	agcaacgaga	acggcggggc	gccgctcacc
5461	cccacgcaga	agcgggaagat	ggccggaaag	atccgctccg	aggtctgaTA	ATATGTTACG
5521	TGCAAAGGTG	ATTGTACACC	CCCCAAAGAC	CATATTGTGA	CACACCCTCA	GTATCACGCC
5581	CAAAACATTA	CAGCCGCGGT	GTCAAAAACC	GCGTGGACGT	GGTTAACATC	CCTGTGGGA
5641	GGATCAGCCG	TAATTATTAT	AATTGGCTTG	GTGCTGGCTA	CTATTGTGGC	CATGTACGTG
5701	CTGACCAACC	AGAAACATAA	TTGAATACAG	CAGCAATTGG	CAAGCTGCTT	ACATAGAACT
5761	CGCGGCGATT	GGCATGCCGC	CTTAAATTTT	TTATTTTATT	TTTCTTTTCT	TTTCCGAATC
5821	GGATTTTGT	TTTAAATATT	CAAAAAAATA	AAAAAATAA	AAAAAATAA	AAAAAATAA
5881	ATAGGGATAA	CAGGGTAATT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC
5941	CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG
6001	CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG
6061	AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT
6121	TCTCCCTTCG	GGAAGCGTGG	CGCTTCTCTA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT
6181	GTAGGTCGTT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTTCAGC	CCGACCGCTG
6241	CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT
6301	GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT
6361	CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGAACA	GTATTTGGTA	TCTGCGCTCT
6421	GCTGAAGCCA	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC
6481	CGCTGGTAGC	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC
6541	TCAAGAAGAT	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	AAAACTCACG
6601	TTAAGGGATT	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA
6661	AAAATGAAGT	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA
6721	ATGCTTAATC	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT	CCATAGTTGC
6781	CTGACTCCCC	GTCGTGTAGA	TAACTACGAT	ACGGGAGGGC	TTACCATCTG	GCCCCAGTGC
6841	TGCAATGATA	CCGCGAGACC	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAAACCAGCC
6901	AGCCGGAAGG	GCCGAGCGCA	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA	TCCAGTCTAT
6961	TAATTGTTGC	CGGGAAGCTA	GAGTAAGTAG	TTCCGCGATT	AATAGTTTGC	GCAACGTTGT
7021	TGCCATTGCT	ACAGGCATCG	TGTTGTACAG	CTCGTCGTTT	GGTATGGCTT	CATTACGCTC

7081	CGGTTCCCAA	CGATCAAGGC	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	AAGCGGTTAG
7141	CTCCTTCGGT	CCTCCGATCG	TTGTGAGAAG	TAAGTTGGCC	GCAGTGTTAT	CACTCATGGT
7201	TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC
7261	TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	GTTGCTCTTG
7321	CCCGGCGTCA	ATACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT
7381	TGGAAAACGT	TCTTCGGGGC	GAAAACCTCT	AAGGATCTTA	CCGCTGTTGA	GATCCAGTTC
7441	GATGTAACCC	ACTCGTGCAC	CCAACCTGATC	TTCAGCATCT	TTTACTTTCA	CCAGCGTTTC
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9961	GATCAGGCAA	GTCTGGCATC	ATTAAGGCG	CAGTCACCAA	AAAAGATCTA	GTGGTGAGCG
10021	CCAAGAAAGA	AACTGTGCA	GAAATTATAA	GGGACGTCAA	GAAAATGAAA	GGGCTGGACG
10081	TCAATGCCAG	AACTGTGGAC	TCAGTGCCTT	TGAATGGATG	CAAACACCCC	GTAGAGACCC
10141	TGTATATTGA	CGAAGCTTTT	GCTTGTCTAG	CAGGTACTCT	CAGAGCGCTC	ATAGCCATTA
10201	TAAGACCTAA	AAAGGCAGTG	CTCTGCGGGG	ATCCCAAACA	GTGCGGTTTT	TTTAACATGA
10261	TGTGCCTGAA	AGTGCATTTT	AACCACGAGA	TTTGCACACA	AGTCTTCCAC	AAAAGCATCT
10321	CTCGCCGTTG	CACTAAATCT	GTGACTTCGG	TCGTCTCAAC	CTTGTTTTAC	GACAAAAAAA
10381	TGAGAACGAC	GAATCCGAAA	GAGACTAAGA	TTGTGATTGA	CACTACCGGC	AGTACCAAAC
10441	CTAAGCAGGA	CGATCTCATT	CTCACTTGTT	TCAGAGGGTG	GGTGAAGCAG	TTGCAAA

II.C. Vectors

[0139] In some aspects, one or more of the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are present in a vector. Any vectors can be used in the compositions and method disclosed herein. In some aspects, the vector comprises a viral vector, a mammalian vector, bacterial vector, or a combination or variant thereof. In some aspects, the vector is selected from the group consisting of an adenoviral vector, a lentivirus, a Sendai virus vector, a baculoviral vector, an Epstein Barr viral vector, a papovaviral vector, a vaccinia viral vector, a herpes simplex viral vector, a hybrid vector, and an adeno associated virus (AAV) vector.

[0140] In some aspects, the vector comprises a replicon. In some aspects, a replicon derived from an alphavirus comprises a positive-strand RNA that encodes RNA-dependent RNA polymerases that simultaneously transcribe therapeutic payloads and self-amplify the replicon on entry into the cytoplasm. In some aspects, the vector comprises a replicon, wherein the replicon comprises (1) UTRs of the parent virus and non-structural proteins and subgenomic promoter (SGP) of the parent virus and (2) the first nucleic acid molecule encoding the influenza NS1 protein, the second nucleic acid molecule encoding the target mRNA, or both the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA. Any replicon can be used in the compositions and methods disclosed herein. In some aspects, the replicon comprises a Venezuelan equine encephalitis (VEE) replicon or a derivative or portion thereof. In some aspects, the replicon comprises one or more point mutation relative to the parent viral replicon, *e.g.*, one or more point mutation relative to the VEE replicon sequence. Modifications to the sequence of the replicon can be used to increase the expression of the target mRNA, to increase the persistence of the target mRNA (*e.g.*, by reducing an immune response against the target mRNA or the polynucleotide encoding the target mRNA and/or by reducing type I interferon activity in a target cell, *e.g.*, a target cancer cell), or both. In some aspects, the vector comprises a VEE replicon, wherein the VEE replicon comprises a Q739L mutation relative to the parent VEE replicon. In some aspects, the first vector, the second vector, or both comprise one or more additional regulatory elements. In some aspects, the first vector, the second vector, or both comprise a tissue specific promoter, a tissue specific enhancer, a tissue specific silencer, or any combination thereof.

[0141] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) the second nucleic acid molecule encoding the target mRNA; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0142] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA; and (vi) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0143] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) the second nucleic acid molecule encoding the target mRNA; (v) an E1 sequence; and (vi) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0144] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0145] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0146] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0147] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) the second nucleic acid molecule encoding the target mRNA; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0148] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0149] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0150] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0151] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE. In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about

75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE. In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 1; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

[0152] In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule encoding an H5N1 NS1; (iv) a P2A linker; (v) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE. In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 2; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE. In some aspects, the vector comprises (i) a 5'UTR from a parent replicon, *e.g.*, a 5'UTR from VEE; (ii) one or more non-structural protein (nsP) from a parent replicon, *e.g.*, one or more nsP from VEE, *e.g.*, nsP2, nsP3, and nsP4 from VEE; (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 2; (iv) the second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.

II.D. Lipid Nanoparticles

[0153] Some aspects of the present disclosure are directed to a lipid nanoparticle comprising, *e.g.*, encapsulating, a polynucleotide or a set of polynucleotides disclosed herein. In some aspects, the polynucleotide or the set of polynucleotides disclosed herein is packaged and/or delivered in a lipid nanoparticle. Accordingly, in some aspects, the present disclosure relates to a polynucleotide described herein encapsulated by lipid nanoparticles, the composition thereof, and use of the composition thereof.

[0154] A "lipid nanoparticle" (LNP), as used herein, refers to a vesicle, such as a spherical vesicle, having a contiguous lipid bilayer. Lipid nanoparticles can be used in methods by which pharmaceutical therapies are delivered to targeted locations. Non-limiting examples of LNPs include liposomes, bolaamphiphiles, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and monolayer membrane structures (*e.g.*, archaeosomes and micelles).

[0155] In some aspects, the lipid nanoparticle comprises one or more types of lipids. A lipid, as used herein, refers to a group of organic compounds that include, but are not limited to, esters of fatty acids and in some aspects are characterized by being insoluble in water, but soluble in many organic solvents. They are usually divided into at least three classes: (1) "simple lipids," which include fats and oils as well as waxes; (2) "compound lipids," which include phospholipids and glycolipids; and (3) "derived lipids" such as steroids. Non-limiting examples of lipids include triglycerides (*e.g.*, tristearin), diglycerides (*e.g.*, glycerol behenate), monoglycerides (*e.g.*, glycerol monostearate), fatty acids (*e.g.*, stearic acid), steroids (*e.g.*, cholesterol), and waxes (*e.g.*, cetyl palmitate). In some aspects, the one or more types of lipids in the LNP comprises a cationic lipid. In some aspects, the one or more types of lipids in the LNP comprises a lipidoid, *e.g.*, TT3.

[0156] Such lipids useful for the present disclosure include, but are not limited to N1,N3,N5-tris(3-(didodecylamino)propyl)benzene-1,3,5-tricarboxamide (TT3), N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTAP); lipofectamine; 1,2-DiLinoleyloxy-N,N-dimethylaminopropane (DLinDMA), 1,2-Dilinolenyloxy-N,N-dimethylaminopropane (DLenDMA); dioctadecyldimethylammonium (DODMA), Distearyldimethylammonium (DSDMA), N,N-dioleoyl-N,N,-dimethylammonium chloride (DODAC); N-(2,3-dioleoyloxy)propyl)-N,N,N-trimethylammonium chloride (DOTMA); N,N-distearyl-N,N-dimethylammonium bromide (DDAB); 3-(N—(N',N'-dimethylaminoethane)-carbamoyl)cholesterol (DC-Chol) and N-(1,2-dimyristyloxprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide (DMRIE).

[0157] In some aspects of the disclosure, the lipids, *e.g.*, lipidoid, is TT3. TT3, as used herein, is capable of forming lipid nanoparticles for delivery of various biologic active agents into the cells.

[0158] In some aspects of the disclosure, the cationic lipid is DOTAP. DOTAP, as used herein, is also capable of forming lipid nanoparticles. DOTAP can be used for the highly efficient transfection of DNA including yeast artificial chromosomes (YACs) into eukaryotic cells for transient or stable gene expression, and is also suitable for the efficient transfer of other negatively charged molecules, such as RNA, oligonucleotides, nucleotides, ribonucleoprotein (RNP) complexes, and proteins into research samples of mammalian cells.

[0159] In some aspects of the disclosure, the cationic lipid is lipofectamine. Lipofectamine, as used herein, is a common transfection reagent, produced and sold by Invitrogen, used in molecular and cellular biology. It is used to increase the transfection efficiency of RNA (including mRNA and siRNA) or plasmid DNA into in vitro cell cultures by lipofection. Lipofectamine contains lipid subunits that can form liposomes or lipid nanoparticles in an aqueous environment, which entrap the transfection payload, *e.g.*, modRNA. The RNA-containing liposomes (positively charged on their surface) can fuse with the negatively charged plasma membrane of living cells, due to the neutral co-lipid mediating fusion of the liposome with the cell membrane, allowing nucleic acid cargo molecules to cross into the cytoplasm for replication or expression.

[0160] In some aspects, LNPs are composed primarily of cationic lipids along with other lipid ingredients. These typically include other lipid molecules belonging but not limited to the phosphatidylcholine (PC) class (*e.g.*, 1,3-Distearoyl-sn-glycero-3-phosphocholine (DSPC), and 1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), sterols (*e.g.*, cholesterol) and Polyethylene glycol (PEG)-lipid conjugates (*e.g.*, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[folate(polyethylene glycol)-2000 (DSPE-PEG2000), and C14-PEG2000. Table 2 shows the formulation of exemplary LNPs, TT3-LNP and DOTAP-LNP.

Table 2.

DOTAP-LNP	DOTAP	DSPC	Cholesterol	DSPE-PEG2000
Molar ratio	40	10	48	2
TT3-LNP	TT3	DOPE	Cholesterol	C14-PEG2000
Molar Ratio	20	30	40	0.75

[0161] In some aspects, the LNP comprises C14-PEG2000. In some aspects, C14-PEG2000 comprises 1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (DMG-PEG2000), 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] (DMPE-PEG2000), or both. In some aspects, the C14-PEG2000 (or other lipid ingredients disclosed herein) can be embedded in the LNP prior to the encapsulation of the polynucleotide. In some aspects, the C14-PEG2000 (or other lipid ingredients disclosed herein) can be added to the LNP after the encapsulation of the polynucleotide.

[0162] Particle size of lipid nanoparticles can affect drug release rate, bio-distribution, mucoadhesion, cellular uptake of water and buffer exchange to the interior of the nanoparticles, and protein diffusion. In some aspects of the disclosure, the diameter of the LNPs ranges from about 30 to about 500 nm. In some aspects of the disclosure, the diameter of the LNPs ranges from about 30 to about 500 nm, about 50 to about 400 nm, about 70 to about 300 nm, about 100 to about 200 nm, about 100 to about 175 nm, or about 100 to about 160 nm. In some aspects of the disclosure, the diameter of the LNPs ranges from 100-160 nm. In some aspects of the disclosure, the diameter of the LNPs can be about 30 nm, about 40 nm, about 50 nm, about 60 nm, about 70 nm, about 80 nm, about 90 nm, about 100 nm, about 101 nm, about 102 nm, about 103 nm, about 104 nm, about 105 nm, about 106 nm, about 107 nm, about 108 nm, about 109 nm, about 110 nm, about 111 nm, about 112 nm, about 113 nm, about 114 nm, about 115 nm, about 116 nm, about 117 nm, about 118 nm, about 119 nm, about 120 nm., about 130 nm, about 140 nm, about 150 nm, or about 160 nm. In some aspects, the lipid nanoparticle has a diameter of about 140 nm.

[0163] Zeta potential is a measure of the effective electric charge on the lipid nanoparticle surface. The magnitude of the zeta potential provides information about particle stability. In some aspects of the disclosure, the zeta potential of the LNPs ranges from about 3 to about 6 mv. In some aspects of the disclosure, the zeta potential of the LNPs can be about 3 mv, about 3.1 mv, about 3.2 mv, about 3.3 mv, about 3.4 mv, about 3.5 mv, about 3.6 mv, about 3.7 mv, about 3.8 mv, about 3.9 mv, about 4 mv, about 4.1 mv, about 4.2 mv, about 4.3 mv, about 4.4 mv, about 4.5 mv, about 4.6 mv, about 4.7 mv, about 4.8 mv, about 4.9 mv, about 5 mv, about 5.1 mv, about 5.2 mv, about 5.3 mv, about 5.4 mv, about 5.5 mv, about 5.6 mv, about 5.7 mv, about 5.8 mv, about 5.9 mv, or about 6 mv.

[0164] In some aspects, the disclosure is related to encapsulated polynucleotide or set of polynucleotides with lipid nanoparticles (LNPs). In some aspects of the disclosure, the mass ratio between the lipid of LNPs and the polynucleotide or set of polynucleotides ranges from about 1:2 to about 15:1. In some aspects, the mass ratio between the lipid and the polynucleotide or set of

polynucleotides can be about 1:2, about 1:1.9, about 1:1.8, about 1:1.7, about 1:1.6, about 1:1.5, about 1:1.4, about 1:1.3, about 1:1.2, about 1:1.1, about 1:1, about 1.1:1, about 1.2:1, about 1.3:1, about 1.4:1, about 1.5:1, about 1.6:1, about 1.7:1, about 1.8:1, about 1.9:1, about 2:1, about 2.5:1, about 3:1, about 3.5:1, about 4:1, about 4.5:1, about 5:1, about 5.5:1, about 6:1, about 6.5:1, about 7:1, about 7.5:1, about 8:1, about 8.5:1, about 9:1, about 9.5:1, about 10:1, about 10.5:1, about 11:1, about 11.5:1, about 12:1, about 12.5:1, about 13:1, about 13.5:1, about 14:1, about 14.5:1, or about 15:1. In some aspects of the disclosure, the mass ratio between the lipid and the polynucleotide or set of polynucleotides is about 10:1.

II.E. Cells

[0165] In some aspects, provided herein are cells that have been modified to comprise a polynucleotide or set of polynucleotides described herein. In some aspects, the cells comprise a vector or a set of vectors that comprise(s) a polynucleotide or set of polynucleotides described herein. In some aspects, the cells comprise a lipid nanoparticle that comprises a polynucleotide or set of polynucleotides described herein. In some aspects, the cell is a cancer cell. In some aspects, the cell is an immune cell. In some aspects, immune cell comprises a T cell (*e.g.*, CD4⁺ T cell, CD8⁺ T cell, or both), a natural killer cell (NK cell), a tumor infiltrating lymphocyte, a dendritic cell, a B cell, a bone marrow cell, a monocyte, or a PBMC.

[0166] In some aspects, the cells described herein (*i.e.*, comprising a polynucleotide or a set of polynucleotides of the present disclosure or a vector or a set of vectors comprising the same) can produce the (i) human influenza NS1 and (ii) the target heterologous mRNA. In some aspects, the cells described herein (*i.e.*, comprising a polynucleotide or a set of polynucleotides of the present disclosure or a vector or a set of vectors comprising the same) can produce the (i) human influenza NS1 and (ii) the target heterologous mRNA *in vivo*. For instance, in some aspects, a polynucleotide or a set of polynucleotides of the present disclosure or a vector or a set of vectors comprising the same can be introduced into a cell *ex vivo* (*e.g.*, via transfection), and then the cell can be administered to a subject (*e.g.*, adoptive cell therapy), wherein the (i) human influenza NS1 and (ii) the target heterologous mRNA are produced in the subject after the administration. In some aspects, a polynucleotide or a set of polynucleotides of the present disclosure or a vector or a set of vectors comprising the same can be administered to a subject, *e.g.*, as part of a gene therapy. In some aspects, the cells described herein (*i.e.*, comprising a polynucleotide or a set of polynucleotides of the present disclosure or a vector or a set of vectors comprising the same) can

produce the (i) human influenza NS1 and (ii) the target heterologous mRNA both *in vitro* and *in vivo*.

[0167] In some aspects, the cell is a host cell. In some aspects, the host cell is a eukaryotic cell. In some aspects, the host cell is selected from the group consisting of a mammalian cell, an insect cell, a yeast cell, a transgenic mammalian cell, a plant cell, and any combination thereof. In some aspects, the host cell is a prokaryotic cell. In some aspects, the prokaryotic cell is a bacterial cell.

[0168] In some aspects, the host cell is a mammalian cell. Non-limiting examples of mammalian host cells that are suitable for the present disclosure include: CHO, VERO, BHK, Hela, MDCK, HEK 293, NIH 3T3, W138, BT483, Hs578T, HTB2, BT2O and T47D, NS0 (a murine myeloma cell line that does not endogenously produce any immunoglobulin chains), CRL703O, COS (*e.g.*, COS1 or COS), PER.C6, VERO, HsS78Bst, HEK-293T, HepG2, SP210, R1.1, B-W, L-M, BSC1, BSC40, YB/20, BMT10, HBK, NSO, HT1080, HsS78Bst cells, and combinations thereof.

II.F. Pharmaceutical compositions

[0169] As is apparent from the present disclosure, any of the polynucleotides, vectors, lipid nanoparticles, and cells described herein (also referred to herein as "active compounds") can be incorporated into pharmaceutical compositions suitable for administration. Accordingly, in some aspects, the pharmaceutical composition comprises the active compound and a pharmaceutically acceptable excipient.

[0170] As used herein, the term "pharmaceutically acceptable excipient" (also referred to herein as "pharmaceutically acceptable carrier") comprises any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active compounds is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0171] In some aspects, disclosed herein is a pharmaceutical composition comprising (a) a polynucleotide or a set of polynucleotides described herein and (b) a pharmaceutically acceptable excipient. In some aspects, disclosed herein is a pharmaceutical composition comprising (a) a vector or a set of vectors as described herein and (b) a pharmaceutically acceptable excipient. In some aspects, disclosed herein is a pharmaceutical composition comprising (a) a lipid nanoparticle

as described herein and (b) a pharmaceutically acceptable excipient. In some aspects, disclosed herein is a pharmaceutical composition comprising (a) a cell as described herein (*e.g.*, modified to comprise a polynucleotide or a set of polynucleotides of the present disclosure) and (b) a pharmaceutically acceptable excipient.

[0172] A pharmaceutical composition of the present disclosure is formulated to be compatible with its intended route of administration. In some aspects, a suitable route of administration that can be used with the present disclosure comprises intramuscular administration. In some aspects, a suitable route of administration includes intranasal administration. Additional examples of suitable routes of administration include parenteral, *e.g.*, intravenous, intradermal, subcutaneous, oral, transdermal (topical), and transmucosal, and any combination thereof. Another route of administration includes pulmonary administration. In addition, it can be desirable to administer a therapeutically effective amount of the pharmaceutical composition locally to an area in need of treatment. This can be achieved by, for example, local or regional infusion or perfusion during surgery, topical application, injection, catheter, suppository, or implant (for example, implants formed from porous, non-porous, or gelatinous materials, including membranes, such as sialastic membranes or fibers), and the like. In some aspects, the therapeutically effective amount of the pharmaceutical composition is delivered in a vesicle, such as liposomes (see, *e.g.*, Langer, *Science* 249:1527-33, 1990 and Treat *et al.*, in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez Berestein and Fidler (eds.), Liss, N.Y., pp. 353-65, 1989).

[0173] In some aspects, a pharmaceutical composition described herein can be delivered in a controlled release system. For instance, in some aspects, a pump can be used (*see, e.g.*, Langer, *Science* 249:1527-33, 1990; Sefton, *Crit. Rev. Biomed. Eng.* 14:201-40, 1987; Buchwald *et al.*, *Surgery* 88:507-16, 1980; Saudek *et al.*, *N Engl. J Med.* 321:574-79, 1989). In some aspects, polymeric materials can be used (*see, e.g.*, Levy *et al.*, *Science* 228:190-92, 1985; During *et al.*, *Ann. Neural.* 25:351-56, 1989; Howard *et al.*, *J Neurosurg.* 71:105-12, 1989). Other controlled release systems, such as those discussed by Langer (*Science* 249:1527-33, 1990), can also be used.

[0174] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or

immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.*, Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

[0175] Pharmaceutically acceptable carriers used in parenteral preparations include aqueous vehicles, nonaqueous vehicles, antimicrobial agents, isotonic agents, buffers, antioxidants, local anesthetics, suspending and dispersing agents, emulsifying agents, sequestering or chelating agents and other pharmaceutically acceptable substances. Examples of aqueous vehicles include Sodium Chloride Injection, Ringers Injection, Isotonic Dextrose Injection, Sterile Water Injection, Dextrose and Lactated Ringers Injection. Nonaqueous parenteral vehicles include fixed oils of vegetable origin, cottonseed oil, corn oil, sesame oil and peanut oil. Antimicrobial agents in bacteriostatic or fungistatic concentrations can be added to parenteral preparations packaged in multiple-dose containers, which include phenols or cresols, mercurials, benzyl alcohol, chlorobutanol, methyl and propyl p-hydroxybenzoic acid esters, thimerosal, benzalkonium chloride and benzethonium chloride. Isotonic agents include sodium chloride and dextrose. Buffers include phosphate and citrate. Antioxidants include sodium bisulfate. Local anesthetics include procaine hydrochloride. Suspending and dispersing agents include sodium carboxymethylcellulose, hydroxypropyl methylcellulose and polyvinylpyrrolidone. Emulsifying agents include Polysorbate 80 (TWEEN® 80). A sequestering or chelating agent of metal ions includes EDTA. Pharmaceutical carriers also include ethyl alcohol, polyethylene glycol and propylene glycol for water miscible vehicles; and sodium hydroxide, hydrochloric acid, citric acid or lactic acid for pH adjustment.

[0176] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes, or multiple dose vials made of glass or plastic.

[0177] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELS (BASF; Parsippany, NJ), or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion, and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride, in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

[0178] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the methods of preparation can be vacuum drying and freeze-drying, which yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0179] For administration by inhalation, the compounds are delivered in the form of an aerosol spray from a pressurized container or dispenser that contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Systemic administration can also be by transmucosal or transdermal means.

[0180] For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic

acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art. The compounds can also be prepared in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[0181] In some aspects, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No. 4,522,811.

[0182] In some aspects, active compounds of the present disclosure can be formulated in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated with each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the disclosure are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such a functional compound for the treatment of individuals. The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

III. Methods of the Disclosure

[0183] Some aspects of the present disclosure are directed to nucleic acid molecules that increase the expression of a target heterologous mRNA. As such, the compositions of the present disclosure can be used in methods to increase expression of a target in a cell, including a cell in a human subject in need of a treatment.

[0184] Some aspects of the present disclosure are directed to a method of expressing a target mRNA in a cell, comprising co-expressing the target mRNA and an influenza NS1 protein in the cell, wherein the target mRNA is not an influenza mRNA. In some aspects, the influenza

NS1 protein is encoded by a first nucleic acid molecule and the target mRNA is encoded by a second nucleic acid molecule, *e.g.*, as described herein.

[0185] Some aspects of the present disclosure are directed to method of treating a disease or condition in a subject in need thereof comprising administering to the subject composition disclosed herein, *e.g.*, a polynucleotide or set of polynucleotides disclosed herein, a vector or a set of vectors disclosed herein, a lipid nanoparticle a cell disclosed herein, or a pharmaceutical composition disclosed herein. For example, a composition described herein can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, *e.g.*, *in vivo*, to induce the selective and persistent expression of an encoded target heterologous mRNA in a cell (*e.g.*, a tumor cell and/or an immune cell), which, in some aspects, can help treat a disease or disorder.

[0186] Accordingly, in some aspects, the present disclosure is directed to therapeutic methods using a composition described herein. In some aspects, disclosed herein is a method of expressing a target heterologous mRNA in a subject in need thereof, comprising administering to the subject a composition of the present disclosure.

[0187] In some aspects, a disease or disorder that can be treated with the present disclosure includes a cancer. In some aspects, the cancer comprises a squamous cell carcinoma, small-cell lung cancer (SCLC), non-small cell lung cancer, squamous non-small cell lung cancer (NSCLC), nonsquamous NSCLC, gastrointestinal cancer, renal cancer (*e.g.*, clear cell carcinoma), ovarian cancer, liver cancer (*e.g.*, hepatocellular carcinoma), colorectal cancer, endometrial cancer, kidney cancer (*e.g.*, renal cell carcinoma (RCC)), prostate cancer (*e.g.*, hormone refractory prostate adenocarcinoma), thyroid cancer, pancreatic cancer, cervical cancer, stomach cancer, bladder cancer, hepatoma, breast cancer, colon carcinoma, and head and neck cancer (or carcinoma), gastric cancer, germ cell tumor, pediatric sarcoma, sinonasal natural killer, melanoma (*e.g.*, metastatic malignant melanoma, such as cutaneous or intraocular malignant melanoma), bone cancer, skin cancer, uterine cancer, cancer of the anal region, testicular cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, cancer of the esophagus (*e.g.*, gastroesophageal junction cancer), cancer of the small intestine, cancer of the endocrine system, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, solid tumors of childhood, cancer of the ureter, carcinoma of the renal pelvis, tumor angiogenesis, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell lymphoma, environmentally-induced cancers including those induced by asbestos, virus-related cancers or cancers of viral origin (*e.g.*, human papilloma virus (HPV-related or -originating tumors)), and

hematologic malignancies derived from either of the two major blood cell lineages, *i.e.*, the myeloid cell line (which produces granulocytes, erythrocytes, thrombocytes, macrophages and mast cells) or lymphoid cell line (which produces B, T, NK and plasma cells), such as all types of leukemias, lymphomas, and myelomas, *e.g.*, acute, chronic, lymphocytic and/or myelogenous leukemias, such as acute leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML), undifferentiated AML (MO), myeloblastic leukemia (M1), myeloblastic leukemia (M2; with cell maturation), promyelocytic leukemia (M3 or M3 variant [M3V]), myelomonocytic leukemia (M4 or M4 variant with eosinophilia [M4E]), monocytic leukemia (M5), erythroleukemia (M6), megakaryoblastic leukemia (M7), isolated granulocytic sarcoma, and chloroma; lymphomas, such as Hodgkin's lymphoma (HL), non-Hodgkin's lymphoma (NHL), B cell hematologic malignancy, *e.g.*, B-cell lymphomas, T-cell lymphomas, lymphoplasmacytoid lymphoma, monocytoid B-cell lymphoma, mucosa-associated lymphoid tissue (MALT) lymphoma, anaplastic (*e.g.*, Ki1⁺) large-cell lymphoma, adult T-cell lymphoma/leukemia, mantle cell lymphoma, angio immunoblastic T-cell lymphoma, angiocentric lymphoma, intestinal T-cell lymphoma, primary mediastinal B-cell lymphoma, precursor T-lymphoblastic lymphoma, T-lymphoblastic; and lymphoma/leukaemia (T-Lbly/T-ALL), peripheral T- cell lymphoma, lymphoblastic lymphoma, post-transplantation lymphoproliferative disorder, true histiocytic lymphoma, primary effusion lymphoma, B cell lymphoma, lymphoblastic lymphoma (LBL), hematopoietic tumors of lymphoid lineage, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, Burkitt's lymphoma, follicular lymphoma, diffuse histiocytic lymphoma (DHL), immunoblastic large cell lymphoma, precursor B-lymphoblastic lymphoma, cutaneous T-cell lymphoma (CTLC) (also called mycosis fungoides or Sezary syndrome), and lymphoplasmacytoid lymphoma (LPL) with Waldenstrom's macroglobulinemia; myelomas, such as IgG myeloma, light chain myeloma, nonsecretory myeloma, smoldering myeloma (also called indolent myeloma), solitary plasmacytoma, and multiple myelomas, chronic lymphocytic leukemia (CLL), hairy cell lymphoma; hematopoietic tumors of myeloid lineage, tumors of mesenchymal origin, including fibrosarcoma and rhabdomyosarcoma; seminoma, teratocarcinoma, tumors of mesenchymal origin, including fibrosarcoma, rhabdomyosarcoma, and osteosarcoma; and other tumors, including melanoma, xeroderma pigmentosum, keratoacanthoma, seminoma, thyroid follicular cancer and teratocarcinoma, hematopoietic tumors of lymphoid lineage, for example T-cell and B-cell tumors, including but not limited to T-cell disorders such as T-prolymphocytic leukemia (T-PLL), including of the small cell and cerebriform cell type; large granular lymphocyte leukemia (LGL)

of the T-cell type; a/d T-NHL hepatosplenic lymphoma; peripheral/post-thymic T cell lymphoma (pleomorphic and immunoblastic subtypes); angiocentric (nasal) T-cell lymphoma; cancer of the head or neck, renal cancer, rectal cancer, cancer of the thyroid gland; acute myeloid lymphoma, or any combination thereof.

[0188] In some aspects, a composition described herein is administered to a subject in need thereof at an amount sufficient to reduce tumor burden or cancer cell growth *in vivo* by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or greater. In some aspects, the composition described herein is administered in an amount effective in increasing immune activity by at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or greater.

[0189] In some aspects, a disease or disorder that can be treated with the present disclosure comprises an autoimmune disease. As used herein, the term "autoimmune disease" refers to a disease caused by an inability of a host's immune system to distinguish foreign molecules from self-molecules, such that the host's immune system attacks and destroys the self-molecules. As used herein, "self-molecules" (*e.g.*, protein or DNA) refer to a molecule that is derived from or is native to a host. As used herein, "foreign molecules" refer to molecules that are derived from another, and are of a non-native origin. Non-limiting examples of autoimmune diseases include: multiple sclerosis, peripheral neuritis, Sjogren's syndrome, rheumatoid arthritis, alopecia, autoimmune pancreatitis, Behcet's disease, Bullous pemphigoid, Celiac disease, Devic's disease (neuromyelitis optica), Glomerulonephritis, IgA nephropathy, assorted vasculitides, scleroderma, diabetes, arteritis, vitiligo, ulcerative colitis, irritable bowel syndrome, psoriasis, uveitis, systemic lupus erythematosus, Graves' disease, myasthenia gravis, pemphigus vulgaris, anti-glomerular basement membrane disease (Goodpasture syndrome), Hashimoto's thyroiditis, autoimmune hepatitis, and combinations thereof.

[0190] In some aspects, where the disease or disorder to be treated comprises an autoimmune disease, administering the composition (*e.g.*, the polynucleotide, vector, lipid nanoparticle, or pharmaceutical composition described herein) to a subject can decrease immune activity, such as T cell activity, in the subject. For instance, as is apparent from the present disclosure, in some aspects, by encoding a target heterologous mRNA that is capable of decreasing immune cell function and/or promoting immune suppressor activity (*e.g.*, promoting the development regulatory T cells), a polynucleotide described herein can be used to decrease

immune activity. In some aspects, the immune activity is decreased by at least about 5%, by at least about 10%, by at least about 20%, by at least about 30%, by at least about 40%, by at least about 50%, by at least about 60%, by at least about 70%, by at least about 80%, by at least about 90% or more, compared to the immune activity of a reference subject (*e.g.*, the subject prior to the administration of the composition or a corresponding subject that did not receive an administration of the composition).

Examples

Example 1

[0191] Replicon plasmids used in the present example included the following: Strand-EGFP (SEQ ID NO: 3; Table 6); Strand-mCherry (SEQ ID NO: 4; Table 6); non-cytopathic-EGFP (SEQ ID NO: 5; FIG. 1A; Table 6); Strand-NS1-H5N1 (SEQ ID NO: 2; Table 1); Strand-NS1-TX91 (SEQ ID NO: 1; Table 1); Strand-NS1-EGFP (SEQ ID NO: 6; Table 6); Strand-NS1-mCherry (SEQ ID NO: 7; Table 6); and non-cytopathic-NS1-EGFP (SEQ ID NO: 8; FIG. 1B; Table 6).

[0192] Methods

[0193] Template preparation

[0194] For replicon RNA, the VEE replicon vector containing the EGFP-encoding mRNA payload was prepared by a suitable plasmid preparation method. The plasmid was further linearized by BspQI treatment. Briefly, 5 µg of replicon plasmid DNA was treated with BspQI in NEB3.1 buffer for 3hr at 50°C. The enzyme was heat inactivated at 80°C for 20 minutes, and the samples proceeded to a DNA cleanup step.

Table 3.

Component	Volume	Concentration
DNA template		1 µg
Cutsmart buffer/NEB 3.1	5	1x
BspQI/IsceI	1	5 units
Water	44	

[0195] For mCherry containing replicon plasmids (Strand-mCherry and Strand-NS1-mCherry) as well as only NS1 containing plasmids (Strand-NS1-H5N1 and Strand-NS1-TX91), IsceI was used to digest the replicon template. The reaction included Cutsmart buffer at 37°C for 3hrs.

[0196] modRNA template generation

[0197] For modified RNA (modRNA) templates, the DNA was generated by PCR using a replicon plasmid with forward primer containing T7 promoter and subgenomic promoter and a reverse primer in the 3'-UTR with a stretch of 120 amino acids.

Table 4A. PCR setup

COMPONENT	25 μ l REACTION	FINAL CONCENTRATION
10 μ M Forward Primer	1.25 μ l	0.5 μ M
10 μ M Reverse Primer	1.25 μ l	0.5 μ M
Template DNA	variable	10 ng
2X Q5 Hot Start Master mix	12.5 μ l	
Nuclease-Free Water	to 25 μ l	

Table 4B. PCR Cycling Conditions

STEP	TEMP	TIME
Initial Denaturation	98°C	30 seconds
30 Cycles	98°C	10 seconds
	72°C	4 minutes
Final Extension	72°C	4 minutes
Hold	4–10°C	

[0198] DpnI digestion

[0199] Plasmid DNA (template) in the PCR reaction was digested by DpnI. Add 1 μ L DpnI per μ g of initial plasmid to PCR sample and incubated for 1 hr at 37°C.

[0200] DNA cleanup

[0201] PCR-amplified DNA samples were cleaned using the NEB PCR cleanup kit, according to the manufacture's protocol, and DNA was eluted in 20 μ L water.

[0202] PCR (modRNA template) and BspQI-treated replicon DNA (repRNA template) were checked on the pre-cast gels to confirm the purity (PCR) and integrity (replicon template). 1 μ g of purified template was employed in each 20 μ L IVT reaction, below.

[0203] Up to 20 ng total DNA was loaded on the 1.2% DNA gels and run at 275V for 7-10 minutes.

[0204] In vitro transcription

[0205] NEB HiScribe High yield T7 kit was used for RNA production. For modified RNA (modRNA) synthesis, the UTP component of the kit was replaced by N1-methylpseudouridine-5' - triphosphate.

[0206] When ready for the IVT reaction, the necessary kit components were thawed on ice, mixed and pulse-spinned in microfuge to collect solutions to the bottom of tubes. Samples were kept on ice, and the enzyme was not vortexed.

[0207] Co-transcriptional capping method

[0208] For production using cap analog replicon plasmids and modRNA templates, the reaction was assembled at room temperature in the following order:

Component	Volume	Final Conc.
Nuclease-free water	X μ l	
10X Reaction Buffer (NEB)	10 μ l	1X
ATP (100 mM)	10 μ l	10 mM final
GTP (100 mM)	10 μ l	10 mM final
UTP or N1-methylpseudoUTP (100 mM)	10 μ l	10 mM final
CTP (100 mM) or 5-methylCTP (100mm)	10 μ l	10 mM final
Cap Analog	5 μ l	
Template DNA	X μ l	1 μ g
T7 RNA Polymerase Mix	10 μ l	
SUPERase Inh.	5 μ l	
Total reaction volume	100 μ l	

[0209] Samples were then mixed thoroughly, pulse-spun in microfuge, and incubated at 37°C for 3 hours in a thermomixer at 400rpm. A 1uL aliquot was reserved for quality control.

[0210] DNase treatment

[0211] Turbo DNase enzyme was used to digest template DNA. 10x buffer was not added as the enzyme is active in IVT reactions. The reaction was diluted to 200 uL with nuclease free water.

[0212] 20 μ L enzyme (2U/ μ L) was used per 100 μ L IVT reaction and incubated for 60 minutes at 37°C. Following the reaction, the RNA was purified using Monarch RNA cleanup kit. A 1 μ L aliquot of RNA was kept for quality control.

[0213] RNA Quality Control

[0214] The concentration of the sample was checked on Nanodrop and quality control on gel with RNA from all intermediate steps. Up to 200 ng RNA was run on a 1.2% RNA gel. 1uL of Lonza RNA ladder was used as a size marker. RNA was denatured by adding 50% formaldehyde sample buffer at 65°C for 5 minutes, and the samples were immediately kept on ice for at least one minute.

[0215] Up to 5 uL total sample was loaded on the gel and visualized. RNA integrity can also be checked by running on a fragment analyzer.

[0216] Prepare conventional TT3 LNPs formulations with T-junction

[0217] The lipid materials were each weighed out and dissolved in ethanol. The ethanol phase was prepared by mixing all the lipid materials according to composition ratio in the form below. The aqueous phase was prepared by diluting the silica column purified repRNA/modRNA with 20 mM Citrate Buffer (pH 4.0), 300 mM NaCl and water so that the final composition of the salt was 10 mM citrate buffer (pH 4.0, 150 mM NaCl). The conventional TT3 LNPs were afforded by mixing the ethanol phase and aqueous phase of the LNPs through T-junction mixing at the flow rate ratio of 3:1 (aqueous phase: ethanol phase).

Table 5. TT3 LNP Composition (mg)

mRNA	1.0
TT3	10.0
DOPE	8.0
Cholesterol	5.6
DMG-PEG-2k	0.7

[0218] Preparation of post-PEG micelles TT3 LNPs formulations with T-junction

[0219] The lipid materials were each weighed out and dissolved in ethanol. The ethanol phase was prepared by mixing all the lipid materials except from DMG-PEG-2K, according to composition ratio in the form above. The aqueous phase was prepared by diluting the silica column purified repRNA/modRNA with 20 mM Citrate Buffer (pH 4.0), 300 mM NaCl and water so that the final composition of the salt was 10 mM citrate buffer (pH 4.0, 150 mM NaCl). PEG micelle phase was prepared by adding the corresponding volume of DMG-PEG-2K into TBS buffer and mixing thoroughly via vortex. Finally, the post-PEG micelles TT3 LNPs were afforded by first mixing the ethanol phase and aqueous phase of the LNPs through a T-junction mixing at the flow

rate ratio of 3:1 (aqueous phase: ethanol phase), and followed by an immediate in-line dilution with the PEG micelle phase via T-junction mixing at the flow rate ratio of 1:1 (LNP phase:PEG phase). The final lipid composition of post-PEG micelle TT3 LNP is described in Table B.

Table A

TT3 LNP Composition Component	Weight Ratio
mRNA	1.0
TT3	10.0
DOPE	8.0
Cholesterol	5.6
PEG-DMG-2k Micelle	Weight Ratio
mRNA	1.0
DMG-PEG-2K	4.2

Table B

TT3 LNP Composition Component	Weight Ratio
mRNA	1.0
TT3	10.0
DOPE	8.0
Cholesterol	5.6
DMG-PEG-2K	4.2

[0220] Buffer exchange and Freeze/Thaw of TT3 LNPs

[0221] The afforded TT3 LNPs were transferred to the dialysis cassettes and dialyze in TBS buffer for 2 hours. Then the TT3 LNPs were concentrated via tangential flow filtration. Subsequently, 40% sucrose (W/V) in TBS stock solution was added into all the prepared TT3 LNPs to make a final solution of TT3 LNPs in 10% sucrose. The final RNA concentrations of LNPs were measured by dissociating the LNPs with 2% TE+Triton and further detected with Qubit

assay. TT3 LNPs were aliquot into 50 µl/tube aliquots and put the at -80 °C for freezing. Before treating cells with LNPs, TT3 LNPs were thawed at room temperature.

[0222] Lipid transfection

[0223] For the cell lines, 50 ng TT3:RNA was added to respective wells. For some experiments, Lipofectamine MessengerMax (Thermo Fisher) was used to deliver payloads according to manufacturer's recommendations.

[0224] FACS sample preparation

[0225] Zombie NIR staining buffer was prepared by diluting the 100X dye stock in PBS. Cells were washed in PBS and transferred to a deep well 96-well plate and centrifuged at 500g, 10 minutes for suspension cells. For adherent cells, cells are trypsinized for 5 minutes followed by centrifugation at 500g, 10 minutes. Cells are resuspended in PBS and transferred to 96 V-bottom plate, followed by live/dead staining using 100 uL of PBS containing the Zombie NIR dye for 10 minutes at RT in the dark. The reaction is stopped by adding 200 uL of FACS buffer (contains BSA to quench the dye) and centrifuged again and resuspended finally in 200 uL FACS buffer. The cells are analyzed on the flow cytometer for reporter (GFP or mCherry) signal and viability.

[0226] Results

[0227] Tandem transfection of NS1 with repRNA improved replicon expression in the 4T1 mouse tumor cell line. 4T1 cells were transfected with Strand-mCherry, mCherry modRNA, NS1 modRNA mRNAs using Lipofectamine MessengerMax. In this tandem transfection, NS1 modRNA was transfected 6hrs prior to transfecting the repRNA. This provides cells the opportunity to provide NS1 protein before the cells experience the replicon. Twenty-four hours following transfection, cells were visualized by microscopy as well as processed by Flow cytometry to look at the quantitative signal (FIGs. 2A-2D). Tandem transfection of NS1 from both H5N1 and TX91 with repRNA improved replicon expression (FIG. 2E).

[0228] Next, 4T1 cells were transfected with Strand-mCherry, mCherry modRNA, NS1 modRNA mRNAs using TT3 lipid nanoparticle. In this co-transfection, NS1 modRNA was transfected together with the repRNA. Twenty-four hours following transfection, cells were visualized by microscopy as well as processed by Flow cytometry to look at the quantitative signal (FIGs. 3A-3F). Tandem NS1 expression (FIG. 3E) and NS1 cotransfection (FIG. 3F) mediated higher MFI from repRNA in 4T1 cells.

[0229] Co-transfection of NS1 with repRNA improves replicon expression in a variety of human cancer cell lines. Hcc38 tumor cells were transfected with NS1 modRNA (FIGs. 4B and 4F), NS1 repRNA (FIGs. 4C and 4G), or NS1-P2A-mCherry mRNA (FIGs. 4D and 4H) using

Lipofectamine MessengerMax. In this experiment, NS1 was provided either as a modRNA (NS1 modRNA; FIGs. 4B and 4F) or as a separate (NS1 repRNA; FIGs. 4C and 4G) or a bi-cistronic repRNA vector (NS1-P2A-mCherry; FIGs. 4D and 4H). Twenty-four (FIGs. 4A-4D) or forty-eight (FIGs. 4E-4H) hours following transfection, cells were visualized by microscopy as well as processed by Flow cytometry to look at the quantitative signal. Similarly, median fluorescence intensity (MFI) was observed to increase with NS1 expression in Scc9 HNSCCs (FIG. 4I) and FaDu HNSCC cells (FIGs. 4J-4K).

[0230] Additive improvements were observed when combining vector engineering with NS1. BT20 cancer cells were transfected using TT3 LNP using EGFP encoding replicon vector containing either the original (Strand) backbone (FIGs. 5A-5B) or one containing a Q739L mutation (non-cytopathic; FIGs. 5C-5D) and the cells were analyzed using Flow cytometry to quantify the reporter signal. An additive improvement was readily apparent when combining NS1 in the Q739L vector (FIGs. 5A-5F).

[0231] Next, B16.F10 (FIGs. 6A-6C) or 4T1 (FIGs. 6D-6F) cells were electroporated with replicon made either using unmodified rNTPs (Unmodified Rep; FIGs. 6A and 6D) or using a ratio of 1:1 (50%; FIGs. 6B and 6E) or 1:3 (25%; FIGs. 6C and 6F) UTP to N1-methyl-pseudoUTP (denoted by psi). While 1:1 ratio leads to significant reduction in payload expression, 1:3 ratio maintains the expression as well as signal intensity (FIG. 6G).

[0232] Additive effects were observed for the combination of vector engineering and the use of modified rNTPs in replicon driven payload expression (FIG. 7). Firefly luciferase (Fluc) encoded replicons were made either using unmodified rNTPs (unmod) or using a 1:3 ratio of certain NTPs- U: Ψ (M1) or U: Ψ; 1:3 C:5me-C (M2), where C refers to Cytidine and 5me-C refers to 5-methyl-cytidine. Luminescence was measured at 24 as 48hrs post transfection via electroporation in 4T1 cells (FIG. 7). These Fluc replicons were next transfected in an interferon inducible cell line, B16-ISG (Invivogen) and payload expression was measured in Luminescence units (FIG. 8A) while Type I IFN activity was measured using the SEAP reporter assay using colorimetry (FIG. 8B). Singly (M1) or doubly modified (M2) replicons showed improvement in payload expression as well as reduced Type I IFN activity.

[0233] The use of modified rNTPs in replicon driven payload expression improved expression and reduced IFN activation (FIGs. 9A-9C). B16-ISG cells were transfected with a Q739L replicon expressing NS1-EGFP (P2A linker) made either using unmodified or singly (M1) or doubly modified rNTPs, as described above. Doubly modified replicons improve payload

expression (FIG. 9A), signal intensity (FIG. 9B), and reduced Type I IFN activity (FIG. 9C) as compared to Poly (I:C) treated cells. Poly (I:C) is a dsRNA analog and a strong Type I IFN agonist.

[0234] LNP delivery in T cells activated for 2 days with Anti-CD3/CD28/CD2 cocktail with high dose IL-2 was improved by addition of a recombinant protein (Enhancer) to the media, leading to robust signal in cells transfected with lipid 2-cholesterol (FIG. 10E) or lipid 2-b-sitosterol (FIG. 10F) as compared to cells transfected with Lipid 1 and cells not exposed to Enhancer (FIGs. 10A-10D).

[0235] Use of modified rNTPs in replicon driven payload expression improved expression and reduced IFN activation. Primary human T cells were activated using IL-2 and anti-CD3/CD28/CD2 for 2 days post thaw and transfected using the Q739L replicon driving NS1-EGFP, as used above, and made using unmodified or singly (M1) or doubly modified (M2) mRNAs. As an addition, unmodified vectors were spiked with 1 or 10% Poly (I:C), the dsRNA analog, and co-encapsulated in the lipid. Twenty-four hours post transfection, the cells were analyzed by flow cytometry to quantify GFP signal, and the cell supernatants were used to analyze pro-inflammatory cytokines, *e.g.*, IFN-gamma. The M2 replicons show the highest level of expression (FIGs. 11A-11B) while maintaining the lowest amount of IFN-gamma secretion (FIG. 11C).

[0236] Human PBMCs were isolated from three healthy donors and either grown with low dose IL-2 (Resting) or with high dose of IL-2 in presence of Anti-CD3/CD28/CD2 cocktail (Activated) for 2 days followed by mRNA:lipid delivery with M2 modified Q739L replicons driving NS1-EGFP or EGFP or with conventional EGFP mRNA (EGFP-mod). Twenty-four hours post-transfection, the cells were stained with a Live-dead viability stain and analyzed for GFP signal (FIG. 12A). The supernatants were collected for cytokine profiling and were subjected to ELISA for IFN-alpha detection. NS1 encoding replicons reduced IFN-alpha activation in both resting and activated states (FIG. 12B).

Sequences

Table 6. Sequences

Strand-EGFP (SEQ ID NO: 3)						
1	gctcttcttaa	gTAATACGAC	TCACTATAAT	GGCGGCGCA	TGAGAGAAGC	CCAGACCAAT
61	TACCTACCCA	AAATGGAGAA	AGTTCACGTT	GACATCGAGG	AAGACAGCCC	ATTCTCTCAGA
121	GCTTTGCAGC	GGAGCTTCCC	GCAGTTTGAG	GTAGAAGCCA	AGCAGGTCAC	TGATAATGAC
181	CATGCTAATG	CCAGAGCGTT	TTCGCATCTG	GCTTCAAAAC	TGATCGAAAC	GGAGGTGGAC
241	CCATCCGACA	CGATCCTTGA	CATTGGAAGT	GCGCCCGCCC	GCAGAAATGTA	TTCTAAGCAC
301	AAGTATCATT	GTATCTGTCC	GATGAGATGT	GCGGAAGATC	CGGACAGATT	GTATAAGTAT
361	GCAACTAAGC	TGAAGAAAAA	CTGTAAGGAA	ATAACTGATA	AGGAATTGGA	CAAGAAAATG
421	AAGGAGCTCG	CCGCCGTCAT	GAGCGACCCT	GACCTGGAAA	CTGAGACTAT	GTGCCCTCCAC
481	GACGACGAGT	CGTGTGCTA	CGAAGGGCAA	GTCGCTGTTT	ACCAGGATGT	ATACGCGGTT
541	GACGGACCGA	CAAGTCTCTA	TCACCAAGCC	AATAAGGGAG	TTAGAGTCGC	CTACTGGATA
601	GGCTTTGACA	CCACCCCTTT	TATGTTTAAG	AACTTGGCTG	GAGCATATCC	ATCATACTCT
661	ACCAACTGGG	CCGACGAAAC	CGTGTTAAGC	GCTCGTAACA	TAGGCCATATG	CAGCTCTGAC
721	GTTATGGAGC	GGTCACGTAG	AGGGATGTCC	ATTCTTAGAA	AGAAGTATTT	GAAACCATCC
781	AACAATGTTC	TATTCTCTGT	TGGCTCGACC	ATCTACCACG	AGAAGAGGGA	CTTACTGAGG
841	AGCTGGCACC	TGCCGTCTGT	ATTTCACTTA	CGTGGCAAGC	AAAATTACAC	ATGTCGGTGT
901	GAGACTATAG	TTAGTTGCGA	CGGGTACGTC	GTTAAAAGAA	TAGCTATCAG	TCCAGGCCTG
961	TATGGGAAGC	CTTCAGGCTA	TGCTGCTACG	ATGCACCGCG	AGGGATTCTT	GTGCTGCAAA
1021	GTGACAGACA	CATTGAACGG	GGAGAGGGTC	TCTTTTCCCG	TGTGCACGTA	TGTGCCAGCT
1081	ACATTGTGTG	ACCAAATGAC	TGGCATACTG	GCAACAGATG	TCAGTGCCGA	CGACGCGCAA
1141	AAACTGCTGG	TTGGGCTCAA	CCAGCGTATA	GTCGTCAACG	GTGCGACCCA	GAGAAACACC
1201	AATACCATGA	AAAATTACCT	TTTGCCCGTA	GTGGCCAGG	CATTTGCTAG	GTGGGCAAAG
1261	GAATATAAGG	AAGATCAAGA	AGATGAAAGG	CCACTAGGAC	TACGAGATAG	ACAGTTAGTC
1321	ATGGGGTGTT	GTTGGGCTTT	TAGAAGGCAC	AAGATAACAT	CTATTTATAA	GCGCCCGGAT
1381	ACCCAAACCA	TCATCAAAGT	GAACAGCGAT	TTCCACTCAT	TCGTGCTGCC	CAGGATAGGC
1441	AGTAACACAT	TGGAGATCGG	GCTGAGAACA	AGAATCAGGA	AAATGTTAGA	GGAGCACAAG
1501	GAGCCGTCAC	CTCTCATTAC	CGCCGAGGAC	GTACAAGAAG	CTAAGTGCGC	AGCCGATGAG
1561	GCTAAGGAGG	TGCGTGAAGC	CGAGGAGTTG	CGCGCAGCTC	TACCACCTTT	GGCAGCTGAT
1621	GTTGAGGAGC	CCACTCTGGA	AGCCGATGTC	GACTTGATGT	TACAAGAGGC	TGGGGCCGGC
1681	TCAGTGGAGA	CACCTCGTGG	CTTGATAAAG	GTTACCAGCT	ACGATGGCGA	GGACAAGATC
1741	GGCTCTTACG	CTGTGCTTTC	TCCGCAAGCT	GTAATCAAGA	GTGAAAAATT	ATCTTGATC
1801	CACCCTCTCG	CTGAACAAGT	CATAGTGATA	ACACACTCTG	GCCGAAAAGG	GCGTTATGCC
1861	GTGGAACCAT	ACCATGGTAA	AGTAGTGGTG	CCAGAGGGAC	ATGCAATACC	CGTCCAGGAC
1921	TTTCAAGCTC	TGAGTGAAAG	TGCCACCATT	GTTGACAACG	AACGTGAGTT	CGTAAACAGG
1981	TACCTGCACC	ATATTGCCAC	ACATGGAGGA	GCGCTGAACA	CTGATGAAGA	ATATTACAAA
2041	ACTGTCAAGC	CCAGCGAGCA	CGACGGCGAA	TACCTGTACG	ACATCGACAG	GAAACAGTGC
2101	GTCAAGAAAG	AACTAGTCAC	TGGGCTAGGG	CTCACAGGCG	AGCTGGTGGA	TCCTCCCTTC
2161	CATGAATTCG	CCTACGAGAG	TCTGAGAACA	CGACCAGCCG	CTCCTTACCA	AGTACCAACC
2221	ATAGGGGTGT	ATGGCGTGCC	AGGATCAGGC	AAGTCTGGCA	TCATTAAAAG	CGCAGTCACC
2281	AAAAAAGATC	TAGTGGTGAG	CGCCAAGAAA	GAAAACGTGT	CAGAAATTAT	AAGGGACGTC
2341	AAGAAAATGA	AAGGGCTGGA	CGTCAATGCC	AGAAGTGTGG	ACTCAGTGCT	CTTGAATGGA
2401	TGCAACACC	CCGTAGAGAC	CCTGTATATT	GACGAAGCTT	TTGCTTGTC	TGCAGGTAAT
2461	CTCAGAGCGC	TCATAGCCAT	TATAAGACCT	AAAAAGGCAG	TGCTCTGCGG	GGATCCCAAA
2521	CAGTGCGGTT	TTTTTAAACAT	GATGTGCCTG	AAAGTGCATT	TTAACCACGA	GATTTCACAC
2581	CAAGTCTTCC	ACAAAAGCAT	CTCTCGCCGT	TGCACTAAAT	CTGTGACTTC	GGTCGTCTCA
2641	ACCTTGTTT	ACGACAAAAA	AATGAGAACG	ACGAATCCGA	AAGAGACTAA	GATTGTGATT
2701	GACACTACCG	GCAGTACCAA	ACCTAAGCAG	GACGATCTCA	TTCTCACTTG	TTTCAGAGGG
2761	TGGGTGAAGC	AGTTGCAAAAT	AGATTACAAA	GGCAACGAAA	TAATGACGGC	AGCTGCCTCT
2821	CAAGGGCTGA	CCCGTAAAGG	TGTGTATGCC	GTTTCGGTACA	AGGTGAATGA	AAATCCTCTG
2881	TACGCACCCA	CCTCAGAACA	TGTGAACGTC	CTACTGACCC	GCACGGAGGA	CCGCATCGTG
2941	TGGAAAACAC	TAGCCGGCGA	CCCATGGATA	AAAACACTGA	CTGCCAAGTA	CCCTGGGAAT
3001	TTCACTGCCA	CGATAGAGGA	GTGGCAAGCA	GAGCATGATG	CCATCATGAG	GCACATCTTG
3061	GAGAGACCGG	ACCCTACCGA	CGTCTTCCAG	AATAAGGCAA	ACGTGTGTTG	GGCCAAGGCT
3121	TTAGTGCCGG	TGCTGAAGAC	CGCTGGCATA	GACATGACCA	CTGAACAATG	GAACACTGTG

3181	GATTATTTTG	AAACGGACAA	AGCTCACTCA	GCAGAGATAG	TATTGAACCA	ACTATGCGTG
3241	AGGTTCTTTG	GACTCGATCT	GGACTCCGGT	CTATTTTCTG	CACCCACTGT	TCCGTTATCC
3301	ATTAGGAATA	ATCACTGGGA	TAACTCCCCG	TCGCCTAACA	TGTACGGGCT	GAATAAAGAA
3361	GTGGTCCGTC	AGCTCTCTCG	CAGGTACCCA	CAACTGCCTC	GGGCAGTTGC	CACTGGAAGA
3421	GTCTATGACA	TGAACACTGG	TACACTGCGC	AATTATGATC	CGCGCATAAA	CCTAGTACCT
3481	GTAACAGAA	GACTGCCTCA	TGCTTTAGTC	CTCCACCATA	ATGAACACCC	ACAGAGTGAC
3541	TTTTCTTCAT	TCGTGAGCAA	ATTGAAGGGC	AGAACTGTCC	TGGTGGTCCG	GGAAAAGTTG
3601	TCCGTCCCAG	GCAAAATGGT	TGACTGGTTG	TCAGACCGGC	CTGAGGCTAC	CTTCAGAGCT
3661	CGGCTGGATT	TAGGCATCCC	AGGTGATGTG	CCCAAATATG	ACATAATATT	TGTTAATGTG
3721	AGGACCCCAT	ATAAATACCA	TCACTATCAG	CAGTGTGAAG	ACCATGCCAT	TAAGCTTAGC
3781	ATGTTGACCA	AGAAAGCTTG	TCTGCATCTG	AATCCCGGCG	GAACCTGTGT	CAGCATAGGT
3841	TATGTTTACG	CTGACAGGGC	CAGCGAAAGC	ATCATTGGTG	CTATAGCGCG	GAGTTCAAG
3901	TTTTCCCGGG	TATGCAAACC	GAAATCCTCA	CTTGAAGAGA	CGGAAGTTCT	GTTTGTATTTC
3961	ATTGGGTACG	ATCGCAAGGC	CCGTACGCAC	AATCCTTACA	AGCTTTTCATC	AACCTTGACC
4021	AACATTTATA	CAGGTTCCAG	ACTCCACGAA	GCCGGATGTG	CACCCTCATA	TCATGTGGTG
4081	CGAGGGGATA	TTGCCACGGC	CACCGAAGGA	GTGATTATAA	ATGCTGCTAA	CAGCAAAGGA
4141	CAACCTGGCG	GAGGGGTGTG	CGGAGCGCTG	TATAAGAAAT	TCCCGGAAAG	CTTCGATTTA
4201	CAGCCGATCG	AAGTAGGAAA	AGCGGACTG	GTCAAAGGTG	CAGCTAAACA	TATCATTCAT
4261	GCCGTAGGAC	CAAACCTCAA	CAAAGTTTCG	GAGGTTGAAG	GTGACAAACA	GTTGGCAGAG
4321	GCTTATGAGT	CCATCGCTAA	GATTGTCAAC	GATAACAATT	ACAAGTCAGT	AGCGATTCCA
4381	CTGTTGTCCA	CCGGCATCTT	TTCCGGGAAC	AAAGATCGAC	TAACCCAATC	ATTGAACCAT
4441	TTGCTGACAG	CTTTAGACAC	CACTGATGCA	GATGTAGCCA	TATACTGCAG	GGACAAGAAA
4501	TGGGAAATGA	CTCTCAAGGA	AGCAGTGGCT	AGGAGAGAAG	CAGTGGAGGA	GATATGCATA
4561	TCCGACGACT	CTTCAGTGAC	AGAACCTGAT	GCAGAGCTGG	TGAGGGTGCA	TCCGAAGAGT
4621	TCTTTGGCTG	GAAGGAAGGG	CTACAGCACA	AGCGATGGCA	AAACTTTCTC	ATATTTGGAA
4681	GGGACCAAGT	TTCACCAGGC	GGCCAAGGAT	ATAGCAGAAA	TTAATGCCAT	GTGGCCCGTT
4741	GCAACGGAGG	CCAATGAGCA	GGTATGCATG	TATATCCTCG	GAGAAAGCAT	GAGCAGTATT
4801	AGGTCGAAAT	GCCCCGTCGA	AGAGTCGGAA	GCCTCCACAC	CACCTAGCAC	GCTGCCTTGC
4861	TTGTGCATCC	ATGCCATGAC	TCCAGAAAGA	GTACAGCGCC	TAAAAGCCTC	ACGTCCAGAA
4921	CAAAATTACTG	TGTGCTCATC	CTTTCCATTG	CCGAAAGTATA	GAATCACTGG	TGTGCAGAAAG
4981	ATCCAATGCT	CCCAGCCTAT	ATTGTTCTCA	CCGAAAGTGC	CTGCGTATAT	TCATCCAAGG
5041	AAGTATCTCG	TGGAAACACC	ACCGGTAGAC	GAGACTCCGG	AGCCATCGGC	AGAGAACCAA
5101	TCCACAGAGG	GGACACCTGA	ACAACCACCA	CTTATAACCG	AGGATGAGAC	CAGGACTAGA
5161	ACGCCTGAGC	CGATCATCAT	CGAAGAGGAA	GAAGAGGATA	GCATAAGTTT	GCTGTCAAGT
5221	GGCCCGACCC	ACCAGGTGCT	GCAAGTCGAG	GCAGACATTC	ACGGGCCGCC	CTCTGTATCT
5281	AGCTCATCCT	GGTCCATTCC	TCATGCATCC	GACTTTGATG	TGGACAGTTT	ATCCATACCT
5341	GACACCCTGG	AGGGAGCTAG	CGTGACCAGC	GGGGCAACGT	CAGCCGAGAC	TAACCTTTAC
5401	TTGCGAAAGA	GTATGGAGTT	TCTGGCGCGA	CCGGTGCCTG	CGCCTCGAAC	AGTATTCAGG
5461	AACCCTCCAC	ATCCCGCTCC	GCGCACAAGA	ACACCGTCAC	TTGCACCCAG	CAGGGCCTGC
5521	TCGAGAACCA	GCCTAGTTTC	CACCCCGCCA	GGCGTGAATA	GGGTGATCAC	TAGAGAGGAG
5581	CTCGAGGCGC	TTACCCCGTC	ACGCACTCCT	AGCAGGTCGG	TCTCGAGAAC	CAGCCTGGTC
5641	TCCAACCCGC	CAGGCGTAAA	TAGGGTGATT	ACAAGAGAGG	AGTTTGAGGC	GTTTCGTAGCA
5701	CAACAACAAT	GACGGTTTGA	TGCGGGTGCA	TACATCTTTT	CCTCCGACAC	CGGTCAAGGG
5761	CATTTACAAC	AAAAATCAGT	AAGGCAAACG	GTGCTATCCG	AAGTGGTGTT	GGAGAGGACC
5821	GAATTGGAGA	TTTCGTATGC	CCCGCGCCTC	GACCAAGAAA	AAGAAGAATT	ACTACGCAAG
5881	AAATTACAGT	TAAATCCAC	ACCTGCTAAC	AGAAGCAGAT	ACCAGTCCAG	GAAGGTGGAG
5941	AACATGAAAG	CCATAACAGC	TAGACGTATT	CTGCAAGGCC	TAGGGCATT	TTTGAAGGCA
6001	GAAGGAAAAG	TGGAGTGCTA	CCGAACCCCTG	CATCCTGTTT	CTTTGTATT	ATCTAGTGTG
6061	AACCGTGCCT	TTTCAAGCCC	CAAGGTGCGA	GTGGAAGCCT	GTAACGCCAT	GTTGAAAGAG
6121	AACCTTCCGA	CTGTGGCTTC	TTACTGTATT	ATTCCAGAGT	ACGATGCCCT	TTTGGACATG
6181	GTTGACGGAG	CTTCATGCTG	CTTAGACACT	GCCAGTTTTT	GCCCTGCAAA	GCTGCGCAGC
6241	TTTCCAAAGA	AACACTCCTA	TTTGGAAACC	ACAATACGAT	CGGCAGTGCC	TTACGCGATC
6301	CAGAACACGC	TCCAGAACGT	CCTGGCAGCT	GCCACAAAAA	GAAATTGCAA	TGTCACGCCAA
6361	ATGAGAGAAT	TGCCCGTATT	GGATTGCGCG	GCCTTTAATG	TGGAATGCTT	CAAGAAATAT
6421	GCGTGTAATA	ATGAATATTG	GGAAACGTTT	AAAGAAAACC	CCATCAGGCT	TACTGAAGAA
6481	AACGTGGTAA	ATTACATTAC	CAAATTAAAA	GGACCAAAAG	CTGCTGCTCT	TTTTGCGAAG
6541	ACACATAATT	TGAATATGTT	GCAGGACATA	CCAATGGACA	GGTTTGTAAAT	GGACTTAAAG
6601	AGAGACGTGA	AAGTGACTCC	AGGAACAAAA	CATACTGAAG	AACGGCCCCA	GGTACAGGTG
6661	ATCCAGGCTG	CCGATCCGCT	AGCAACAGCG	TATCTGTGCG	GAATCCACCG	AGAGCTGGTT

6721	AGGAGATTAA	ATGCGGTCCT	GCTTCCGAAC	ATTCATACAC	TGTTTGATAT	GTCGGCTGAA
6781	GACTTTGACG	CTATTATAGC	CGAGCACTTC	CAGCCTGGGG	ATTGTGTTCT	GGAAACTGAC
6841	ATCGCGTCGT	TTGATAAAAG	TGAGGACGAC	GCCATGGCTC	TGACCGCGTT	AATGATTCTG
6901	GAAGACTTAG	GTGTGGACGC	AGAGCTGTTG	ACGCTGATTG	AGGCGGCTTT	CGGCGAAATT
6961	TCATCAATAC	ATTTGCCCCA	TAAAACTAAA	TTTAAATTCT	GAGCCATGAT	GAAATCTGGA
7021	ATGTTCCCTCA	CACTGTTTGT	GAACACAGTC	ATTAACATTG	TAATCGCAAG	CAGAGTGTTG
7081	AGAGAACGGC	TAACCGGATC	ACCATGTGCA	GCATTCAATTG	GAGATGACAA	TATCGTGAAA
7141	GGAGTCAAAT	CGGACAAATT	AATGGCAGAC	AGGTGCGCCA	CCTGGTTGAA	TATGGAAGTC
7201	AAGATTATAG	ATGCTGTGGT	GGGCGAGAAA	GCGCCTTATT	TCTGTGGAGG	GTTTATTTTG
7261	TGTGACTCCG	TGACCGGCAC	AGCGTGCCGT	GTGGCAGACC	CCCTAAAAAG	GCTGTTTAAG
7321	CTTGGCAAAC	CTCTGGCAGC	AGACGATGAA	CATGATGATG	ACAGGAGAA	GGCATTGCAT
7381	GAAGAGTCAA	CACGCTGGAA	CCGAGTGGGT	ATTCTTTTCT	AGCTGTGCA	GGCAGTAGAA
7441	TCAAGGTATG	AAACCGTAGG	AACTTCCATC	ATAGTTATGG	CCATGACTAC	TCTAGCTAGC
7501	AGTGTTAAAT	CATTGAGCTA	CCTGAGAGGG	GCCCCATAAA	CTCTCTACGG	CTAACCTGAA
7561	TGGACTACGA	CATAGTCTAG	TCCGCCAAGG	CCACCatggt	gagcaagggc	gaggagctgt
7621	tcaccgggggt	ggtgcccac	ctggctcgagc	tggacggcga	cgtaaaggcc	cacaagttca
7681	gcgtgtccgg	cgaggcgag	ggcgatgcc	cctacggcaa	gctgacctg	aagttcatct
7741	gcacaccgg	caagctgccc	gtgccctggc	ccacctcgt	gaccacctg	acctacggcg
7801	tgccagtgtt	cagccgctac	cccgaccaca	tgaagcagca	cgacttcttc	aagtcggcca
7861	tgcgcgaagg	ctacgtccag	gagcgaccca	tcttcttcaa	ggacgacggc	aactacaaga
7921	cccgcgccga	ggtgaagttc	gagggcgaca	ccctggtgaa	ccgcatcgag	ctgaagggca
7981	tgcacttcaa	ggaggacggc	aacatcctgg	ggcacaagct	ggagtacaac	tacaacagcc
8041	acaacgtcta	tatcatggcc	gacaagcaga	agaacggcat	caaggtgaac	ttcaagatcc
8101	gccacaacat	cgaggacggc	agcgtgcagc	tcgccgacca	ctaccagcag	aacaccccca
8161	tcggcgacgg	ccccgtgctg	ctgcccgcaca	accactacct	gagcaccagc	tccgcctga
8221	gcaaagaccc	caacgagaag	cgcgatcaca	tggctcctgt	ggagttcgtg	accgccgcgg
8281	ggatcactct	cggcatggac	gagctgtaca	agtaaTGATA	ATATGTTACG	TGCAAAAGGTG
8341	ATTGTCACCC	CCCGAAAGAC	CATATTGTGA	CACACCCTCA	GTATCACGCC	CAAACATTTA
8401	CAGCCGCGGT	GTCAAAAACC	CGTGAGACGT	GGTTAACATC	CCTGCTGGGA	GGATCAGCCG
8461	TAATTATATT	AATTGGCTTG	GTGCTGGCTA	CTATTGTGGC	CATGTACCTG	TGACCAACC
8521	AGAAACATAA	TTGAATACAG	CAGCAATTGG	CAAGCTGCTT	ACATAGAACT	CGCGGCGATT
8581	GGCATGCCGC	CTTAAAATTT	TTATTTTATT	TTTCTTTTCT	TTTCCGAATC	GGATTTTGTT
8641	TTTAATATTT	CAAAAAAATA	AAAAAATAAA	AAAAAATAAA	AAAAAATAAA	AAaaagaaga
8701	gcGCAGCTCT	GGCCCGTGTC	TCAAAATCTC	TGATGTTACA	TTGCACAAGA	TAAAAATATA
8761	TCATCATGAA	CAATAAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC
8821	CATATTCAAC	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATCG	TGATTTATAT
8881	GGGTATAAAT	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGAT
8941	GGGAAGCCCG	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT
9001	GTTACAGATG	AGATGGTCAG	ACTAAACTGG	CTGACGGAAT	TTATGCCTCT	TCCGACCATC
9061	AAGCATTTTA	TCCGTACTCC	TGATGATGCA	TGGTTACTCA	CCACTGCGAT	CCCCGAAAAA
9121	ACAGCATTCC	AGGTATTAGA	AGAATATCCT	GATTGAGGTG	AAAATATTGT	TGATGCGCTG
9181	GAGTGTTC	TGCGCCGGTT	GCAATCGATT	CCTGTTTGTA	ATTGTCTTTT	TAACAGCGAT
9241	CGCGTATTTT	GTCTCGCTCA	GGCGCAATCA	CGAATGAATA	ACGGTTTGGT	TGATGCGAGT
9301	GATTTTGATG	ACGAGCGTAA	TGGCTGGCCT	GTTGAACAAG	TCTGGAAAGA	AATGCATAAA
9361	CTTTTGCCAT	TCTCACCGBA	TTCAGTCGTC	ACTCATGGTG	ATTTCTCACT	TGATAACCTT
9421	ATTTTGTGACG	AGGGGAAATT	AATAGGTTGT	ATTGATGTTG	GACGAGTCGG	AATCGCAGAC
9481	CGATACCAGG	ATCTTGCCAT	CCTATGGAAC	TGCCTCGGTG	AGTTTTCTCC	TTCATTACAG
9541	AAACGGCTTT	TTCAAAAATA	TGGTATTGAT	AATCCTGATA	TGAATAAATT	GCAGTTTCAT
9601	TTGATGCTCG	ATGAGTTTTT	CTAATCAGAA	TTGGTTAATT	GGTTGTAACA	CTGGCAGAGC
9661	ATTACGCTGA	CTTGACGGGA	CGGCGCAAGC	TCATGACCAA	AATCCCTTAA	CGTGAGTTAC
9721	GCGTCGTTCC	ACTGAGCGTC	AGACCCCGTA	GAAAAGATCA	AAGGATCTTC	TTGAGATCCT
9781	TTTTTTCTGC	GCGTAATCTG	CTGCTTGCAA	ACAAAAAAC	CACCGCTACC	AGCGGTGGTT
9841	TGTTTGCCGG	ATCAAGAGCT	ACCAACTCTT	TTTCCGAAGG	TAAGTGGCTT	CAGCAGAGCG
9901	CAGATACCAA	ATACTGTTCT	TCTAGTGTA	CCGTAGTTAG	GCCACCATT	CAAGAACTCT
9961	GTAGCACCAG	CTACATACCT	CGCTCTGCTA	ATCCTGTTAC	CAGTGGCTGC	TGCCAGTGGC
10021	GATAAGTCGT	GTCTTACCGG	GTTGGACTCA	AGACGATAGT	TACCGGATAA	GGCGCAGCGG
10081	TCGGGCTGAA	CGGGGGGTTT	GTGCACACAG	CCCAGCTTGG	AGCGAACGAC	CTACACCGAA
10141	CTGAGATACC	TACAGCGTGA	GCTATGAGAA	AGCGCCACGC	TTCCCGAAGG	GAGAAAGGCG
10201	GACAGGTATC	CGGTAAGCGG	CAGGTCGGA	ACAGGAGAGC	GCACGAGGGA	GCTTCCAGGG

10261	GGAAACGCCT	GGTATCTTTA	TAGTCCTGTC	GGGTTTCGCC	ACCTCTGACT	TGAGCGTCGA
10321	TTTTTGTGAT	GCTCGTCAGG	GGGGCGGAGC	CTATGGAAAA	ACGCCAGCAA	CGCGGCCTTT
10381	TTACGGTTCC	TGGCCTTTTG	CTGGCCTTTT	GCTCACAT		
Strand-mCherry (SEQ ID NO: 4)						
1	CGATACGGGA	GGGCTTACCA	TCTGGCCCCA	GTGCTGCAAT	GATACCGCGA	GACCCACGCT
61	CACCGGCTCC	AGATTTATCA	GCAATAAACC	AGCCAGCCGG	AAGGGCCGAG	CGCAGAAGTG
121	GTCCTGCAAC	TTTATCCGCC	TCCATCCAGT	CTATTAATTG	TTGCCGGGAA	GCTAGAGTAA
181	GTAGTTCGCC	AGTTAATAGT	TTGCGCAACG	TTGTTGCCAT	TGCTACAGGC	ATCGTGGTGT
241	CACGCTCGTC	GTTTGGTATG	GCTTCATTCA	GCTCCGGTTC	CCAACGATCA	AGGCGAGTTA
301	CATGATCCCC	CATGTTGTGC	AAAAAAGCGG	TTAGCTCCTT	CGGTCCTCCG	ATCGTTGTCA
361	GAAGTAAGTT	GGCCGCAGTG	TTATCACTCA	TGGTTATGGC	AGCACTGCAT	AATTCTCTTA
421	CTGTCATGCC	ATCCGTAAGA	TGCTTTTCTG	TGACTGGTGA	GTAACAACC	AAGTCAATCT
481	GAGAATAGTG	TATGCGGCGA	CCGAGTTGCT	CTTGCCCCGC	GTCAATACGG	GATAATACCG
541	CGCCACATAG	CAGAACTTTA	AAAGTGCTCA	TCATTGGAAG	ACGTTCTTCG	GGGCGAAAAAC
601	TCTCAAGGAT	CTTACCGCTG	TTGAGATCCA	GTTCGATGTA	ACCCACTCGT	GCACCCAAC
661	GATCTTCAGC	ATCTTTTACT	TTCAACAGCG	TTTCTGGGTG	AGCAAAAAACA	GGAAGGCAAA
721	ATGCCGCAAA	AAAGGAATA	AGGGCGACAC	GGAAATGTTG	AATACTCATA	CTCTTCTTTT
781	TTCAATATTA	TTGAAGCATT	TATCAGGGTT	ATTGTCTCAT	GAGCGGATAC	ATATTTGAAT
841	GTATTTAGAA	AAATAAACAA	ATAGGGGTTT	CGCGCACATT	TCCCCGAAAA	GTGCCACCTG
901	ACGTTAGGGA	TAACAGGGTA	ATTAATACGA	CTCACTATAA	TGGGCGGCGC	ATGAGAGAA
961	CCGAGACCAA	TTACCTACCC	AAAATGGAGA	AAGTTACAGT	TGACATCGAG	GAAGACAGCC
1021	CATTCTCAG	AGCTTTGCAG	CGGAGCTTCC	CGCAGTTTGA	GGTAGAAGCC	AAGCAGGTCA
1081	CTGATAATGA	CCATGCTAAT	GCCAGAGCGT	TTTCGCATCT	GGCTTCAAAA	CTGATCGAAA
1141	CGGAGGTGGA	CCCATCCGAC	ACGATCCTTG	ACATTGGAAG	TGCGCCCCGC	CGCAGAAATG
1201	ATTCTAAGCA	CAAGTATCAT	TGTATCTGTC	CGATGAGATG	TGCGGAAGAT	CCGGACAGAT
1261	TGTATAAGTA	TGCAACTAAG	CTGAAGAAAA	ACTGTAAGGA	AATAACTGAT	AAGGAATTGG
1321	ACAAGAAAAAT	GAAGGAGCTC	GCCGCCGTCA	TGAGCGACCC	TGACCTGGAA	ACTGAGACTA
1381	TGTGCCTCCA	CGACGACGAG	TCGTGTCGCT	ACGAAGGGCA	AGTCGCTGTT	TACCAGGATG
1441	TATACGCGGT	TGACGGACCG	ACAAGTCTCT	ATCACCAAGC	CAATAAGGGA	GTTAGAGTCG
1501	CCTACTGGAT	AGGCTTTGAC	ACCACCCCTT	TTATGTTTAA	GAACCTGGCT	GGAGCATATC
1561	CATCATACTC	TACCAACTGG	GCCGACGAAA	CCGTGTTAAC	GGCTCGTAAC	ATAGGCCTAT
1621	GCAGCTCTGA	CGTTATGGAG	CGGTCACGTA	GAGGGATGTC	CATTCTTAGA	AAGAAGTATT
1681	TGAAACCAATC	CAACAATGTT	CTATTCTCTG	TTGGCTCGAC	CATCTACCAC	GAGAAGAGGG
1741	ACTTACTGAG	GAGCTGGCAC	CTGCCGTCTG	TATTTCACTT	ACGTGGCAAG	CAAAATTACA
1801	CATGTCGGTG	TGAGACTATA	GTTAGTTGCG	ACGGGTACGT	CGTTAAAAGA	ATAGCTATCA
1861	GTCCAGGCCT	GTATGGGAAG	CCTTCAGGCT	ATGCTGCTAC	GATGCACCCG	GAGGGATTCT
1921	TGTGCTGCAA	AGTGACAGAC	ACATTGAACG	GGGAGAGGGT	CTCTTTTCCC	GTGTGCACGT
1981	ATGTGCCAGC	TACATTGTGT	GACCAAATGA	CTGGCATACT	GGCAACAGAT	GTCACTGCGG
2041	ACGACGCGCA	AAAACCTGCT	GTTGGGCTCA	ACCAGCGTAT	AGTCGTCAAC	GGTCGCACCC
2101	AGAGAAACAC	CAATACCATG	AAAAATTACC	TTTTGCCCGT	AGTGGCCCGG	GCATTTGCTA
2161	GGTGGGCAAA	GGAATATAAG	GAAGATCAAG	AAGATGAAAG	GCCACTAGGA	CTACGAGATA
2221	GACAGTTAGT	CATGGGGTGT	TGTTGGGCTT	TTAGAAGGCA	CAAGATAACA	TCTATTTATA
2281	AGCGCCCGGA	TACCCAAACC	ATCATCAAAG	TGAACAGCGA	TTTCCACTCA	TTCTGTCTGC
2341	CCAGGATAGG	CAGTAACACA	TTGGAGATCG	GGCTGAGAAC	AAGAATCAGG	AAAATGTTAG
2401	AGGAGCACAA	GGAGCCGTCA	CCTCTCATTA	CCGCCGAGGA	CGTACAAGAA	GCTAAGTGCG
2461	CAGCCGATGA	GGCTAAGGAG	GTGCGTGAAG	CCGAGGAGTT	GCGCGCAGCT	CTACCACCTT
2521	TGGCAGCTGA	TGTTGAGGAG	CCCACTCTGG	AAGCCGATGT	CGACTTGATG	TTACAAGAGG
2581	CTGGGGCCGG	CTCAGTGGAG	ACACCTCGTG	GCTTGATAAA	GGTTACCAGC	TACGATGGCG
2641	AGGACAAGAT	CGGCTCTTAC	GCTGTGCTTT	CTCCGCAGGC	TGTAACAAG	AGTGAAAAAT
2701	TATCTTGCA	CCACCTCTC	GCTGAACAAG	TCATAGTGAT	AACACACTCT	GGCCGAAAAG
2761	GGCGTTATGC	CGTGGAACCA	TACCATGGTA	AAGTAGTGGT	GCCAGAGGGA	CATGCAATAC
2821	CCGTCCAGGA	CTTTCAAGCT	CTGAGTGAAA	GTGCCACCAT	TGTGTACAAC	GAACGTGAGT
2881	TCGTAAACAG	GTACCTGCAC	CATATTGCCA	CACATGGAGG	AGCGCTGAAC	ACTGATGAAG
2941	AATATTACAA	AACTGTCAAG	CCCAGCGAGC	ACGACGGCGA	ATACCTGTAC	GACATCGACA
3001	GGAAACAGTG	CGTCAAGAAA	GAAGTAGTCA	CTGGGCTAGG	GCTCACAGGC	GAGCTGGTGG
3061	ATCTCCCTT	CCATGAATTC	GCCTAGAGAG	GTCTGAGAAC	ACGACCAGCC	GCTCCTTACC
3121	AAGTACCAAC	CATAGGGGTG	TATGGCGTGC	CAGGATCAGG	CAAGTCTGGC	ATCATTAATA
3181	GCGCAGTCAC	CAAAAAAGAT	CTAGTGGTGA	GCGCCAAGAA	AGAAAACTGT	GCAGAAATTA
3241	TAAGGGACGT	CAAGAAAATG	AAAGGGCTGG	ACGTCAATGC	CAGAACTGTG	GACTCAGTGC

3301	TCTTGAATGG	ATGCAAACAC	CCCGTAGAGA	CCCTGTATAT	TGACGAAGCT	TTTGCTTGTC
3361	ATGCAGGTAC	TCTCAGAGCG	CTCATAGCCA	TTATAAGACC	TAAAAAGGCA	GTGCTCTGCG
3421	GGGATCCCAA	ACAGTGCGGT	TTTTTTAACA	TGATGTGCCT	GAAAGTGCAT	TTTAACCAAG
3481	AGATTTGCAC	ACAAGTCTTC	CACAAAAGCA	TCTCTCGCCG	TTGCACTAAA	TCTGTGACTT
3541	CGGTCGTCTC	AACCTTGTTT	TACGACAAAA	AAATGAGAAC	GACGAATCCG	AAAGAGACTA
3601	AGATTGTGAT	TGACACTACC	GGCAGTACCA	AACCTAAGCA	GGACGATCTC	ATTCTCACTT
3661	GTTTCAGAGG	GTGGGTGAAG	CAGTTGCAAA	TAGATTACAA	AGGCAACGAA	ATAATGACGG
3721	CAGCTGCCTC	TCAAGGGCTG	ACCCGTAAAG	GTGTGTATGC	CGTTCGGTAC	AAGGTGAATG
3781	AAAATCCTCT	GTACGCACCC	ACCTCAGAAC	ATGTGAACGT	CCTACTGACC	CGCACGGAGG
3841	ACCGCATCGT	GTGGAAAACA	CTAGCCGGCG	ACCCATGGAT	AAAAACACTG	ACTGCCAAGT
3901	ACCCTGGGAA	TTTCACTGCC	ACGATAGAGG	AGTGGCAAGC	AGAGCATGAT	GCCATCATGA
3961	GGCATCTCTT	GGAGAGACCG	GACCTTACCG	ACGTCTTCCA	GAATAAGGCA	AACTGTGTTT
4021	GGGCCAAGGC	TTTAGTGCCG	GTGCTGAAGA	CCGCTGGCAT	AGACATGACC	ACTGAACAAT
4081	GGAACACTGT	GGATTATTTT	GAAACGGACA	AAGCTCACTC	AGCAGAGATA	GTATTGAACC
4141	AACTATGCGT	GAGGTTCTTT	GGACTCGATC	TGGACTCCGG	TCTATTTTCT	GCACCCACTG
4201	TTCCGTTATC	CATTAGGAAT	AATCACTGGG	ATAACTCCCC	GTGCGCTAAC	ATGTACGGGC
4261	TGAATAAAGA	AGTGGTCCGT	CAGCTCTCTC	GCAGGTACCC	ACAACTGCCT	CGGGCAGTTG
4321	CCACTGGAAG	AGTCTATGAC	ATGAACACTG	GTACACTGCG	CAATTATGAT	CCGCGCAATA
4381	ACCTAGTACC	TGTAAACAGA	AGACTGCCTC	ATGCTTTAGT	CCTCCACCAT	AATGAACACC
4441	CACAGAGTGA	CTTTTCTTCA	TTCGTACGCA	AATTGAAGGG	CAGAACTGTC	CTGGTGGTCG
4501	GGGAAAAGTT	GTCCGTCCCA	GGCAAAATGG	TTGACTGGTT	GTCAGACCCG	CCTGAGGCTA
4561	CCTTCAGAGC	TCGGCTGGAT	TTAGGCATCC	CAGGTGATGT	GCCCAAATAT	GACATAATAT
4621	TTGTTAATGT	GAGGACCCCA	TATAAATACC	ATCACTATCA	GCAGTGTGAA	GACCATGCCA
4681	TTAAGCTTAG	CATGTTGACC	AAGAAAGCTT	GTCTGCATCT	GAATCCCGGC	GGAACCTGTG
4741	TCAGCATAGG	TTATGGTTAC	GCTGACAGGG	CCAGCGAAAG	CATCATTTGGT	GCTATAGCGC
4801	GGCAGTTCAA	GTTTTCCCGG	GTATGCAAAC	CGAAATCCTC	ACTTGAAGAG	ACGGAAGTTC
4861	TGTTTGTATT	CATTGGGTAC	GATCGCAAGG	CCCGTACGCA	CAATCCTTAC	AAGCTTTTCAT
4921	CAACCTTGAC	CAACATTTAT	ACAGGTTCCA	GACTCCACGA	AGCCGGATGT	GCACCCTCAT
4981	ATCATGTGGT	GCGAGGGGAT	ATTGCCACGG	CCACCGAAGG	AGTGATTATA	AATGCTGCTA
5041	ACAGCAAAGG	ACAACCTGGC	GGAGGGGTGT	GCGGAGCGCT	GTATAAGAAA	TTCCCGGAAA
5101	GCTTCGATTT	ACAGCCGATC	GAAGTAGGAA	AAGCGCGACT	GGTCAAAGGT	GCAGCTAAAC
5161	ATATCATTTA	TGCCGTAGGA	CCAAACTTCA	ACAAAGTTTC	GGAGGTTGAA	GGTGACAAAC
5221	AGTTGGCAGA	GGCTTATGAG	TCCATCGCTA	AGATTGTCAA	CGATAACAAAT	TACAAGTCAG
5281	TAGCGATTCC	ACTGTTGTCC	ACCGGCATCT	TTTCCGGGAA	CAAAGATCGA	CTAACCCAAT
5341	CATTGAACCA	TTTGTCTGACA	GCTTTAGACA	CCACTGATGC	AGATGTAGCC	ATATACTGCA
5401	GGGACAAAGAA	ATGGGAAATG	ACTCTCAAGG	AAGCAGTGCG	TAGGAGAGAA	GCAGTGGAGG
5461	AGATATGCAT	ATCCGACGAC	TCTTCAGTGA	CAGAACCTGA	TGCAGAGCTG	GTGAGGGTGC
5521	ATCCGAAGAG	TTCTTTGGCT	GGAAGGAAGG	GCTACAGCAC	AAGCGATGGC	AAAACTTTCT
5581	CATATTTGGA	AGGGACCAAG	TTTCACCAGG	CGGCCAAGGA	TATAGCAGAA	ATTAATGCCA
5641	TGTGGCCCGT	TGCAACGGAG	GCCAAATGAGC	AGGTATGCAT	GTATATCCTC	GGAGAAAGCA
5701	TGAGCAGTAT	TAGGTCGAAA	TGCCCCGTGC	AAGAGTCGGA	AGCCTCCACA	CCACCTAGCA
5761	CGCTGCCTTG	CTTGTGCATC	CATGCCATGA	CTCCAGAAAG	AGTACAGCGC	CTAAAAGCCT
5821	CACGTCCAGA	ACAAATTACT	GTGTGCTCAT	CCTTTCCATT	GCCGAAGTAT	AGAATCACTG
5881	GTGTGCAGAA	GATCCAATGC	TCCCAGCCTA	TATTGTTCTC	ACCGAAAGTG	CCTGCGTATA
5941	TTCATCCAAG	GAAGTATCTC	GTGGAAACAC	CACCGGTAGA	CGAGACTCCG	GAGCCATCGG
6001	CAGAGAACCA	ATCCACAGAG	GGGACACCTG	AACAACCACC	ACTTATAACC	GAGGATGAGA
6061	CCAGGACTAG	AACGCCTGAG	CCGATCATCA	TCGAAGAGGA	AGAAGAGGAT	AGCATAAGTT
6121	TGCTGTCAGA	TGGCCCCGACC	CACCAGGTGC	TGCAAGTCGA	GGCAGACATT	CACGGGCCGC
6181	CCTCTGTATC	TAGCTCATCC	TGGTCCATTTC	CTCATGCATC	CGACTTTGAT	GTGGACAGTT
6241	TATCCATACT	TGACACCCTG	GAGGGAGCTA	GCGTGACCAG	CGGGGCAACG	TCAGCCGAGA
6301	CTAACTCTTA	CTTCGCAAAG	AGTATGGAGT	TTCTGGCGCG	ACCGGTGCCT	GCGCCTCGAA
6361	CAGTATTCAG	GAACCTTCCA	CATCCCGCTC	CGCGCACAAAG	AACACCGTCA	CTTGCAACCA
6421	GCAGGGCCTG	CTCGAGAACC	AGCCTAGTTT	CCACCCCGCC	AGGCGTGAAT	AGGGTGATCA
6481	CTAGAGAGGA	GCTCGAGGCG	CTTACCCCGT	CACGCACTCC	TAGCAGGTGC	GTCTCGAGAA
6541	CCAGCCTGGT	CTCCAACCCG	CCAGGCGTAA	ATAGGGTGAT	TACAAGAGAG	GAGTTTGAGG
6601	CGTTCTGTAGC	ACAACAACAA	TGACGGTTTG	ATGCGGGTGC	ATACATCTTT	TCCTCCGACA
6661	CCGGTCAAGG	GCATTTACAA	CAAAAATCAG	TAAGGCAAAC	GGTGCTATCC	GAAGTGGTGT
6721	TGGAGAGGAC	CGAATTGGAG	ATTTCTGTATG	CCCCGCGCCT	CGACCAAGAA	AAAGAAGAAT
6781	TACTACGCAA	GAAATTACAG	TTAAATCCCA	CACCTGCTAA	CAGAAGCAGA	TACCAGTCCA

6841	GGAAGGTGGA	GAACATGAAA	GCCATAACAG	CTAGACGTAT	TCTGCAAGGC	CTAGGGCATT
6901	ATTTGAAGGC	AGAAGGAAAA	GTGGAGTGCT	ACCGAACCCT	GCATCCTGTT	CCTTTGTATT
6961	CATCTAGTGT	GAACCGTGCC	TTTTCAAGCC	CCAAGGTCGC	AGTGGAAGCC	TGTAACGCCA
7021	TGTTGAAAGA	GAACTTTCCG	ACTGTGGCTT	CTTACTGTAT	TATTCCAGAG	TACGATGCCT
7081	ATTTGGACAT	GGTTGACGGA	GCTTCATGCT	GCTTAGACAC	TGCCAGTTTT	TGCCCTGCAA
7141	AGCTGCGCAG	CTTTCCAAAG	AAACACTCCT	ATTTGGAACC	CACAATACGA	TCGGCAGTGC
7201	CTTCAGCGAT	CCAGAACACG	CTCCAGAACG	TCCTGGCAGC	TGCCACAAAA	AGAAATTGCA
7261	ATGTCACGCA	AATGAGAGAA	TTGCCCGTAT	TGGATTTCGGC	GGCCTTTAAT	GTGGAATGCT
7321	TCAAGAAATA	TGCGTGTAAT	AATGAATATT	GGGAAACGTT	TAAAGAAAA	CCCATCAGGC
7381	TTACTGAAGA	AAACGTGGTA	AATTACATTA	CCAAATTAAA	AGGACCAAAA	GCTGCTGCTC
7441	TTTTTGCAG	GACACATAAT	TTGAATATGT	TGCAGGACAT	ACCAATGGAC	AGGTTTGTAA
7501	TGGACTTAAA	GAGAGACGTG	AAAGTGACTG	CAGGAACAAA	ACATATGTA	GAACGGACCA
7561	AGGTACAGGT	GATCCAGGCT	GCCGATCCGC	TAGCAACAGC	GTATCTGTGC	GGAATCCACC
7621	GAGAGCTGGT	TAGGAGATTA	AATGCGGTCC	TGCTTCCGAA	CATTTCATACA	CTGTTTGATA
7681	TGTCGGCTGA	AGACTTTGAC	GCTATTATAG	CCGAGCACTT	CCAGCCTGGG	GATTGTGTTC
7741	TGGAAACTGA	CATCGCGTCG	TTTGATAAAA	GTGAGGACGA	CGCCATGGCT	CTGACCGCGT
7801	TAATGATTCT	GGAAGACTTA	GGTGTGGACG	CAGAGCTGTT	GACGCTGATT	GAGGCGGCTT
7861	TGCGCGAAAT	TTCATCAATA	CATTTGCCCA	CTAAAACTAA	ATTTAAATTC	GGAGCTGCA
7921	TGAAATCTGG	AATGTTCTCT	ACACTGTTTG	TGAACACAGT	CATTAAACATT	GTAATCGCAA
7981	GCAGAGTGTT	GAGAGAACGG	CTAACCGGAT	CACCATGTGC	AGCATTTCATT	GGAGATGACA
8041	ATATCGTGAA	AGGAGTCAAA	TCGGACAAAT	TAATGGCAGA	CAGGTGCGCC	ACCTGGTTGA
8101	ATATGGAAGT	CAAGATTATA	GATGCTGTGG	TGGGCGAGAA	AGCGCCTTAT	TTCTGTGGAG
8161	GGTTTATTTT	GTGTGACTCC	GTGACCGGCA	CAGCGTGCCG	TGTGGCAGAC	CCCCTAAAAA
8221	GGCTGTTTAA	GCTTGGCAAA	CCTCTGGCAG	CAGACGATGA	ACATGATGAT	GACAGGAGAA
8281	GGGCATTGCA	TGAAGAGTCA	ACACGCTGGA	ACCGAGTGGG	TATTCTTTCA	GAGCTGTGCA
8341	AGGCAGTAGA	ATCAAGGTAT	GAAACCGTAG	GAACTTCCAT	CATAGTTATG	GCCATGACTA
8401	CTCTAGCTAG	CAGTGTTAAA	TCATTTCAGCT	ACCTGAGAGG	GGCCCCCTATA	ACTCTCTACG
8461	GCTAACCTGA	ATGGACTACG	ACATAGTCTA	GTCCGCCAAG	GCCACCATGG	TGAGCAAGGG
8521	CGAGGAGGAT	AACATGGCCA	TCATCAAGGA	GTTTCATGCG	TTCAAGGTGC	ACATGGAGGG
8581	CTCCGTGAAC	GGCCACGAGT	TCGAGATCGA	GGGCGAGGGC	GAGGGCCGCC	CCTACGAGGG
8641	CACCCAGACC	GCCAAGCTGA	AGGTGACCAA	GGGTGGTCCC	CTGCCCTTCG	CCTGGGACAT
8701	CCTGTCCCCT	CAGTTCATGT	ACGGCTCCAA	GGCCTACGTG	AAGCACCCCG	CCGACATCCC
8761	CGACTACTTG	AAGCTGTCC	TCCCCGAGGG	CTTCAAGTGG	GAGCGCGTGA	TGAACCTCGA
8821	GGACGGCGGC	GTGGTGACCG	TGACCCAGGA	CTCCTCCCTG	CAGGACGGCG	AGTTCATCTA
8881	CAAGGTGAAG	CTGCGCGGCA	CCAACCTCCC	CTCCGACGGC	CCCCTAATGC	AGAAGAAGAC
8941	CATGGGCTGG	GAGGCCTCCT	CCGAGCGGAT	GTACCCCGAG	GACGGCCGCC	TGAAGGGCGA
9001	GATCAAGCAG	AGGCTGAAGC	TGAAGGACGG	CGGCCACTAC	GACGCTGAGG	TCAAGACCAC
9061	CTACAAGGCC	AAGAAGCCCG	TGCAGCTGCC	CGGCGCCTAC	AACGTCAACA	TCAAGTTGGA
9121	CATCACCTCC	CACAACGAGG	ACTACACCAT	CGTGGAACAG	TACGAACGCG	CCGAGGGCCG
9181	CCACTCCACC	GGCGGCATGG	ACGAGCTGTA	CAAGTAGATA	ATATGTTACG	TGCAAAGGTG
9241	ATTGTCACCC	CCCGAAAGAC	CATATTGTGA	CACACCCTCA	GTATCACGCC	CAAACATTTA
9301	CAGCCGCGGT	GTCAAAAACC	GCGTGGACGT	GGTTAACATC	CCTGTGGGGA	GGATCAGCCG
9361	TAATTATTAT	AATTGGCTTG	GTGCTGGCTA	CTATTGTGGC	CATGTACGTG	CTGACCAACC
9421	AGAAACATAA	TTGAATACAG	CAGCAATTGG	CAAGCTGCTT	ACATAGAAGT	CGCGGCGATT
9481	GGCATGCCGC	CTTAAATTTT	TTATTTTATT	TTTCTTTTCT	TTTCCGAATC	GGATTTTGTT
9541	TTTAATATTT	CAAAAAA	AAAAA	AAAAA	AAAAA	ATAGGGATAA
9601	CAGGTAATT	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG
9661	GCGTTTTTCC	ATAGGCTCCG	CCCCCTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG
9721	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC
9781	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT	TCTCCCTTCG
9841	GGAAGCGTGG	CGCTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT
9901	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTTCAGC	CCGACCGCTG	CGCCTTATCC
9961	GGTAACATATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC
10021	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG
10081	TGGCCTAACT	ACGGCTACAC	TAGAAGAACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA
10141	GTTACCTTCG	GAAAAAGAGT	TGGTAGCTCT	TGATCCGCGA	AACAAACCAC	CGCTGTTAGC
10201	GGTGGTTTTT	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT
10261	CCTTTGATCT	TTTCTACGGG	GTCTGACGCT	CAGTGGAACG	AAAACCTCACG	TTAAGGGATT
10321	TTGGTCATGA	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTTAAATTA	AAAATGAAGT

10381	TTTAAATCAA	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC
10441	AGTGAGGCAC	CTATCTCAGC	GATCTGTCTA	TTTCGTTCAT	CCATAGTTGC	CTGACTCCCC
10501	GTCGTGTAGA	TAAC TA				
Non-cytopathic-EGFP (SEQ ID NO: 5)						
1	gctcttctaa	gTAATACGAC	TCACTATAAT	GGGCGGCGCA	TGAGAGAAGC	CCAGACCAAT
61	TACCTACCCA	AAATGGAGAA	AGTTCACGTT	GACATCGAGG	AAGACAGCCC	ATTCTCTCAGA
121	GCTTTGCAGC	GGAGCTTCCC	GCAGTTTGAG	G TAGAAGCCA	AGCAGGTCAC	TGATAATGAC
181	CATGCTAATG	CCAGAGCGTT	TTCGCATCTG	GCTTCAAAAC	TGATCGAAAC	GGAGGTGGAC
241	CCATCCGACA	CGATCCTTGA	CATTGGAAGT	GCGCCCGCCC	GCAGAAATGTA	TTCTAAGCAC
301	AAGTATCATT	GTATCTGTCC	GATGAGATGT	GCGGAAGATC	CGGACAGATT	GTATAAGTAT
361	GCAACTAAGC	TGAAGAAAAA	CTGTAAGGAA	ATAACTGATA	AGGAATTGGA	CAAGAAAATG
421	AAGGAGCTCG	CCGCCGTCAT	GAGCGACCCT	GACCTGGAAA	CTGAGACTAT	GTGCCCTCCAC
481	GACGACGAGT	CGTGTGCTA	CGAAGGGCAA	GTCGCTGTTT	ACCAGGATGT	ATACGCGGTT
541	GACGGACCGA	CAAGTCTCTA	TCACCAAGCC	AATAAGGGAG	TTAGAGTCGC	CTACTGGATA
601	GGCTTTGACA	CCACCCCTTT	TATGTTTAAG	AACTTGGCTG	GAGCATATCC	ATCATACTCT
661	ACCAACTGGG	CCGACGAAAC	CGTGTTAAGC	GCTCGTAACA	TAGGCCATATG	CAGCTCTGAC
721	GTTATGGAGC	GGTCACGTAG	AGGGATGTCC	ATTCTTAGAA	AGAAGTATTT	GAAACCATCC
781	AACAATGTTC	TATTCTCTGT	TGGCTCGACC	ATCTACCACG	AGAAGAGGGA	CTTACTGAGG
841	AGCTGGCACC	TGCCGTCTGT	ATTTCACTTA	CGTGGCAAGC	AAAATTACAC	ATGTCGGTGT
901	GAGACTATAG	TTAGTTGCGA	CGGGTACGTC	GTTAAAAGAA	TAGCTATCAG	TCCAGGCCTG
961	TATGGGAAGC	CTTCAGGCTA	TGCTGCTACG	ATGCACCGCG	AGGGATTCTT	GTGCTGCAAA
1021	GTGACAGACA	CATTGAACGG	GGAGAGGGTC	TCTTTTCCCG	TGTGCACGTA	TGTGCCAGCT
1081	ACATTGTGTG	ACCAAATGAC	TGGCATACTG	GCAACAGATG	TCAGTGCCGA	CGACGCGCAA
1141	AAACTGCTGG	TTGGGCTCAA	CCAGCGTATA	GTCGTCAACG	GTCGCACCCA	GAGAAACACC
1201	AATACCATGA	AAAATTACCT	TTTGCCCGTA	GTGGCCAGG	CATTTGCTAG	GTGGGCAAAG
1261	GAATATAAGG	AAGATCAAGA	AGATGAAAGG	CCACTAGGAC	TACGAGATAG	ACAGTTAGTC
1321	ATGGGGTGTT	GTTGGGCTTT	TAGAAGGCAC	AAGATAACAT	CTATTTATATA	GCGCCCGGAT
1381	ACCCAAACCA	TCATCAAAGT	GAACAGCGAT	TTCCACTCAT	TCGTGCTGCC	CAGGATAGGC
1441	AGTAACACAT	TGGAGATCGG	GCTGAGAACA	AGAATCAGGA	AAATGTTAGA	GGAGCAACAAG
1501	GAGCCGTCAC	CTCTCATTAC	CGCCGAGGAC	GTACAAGAAG	CTAAGTGCGC	AGCCGATGAG
1561	GCTAAGGAGG	TGCGTGAAGC	CGAGGAGTTG	CGCGCAGCTC	TACCACCTTT	GGCAGCTGAT
1621	GTTGAGGAGC	CCACTCTGGA	AGCCGATGTC	GACTTGATGT	TACAAGAGGC	TGGGGCCGGC
1681	TCAGTGGAGA	CACCTCGTGG	CTTGATAAAG	GTTACCAGCT	ACGATGGCGA	GGACAAGATC
1741	GGCTCTTACG	CTGTGCTTTC	TCCGAGGCT	GTA CTCAAGA	GTGAAAAAAT	ATCTTGCA TC
1801	CACCCTCTCG	CTGAACAAGT	CATAGTGATA	ACACACTCTG	GCCGAAAAGG	GC GTTATGCC
1861	GTGGAACCAT	ACCATGGTAA	AGTAGTGGTG	CCAGAGGGAC	ATGCAATACC	CGTCCAGGAC
1921	TTTCAAGCTC	TGAGTGAAAG	TGCCACCATT	G GTGTACAACG	AACGTGAGTT	CGTAAACAGG
1981	TACCTGCACC	ATATTGCCAC	ACATGGAGGA	GCGCTGAACA	CTGATGAAGA	ATATTACAAA
2041	ACTGTCAAGC	CCAGCGAGCA	CGACGGCGAA	TACCTGTACG	ACATCGACAG	GAAACAGTGC
2101	GTCAAGAAAG	AACTAGTCAC	TGGGCTAGGG	CTCACAGGCG	AGCTGGTGGA	TCCTCCCTTC
2161	CATGAATTCTG	CCTACGAGAG	TCTGAGAACA	CGACCAGCCG	CTCCTTACCA	AGTACCAACC
2221	ATAGGGGTGT	ATGGCGTGCC	AGGATCAGGC	AAGTCTGGCA	TCATTAAAAG	CGCAGTCACC
2281	AAAAAAGATC	TAGTGGTGAG	CGCCAAGAAA	GAAAACTGTG	CAGAAATTAT	AAGGGACGTC
2341	AAGAAAATGA	AAGGGCTGGA	CGTCAATGCC	AGAACTGTGG	ACTCAGTGCT	CTTGAATGGA
2401	TGCAAAACACC	CCGTAGAGAC	CCTGTATATT	GACGAAGCTT	TTGCTTGTC	TGCAGGTACT
2461	CTCAGAGCGC	TCATAGCCAT	TATAAGACCT	AAAAAAGCAG	TGCTCTGCGG	GGATCCCAAA
2521	CAGTGCGGTT	TTTTTAAACAT	GATGTGCCTG	AAAGTGCATT	TTAACCACGA	GATTTCACACA
2581	CAAGTCTTCC	ACAAAAGCAT	CTCTCGCCGT	TGCACTAAAT	CTGTGACTTC	GGTCGTCTCA
2641	ACCTTGTTT	ACGACAAAAA	AATGAGAACG	ACGAATCCGA	AAGAGACTAA	GATTGTGATT
2701	GACACTACCG	GCAGTACCAA	ACCTAAGCAG	GACGATCTCA	TTCTCACTTG	TTTCAGAGGG
2761	TGGGTGAAGC	AGTTGCAAA	AGATTACAAA	GGCAACGAAA	TAATGACGGC	AGCTGCCTCT
2821	CAAGGGCTGA	CCCGTAAAGG	TGTGTATGCC	GTTTCGGTACA	AGGTGAATGA	AAATCCTCTG
2881	TACGCACCCA	CCTCAGAACA	TGTGAACGTC	CTACTGACCC	GCACGGAGGA	CCGCATCGTG
2941	TGGAAAACAC	TAGCCGGCGA	CCCATGGATA	AAAACACTGA	CTGCCAAGTA	CCCTGGGAAT
3001	TTCACTGCCA	CGATAGAGGA	GTGGCAAGCA	GAGCATGATG	CCATCATGAG	GCACATCTTG
3061	GAGAGACCGG	ACCTTACCGA	CGTCTTCCAG	AATAAGGCAA	ACGTGTGTTG	GGCCAAGGCT
3121	TTAGTGCCGG	TGCTGAAGAC	CGCTGGCATA	GACATGACCA	CTGAACAATG	GAACACTGTG

3181	GATTATTTTG	AAACGGACAA	AGCTCACTCA	GCAGAGATAG	TATTGAACCA	ACTATGCGTG
3241	AGGTTCTTTG	GACTCGATCT	GGACTCCGGT	CTATTTTCTG	CACCCACTGT	TCCGTTATCC
3301	ATTAGGAATA	ATCACTGGGA	TAACTCCCCG	TCGCCTAACA	TGTACGGGCT	GAATAAAGAA
3361	GTGGTCCGTC	AGCTCTCTCG	CAGGTACCCA	CAACTGCCTC	GGGCAGTTGC	CACTGGAAGA
3421	GTCTATGACA	TGAACACTGG	TACACTGCGC	AATTATGATC	CGCGCATAAA	CCTAGTACCT
3481	GTAACAGAA	GACTGCCTCA	TGCTTTAGTC	CTCCACCATA	ATGAACACCC	ACAGAGTGAC
3541	TTTTCTTCAT	TCGTCAGCAA	ATTGAAGGGC	AGAACTGTCC	TGGTGGTCCG	GGAAAAGTTG
3601	TCCGTCCCAG	GCAAAATGGT	TGACTGGTTG	TCAGACCGGC	CTGAGGCTAC	CTTCAGAGCT
3661	CGGCTGGATT	TAGGCATCCC	AGGTGATGTG	CCCAAATATG	ACATAATATT	TGTTAATGTG
3721	AGGACCCCAT	ATAAATACCA	TCACTATCAG	CAGTGTGAAG	ACCATGCCAT	TAAGCTTAGC
3781	ATGTTGACCA	AGAAAGCTTG	TCTGCATCTG	AATCCCGGCG	GAACCTGTGT	CAGCATAGGT
3841	TATGTTTACG	CTGACAGGGC	CAGCGAAAGC	ATCATTGGTG	CTATAGCGCG	CTGTGTTCAAG
3901	TTTTCCCGGG	TATGCAAACC	GAAATCCTCA	CTTGAAGAGA	CGGAAGTTCT	GTTTGTATTTC
3961	ATTGGGTACG	ATCGCAAGGC	CCGTACGCAC	AATCCTTACA	AGCTTTTCATC	AACCTTGACC
4021	AACATTTATA	CAGGTTCCAG	ACTCCACGAA	GCCGGATGTG	CACCCTCATA	TCATGTGGTG
4081	CGAGGGGATA	TTGCCACGGC	CACCGAAGGA	GTGATTATAA	ATGCTGCTAA	CAGCAAAGGA
4141	CAACCTGGCG	GAGGGGTGTG	CGGAGCGCTG	TATAAGAAAT	TCCCGGAAAG	CTTCGATTTA
4201	CAGCCGATCG	AAGTAGGAAA	AGCGGACTG	GTCAAAGGTG	CAGCTAAACA	TATCATTCATG
4261	GCCGTAGGAC	CAAACCTCAA	CAAAGTTTCG	GAGGTTGAAG	GTGACAAACA	GTTGGCAGAG
4321	GCTTATGAGT	CCATCGCTAA	GATTGTCAAC	GATAACAATT	ACAAGTCAGT	AGCGATTCCA
4381	CTGTTGTCCA	CCGGCATCTT	TTCCGGGAAC	AAAGATCGAC	TAACCCAATC	ATTGAACCAT
4441	TTGCTGACAG	CTTTAGACAC	CACTGATGCA	GATGTAGCCA	TATACTGCAG	GGACAAGAAA
4501	TGGGAAATGA	CTCTCAAGGA	AGCAGTGGCT	AGGAGAGAAG	CAGTGGAGGA	GATATGCATA
4561	TCCGACGACT	CTTCAGTGAC	AGAACCTGAT	GCAGAGCTGG	TGAGGGTGCA	TCCGAAGAGT
4621	TCTTTGGCTG	GAAGGAAGGG	CTACAGCACA	AGCGATGGCA	AAACTTTCTC	ATATTTGGAA
4681	GGGACCAAGT	TTCACCAGGC	GGCCAAGGAT	ATAGCAGAAA	TTAATGCCAT	GTGGCCCGTT
4741	GCAACGGAGG	CCAATGAGCA	GGTATGCATG	TATATCCTCG	GAGAAAGCAT	GAGCAGTATT
4801	AGGTCGAAAT	GCCCCGTCGA	AGAGTCGGAA	GCCTCCACAC	CACCTAGCAC	GCTGCCTTGC
4861	TTGTGCATCC	ATGCCATGAC	TCCAGAAAGA	GTACAGCGCC	TAAAAGCCTC	ACGTCCAGAA
4921	CAAAATTACTG	TGTGCTCATC	CTTTCCATTG	CCGAAAGTATA	GAATCACTGG	TGTGCAGAAAG
4981	ATCCAATGCT	CCCAGCCTAT	ATTGTTCTCA	CCGAAAGTGC	CTGCGTATAT	TCATCCAAGG
5041	AAGTATCTCG	TGGAAACACC	ACCGGTAGAC	GAGACTCCGG	AGCCATCGGC	AGAGAACCAA
5101	TCCACAGAGG	GGACACCTGA	ACAACCACCA	CTTATAACCG	AGGATGAGAC	CAGGACTAGA
5161	ACGCCTGAGC	CGATCATCAT	CGAAGAGGAA	GAAGAGGATA	GCATAAGTTT	GCTGTCAAGT
5221	GGCCCGACCC	ACCAGGTGCT	GCAAGTCGAG	GCAGACATTC	ACGGGCCGCC	CTCTGTATCT
5281	AGCTCATCCT	GGTCCATTCC	TCATGCATCC	GACTTTGATG	TGGACAGTTT	ATCCATACCT
5341	GACACCCTGG	AGGGAGCTAG	CGTGACCAGC	GGGGCAACGT	CAGCCGAGAC	TAACCTTTAC
5401	TTGCGAAAGA	GTATGGAGTT	TCTGGCGCGA	CCGGTGCCTG	CGCCTCGAAC	AGTATTCAGG
5461	AACCCTCCAC	ATCCCGCTCC	GCGCACAAGA	ACACCGTCAC	TTGCACCCAG	CAGGGCCTGC
5521	TCGAGAACCA	GCCTAGTTTC	CACCCCGCCA	GGCGTGAATA	GGGTGATCAC	TAGAGAGGAG
5581	CTCGAGGCGC	TTACCCCGTC	ACGCACTCCT	AGCAGGTCGG	TCTCGAGAAC	CAGCCTGGTC
5641	TCCAACCCGC	CAGGCGTAAA	TAGGGTGATT	ACAAGAGAGG	AGTTTGAGGC	GTTTCGTAGCA
5701	CAACAACAAT	GACGGTTTGA	TGCGGGTGCA	TACATCTTTT	CCTCCGACAC	CGGTCAAGGG
5761	CATTTACAAC	AAAAATCAGT	AAGGCAAACG	GTGCTATCCG	AAGTGGTGTT	GGAGAGGACC
5821	GAATTGGAGA	TTTCGTATGC	CCCGCGCCTC	GACCAAGAAA	AAGAAGAATT	ACTACGCAAG
5881	AAATTACAGT	TAAATCCAC	ACCTGCTAAC	AGAAGCAGAT	ACCAGTCCAG	GAAGGTGGAG
5941	AACATGAAAAG	CCATAACAGC	TAGACGTATT	CTGCAAGGCC	TAGGGCATTAT	TTTGAAGGCA
6001	GAAGGAAAAG	TGGAGTGCTA	CCGAACCCCTG	CATCCTGTTT	CTTTGTATTTC	ATCTAGTGTG
6061	AACCGTGCCT	TTTCAAGCCC	CAAGGTGCGA	GTGGAAGCCT	GTAACGCCAT	GTTGAAAGAG
6121	AACCTTCCGA	CTGTGGCTTC	TTACTGTATT	ATTCCAGAGT	ACGATGCCCTA	TTTGGACATG
6181	GTTGACGGAG	CTTCATGCTG	CTTAGACACT	GCCAGTTTTT	GCCCTGCAAA	GCTGCGCAGC
6241	TTTCCAAAGA	AACACTCCTA	TTTGGAAACC	ACAATACGAT	CGGCAGTGCC	TTACGCGATC
6301	CAGAACACGC	TCCAGAACGT	CCTGGCAGCT	GCCACAAAAA	GAAATTGCAA	TGTCACGCCAA
6361	ATGAGAGAAT	TGCCCGTATT	GGATTGCGCG	GCCTTTAATG	TGGAATGCTT	CAAGAAATAT
6421	GCGTGTAATA	ATGAATATTG	GGAACGTTT	AAAGAAAACC	CCATCAGGCT	TACTGAAGAA
6481	AACGTGGTAA	ATTACATTAC	CAAATTAAAA	GGACCAAAAG	CTGCTGCTCT	TTTTGCGAAG
6541	ACACATAATT	TGAATATGTT	GCAGGACATA	CCAATGGACA	GGTTTGTAAAT	GGACTTAAAG
6601	AGAGACGTGA	AAGTGACTCC	AGGAACAAAA	CATACTGAAG	AACGGCCCCA	GGTACAGGTG
6661	ATCCAGGCTG	CCGATCCGCT	AGCAACAGCG	TATCTGTGCG	GAATCCACCG	AGAGCTGGTT

6721	AGGAGATTAA	ATGCGGTCCT	GCTTCCGAAC	ATTCATACAC	TGTTTGATAT	GTCGGCTGAA
6781	GACTTTGACG	CTATTATAGC	CGAGCACTTC	CAGCCTGGGG	ATTGTGTCT	GGAAACTGAC
6841	ATCGCGTCGT	TTGATAAAAG	TGAGGACGAC	GCCATGGCTC	TGACCGCGTT	AATGATTCTG
6901	GAAGACTTAG	GTGTGGACGC	AGAGCTGTTG	ACGCTGATTG	AGGCGGCTTT	CGGCGAAATT
6961	TCATCAATAC	ATTTGCCCC	TAAAACTAAA	TTTAAATTTCG	GAGCCATGAT	GAAATCTGGA
7021	ATGTTCTCTCA	CACTGTTTGT	GAACACAGTC	ATTAACATTG	TAATCGCAAG	CAGAGTGTTG
7081	AGAGAACGGC	TAACCGGATC	ACCATGTGCA	GCATTCAATTG	GAGATGACAA	TATCGTGAAA
7141	GGAGTCAAAT	CGGACAAATT	AATGGCAGAC	AGGTGCGCCA	CCTGGTTGAA	TATGGAAGTC
7201	AAGATTATAG	ATGCTGTGGT	GGGCGAGAAA	GCGCCTTATT	TCTGTGGAGG	GTTTATTTTG
7261	TGTGACTCCG	TGACCGGCAC	AGCGTGCCGT	GTGGCAGACC	CCCTAAAAAG	GCTGTTTAAG
7321	CTTGGCAAAC	CTCTGGCAGC	AGACGATGAA	CATGATGATG	ACAGGAGAA	GGCATTGCAT
7381	GAAGAGTCAA	CACGCTGGAA	CCGAGTGGGT	ATTCTTTTCAG	AGCTGTGCA	GGCAGTAGAA
7441	TCAAGGTATG	AAACCGTAGG	AACTTCCATC	ATAGTTATGG	CCATGACTAC	TCTAGCTAGC
7501	AGTGTTAAAT	CATTGAGCTA	CCTGAGAGGG	GCCCCATAAA	CTCTCTACGG	CTAACCTGAA
7561	TGGACTACGA	CATAGTCTAG	TCCGCCAAGG	CCACCatggt	gagcaagggc	gaggagctgt
7621	tcaccgggggt	ggtgcccac	ctggctcgagc	tggacggcga	cgtaaaggcc	cacaagttca
7681	gcgtgtccgg	cgaggggcgag	ggcgatgcc	cctacggcaa	gctgacctg	aagttcatct
7741	gcacaccgg	caagctgccc	gtgccctggc	ccacctcgt	gaccacctg	acctacggcg
7801	tgccagtgtt	cagccgctac	cccgaccaca	tgaagcagca	cgacttcttc	aagtcggcca
7861	tgcgcgaagg	ctacgtccag	gagcgaccca	tcttcttcaa	ggacgacggc	aactacaaga
7921	cccgcgccga	ggtgaagttc	gagggcgaca	ccctggtgaa	ccgcatcgag	ctgaagggca
7981	tgcacttcaa	ggaggacggc	aacatcctgg	ggcacaagct	ggagtacaac	tacaacagcc
8041	acaacgtcta	tatcatggcc	gacaagcaga	agaacggcat	caaggtgaac	ttcaagatcc
8101	gccacaacat	cgaggacggc	agcgtgcagc	tcgcccacca	ctaccagcag	aacaccccca
8161	tcggcgacgg	ccccgtgctg	ctgcccgcaca	accactacct	gagcaccag	tccgcctga
8221	gcaaagaccc	caacgagaag	cgcgatcaca	tggctctgct	ggagtctgct	accgccgccc
8281	ggatcactct	cggcatggac	gagctgtaca	agtaaTGATA	ATATGTTACG	TGCAAAAGGTG
8341	ATTGTCACCC	CCCGAAAGAC	CATATTGTGA	CACACCCTCA	GTATCACGCC	CAAACATTTA
8401	CAGCCGCGGT	GTCAAAAACC	CGTGAGACGT	GGTTAACATC	CCTGCTGGGA	GGATCAGCCG
8461	TAATTATAT	AATTGGCTTG	GTGCTGGCTA	CTATTGTGGC	CATGTACGTG	TGACCAACC
8521	AGAAACATAA	TTGAATACAG	CAGCAATTGG	CAAGCTGCTT	ACATAGAACT	CGCGGCGATT
8581	GGCATGCCGC	CTTAAAATTT	TTATTTTATT	TTTCTTTTCT	TTTCCGAATC	GGATTTTGTT
8641	TTTAATATTT	CAAAAAA	AAAAA	AAAAA	AAAAA	AAaaagaaga
8701	gcGCAGCTCT	GGCCCGTGTC	TCAAAATCTC	TGATGTTACA	TTGCACAAGA	TAAAAATATA
8761	TCATCATGAA	CAATAAACT	GTCTGCTTAC	ATAAACAGTA	ATACAAGGGG	TGTTATGAGC
8821	CATATTC AAC	GGGAAACGTC	GAGGCCGCGA	TTAAATTCCA	ACATGGATCG	TGATTTATAT
8881	GGGTATAAAT	GGGCTCGCGA	TAATGTCGGG	CAATCAGGTG	CGACAATCTA	TCGCTTGAT
8941	GGGAAGCCCG	ATGCGCCAGA	GTTGTTTCTG	AAACATGGCA	AAGGTAGCGT	TGCCAATGAT
9001	GTTACAGATG	AGATGGTCAG	ACTAACTGG	CTGACGGAAT	TTATGCCTCT	TCCGACCATC
9061	AAGCATTTTA	TCCGTACTCC	TGATGATGCA	TGGTTACTCA	CCACTGCGAT	CCCCGAAAA
9121	ACAGCATTC	AGGTATTAGA	AGAATATCCT	GATTGAGGTG	AAAATATTGT	TGATGCGCTG
9181	GAGTGTTC	TGCGCCGTT	GCAATCGATT	CCTGTTTGTA	ATTGTCTTTT	TAACAGCGAT
9241	CGCGTATTT	GTCTCGCTCA	GGCGCAATCA	CGAATGAATA	ACGGTTTGGT	TGATGCGAGT
9301	GATTTTGATG	ACGAGCGTAA	TGGCTGGCCT	GTTGAACAAG	TCTGGAAAGA	AATGCATAAA
9361	CTTTTGCCAT	TCTCACC GGA	TTCAGTCGTC	ACTCATGGTG	ATTTCTCACT	TGATAACCTT
9421	ATTTTGTGACG	AGGGGAAATT	AATAGGTTGT	ATTGATGTTG	GACGAGTCGG	AATCGCAGAC
9481	CGATACCAGG	ATCTTGCCAT	CCTATGGAAC	TGCCTCGGTG	AGTTTTCTCC	TTCATTACAG
9541	AAACGGCTTT	TTCAAAAATA	TGGTATTGAT	AATCCTGATA	TGAATAAATT	GCAGTTTCAT
9601	TTGATGCTCG	ATGAGTTTTT	CTAATCAGAA	TTGGTTAATT	GGTTGTAACA	CTGGCAGAGC
9661	ATTACGCTGA	CTTGACGGGA	CGGCGCAAGC	TCATGACCAA	AATCCCTTAA	CGTGAGTTAC
9721	GCGTCGTTCC	ACTGAGCGTC	AGACCCCGTA	GAAAAGATCA	AAGGATCTTC	TTGAGATCCT
9781	TTTTTTCTGC	GCGTAATCTG	CTGCTTGCAA	ACAAAAAAC	CACCGCTACC	AGCGGTGGTT
9841	TGTTTGCCGG	ATCAAGAGCT	ACCAACTCTT	TTTCCGAAGG	TAAGTGGCTT	CAGCAGAGCG
9901	CAGATACCAA	ATACTGTTCT	TCTAGTG TAG	CCGTAGTTAG	GCCACCATT	CAAGAACTCT
9961	GTAGCACC GC	CTACATACCT	CGCTCTGCTA	ATCCTGTTAC	CAGTGGCTGC	TGCCAGTGGC
10021	GATAAGTCGT	GTCTTACCGG	GTTGGACTCA	AGACGATAGT	TACCGGATAA	GGCGCAGCGG
10081	TCGGGCTGAA	CGGGGGTTC	GTGCACACAG	CCCAGCTTGG	AGCGAACGAC	CTACACCGAA
10141	CTGAGATACC	TACAGCGTGA	GCTATGAGAA	AGCGCCACGC	TTCCCGAAGG	GAGAAAGGCG
10201	GACAGGTATC	CGGTAAGCGG	CAGGTCGGA	ACAGGAGAGC	GCACGAGGGA	GCTTCCAGGG

10261	GGAAACGCCT	GGTATCTTTA	TAGTCCTGTC	GGGTTTCGCC	ACCTCTGACT	TGAGCGTCGA
10321	TTTTTGTGAT	GCTCGTCAGG	GGGGCGGAGC	CTATGGAAAA	ACGCCAGCAA	CGCGGCCTTT
10381	TTACGGTTCC	TGGCCTTTTG	CTGGCCTTTT	GCTCACAT		
Strand-NS1-EGFP (SEQ ID NO: 6)						
1	gctcttctaa	gTAATACGAC	TCACTATAAT	GGGCGGCGCA	TGAGAGAAGC	CCAGACCAAT
61	TACCTACCCA	AAATGGAGAA	AGTTCACGTT	GACATCGAGG	AAGACAGCCC	ATTCTCTAGA
121	GCTTTGCAGC	GGAGCTTCCC	GCAGTTTGAG	GTAGAAGCCA	AGCAGGTCAC	TGATAATGAC
181	CATGCTAATG	CCAGAGCGTT	TTCGCATCTG	GCTTCAAAAC	TGATCGAAAC	GGAGGTGGAC
241	CCATCCGACA	CGATCCTTGA	CATTGGAAGT	GCGCCCGCCC	GCAGAATGTA	TTCTAAGCAC
301	AAGTATCATT	GTATCTGTCC	GATGAGATGT	GCGGAAGATC	CGGACAGATT	GTATAAGTAT
361	GCAACTAAGC	TGAAGAAAAA	CTGTAAGGAA	ATAACTGATA	AGGAATTGGA	CAAGAAAATG
421	AAGGAGCTCG	CGCCCGTCAT	GAGCGACCCT	GACCTGGAAA	CTGAGACTAT	GTGCCTCCAC
481	GACGACGAGT	CGTGTCGCTA	CGAAGGGCAA	GTGCTGTTT	ACCAGGATGT	ATACGCGGTT
541	GACGGACCGA	CAAGTCTCTA	TCACCAAGCC	AATAAGGGAG	TTAGAGTCGC	CTACTGGATA
601	GGCTTTTGACA	CCACCCCTTT	TATGTTTAAAG	AACCTTGGCTG	GAGCATATCC	ATCATACTCT
661	ACCAACTGGG	CCGACGAAAC	CGTGTTAACG	GCTCGTAACA	TAGGCCTATG	CAGCTCTGAC
721	GTATATGGAGC	GGTCACGTAG	AGGGATGTCC	ATTCTTAGAA	AGAAGTATTT	GAAACCATCC
781	AACATGTTC	TATTCTCTGT	TGGCTCGACC	ATCTACCACG	AGAAGAGGGA	CTTACTGAGG
841	AGCTGGCACC	TGCCGTCTGT	ATTTCACTTA	CGTGGCAAGC	AAAATTACAC	ATGTCGGTGT
901	GAGACTATAG	TTAGTTGCGA	CGGGTACGTC	GTTAAAAGAA	TAGCTATCAG	TCCAGGCCCTG
961	TATGGGAAGC	CTTCAGGCTA	TGCTGCTACG	ATGCACCGCG	AGGGATTCTT	GTGCTGCAAA
1021	GTGACAGACA	CATTGAACGG	GGAGAGGGTC	TCTTTTCCCG	TGTGCACGTA	TGTGCCAGCT
1081	ACATTGTGTG	ACCAAATGAC	TGGCATACTG	GCAACAGATG	TCAGTGCGGA	CGACGCGCAA
1141	AAACTGCTGG	TTGGGCTCAA	CCAGCGTATA	GTGCTCAACG	GTGCGACCCA	GAGAAACACC
1201	AATACCATGA	AAAATTACCT	TTTGCCCGTA	GTGGCCCAGG	CATTTGCTAG	GTGGGCAAAG
1261	GAATATAAGG	AAGATCAAGA	AGATGAAAGG	CCACTAGGAC	TACGAGATAG	ACAGTTAGTC
1321	ATGGGGTGTT	GTTGGGCTTT	TAGAAGGCAC	AAGATAACAT	CTATTTATAA	GCGCCCGGAT
1381	ACCCAAACCA	TCATCAAAGT	GAACAGCGAT	TTCCACTCAT	TCGTGCTGCC	CAGGATAGGC
1441	AGTAACACAT	TGGAGATCGG	GCTGAGAACA	AGAATCAGGA	AAATGTTAGA	GGAGCACAAG
1501	GAGCCGTAC	CTCTCATTAC	CGCCGAGGAC	GTACAAGAA	CTAAGTGCGC	AGCCGATGAG
1561	GCTAAGGAGG	TGCGTGAAGC	CGAGGAGTTG	CGCGCAGCTC	TACCACCTTT	GGCAGCTGAT
1621	GTTGAGGAGC	CCACTCTGGA	AGCCGATGTC	GACTTGATGT	TACAAGAGGC	TGGGGCCGGC
1681	TCAGTGGAGA	CACCTCGTGG	CTTGATAAAG	GTTACCAGCT	ACGATGGCGA	GGACAAGATC
1741	GGCTCTTACG	CTGTGCTTTC	TCCGCAGGCT	GTACTCAAGA	GTGAAAAATT	ATCTTGCATC
1801	CACCCTCTCG	CTGAACAAGT	CATAGTGATA	ACACACTCTG	GCCGAAAAGG	GCGTTATGCC
1861	GTGGAACCAT	ACCATGGTAA	AGTAGTGGTG	CCAGAGGGAC	ATGCAATACC	CGTCCAGGAC
1921	TTTCAAGCTC	TGAGTGAAAG	TGCCACCATT	GTGTACAACG	AACGTGAGTT	CGTAAACAGG
1981	TACCTGCACC	ATATTGCCAC	ACATGGAGGA	GCGCTGAACA	CTGATGAAGA	ATATTACAAA
2041	ACTGTCAAGC	CCAGCGAGCA	CGACGGCGAA	TACCTGTACG	ACATCGACAG	GAAACAGTGC
2101	GTCAAGAAAG	AACTAGTCAC	TGGGCTAGGG	CTCACAGGCG	AGCTGGTGGA	TCCTCCCTTC
2161	CATGAATTCG	CCTACGAGAG	TCTGAGAACA	CGACCAGCCG	CTCCTTACCA	AGTACCAACC
2221	ATAGGGGTGT	ATGGCGTGCC	AGGATCAGGC	AAGTCTGGCA	TCATTAAAAG	CGCAGTCACC
2281	AAAAAAGATC	TAGTGGTGAG	CGCCAAGAAA	GAAAACTGTG	CAGAAATTAT	AAGGGACGTC
2341	AAGAAAATGA	AAGGGCTGGA	CGTCAATGCC	AGAACTGTGG	ACTCAGTGCT	CTTGAATGGA
2401	TGCAAAACACC	CCGTAGAGAC	CCTGTATATT	GACGAAGCTT	TTGCTTGCTA	TGCAGGTACT
2461	CTCAGAGCGC	TCATAGCCAT	TATAAGACCT	AAAAAGGCAG	TGCTCTGCGG	GGATCCCAAA
2521	CAGTGCGGTT	TTTTTAACAT	GATGTGCCTG	AAAGTGCAAT	TTAACCACGA	GATTTGCACA
2581	CAAGTCTTCC	ACAAAAGCAT	CTCTCGCCGT	TGCACTAAAT	CTGTGACTTC	GGTCGTCTCA
2641	ACCTTGTTTT	ACGACAAAAA	AATGAGAACG	ACGAATCCGA	AAGAGACTAA	GATTGTGATT
2701	GACACTACCG	GCAGTACCAA	ACCTAAGCAG	GACGATCTCA	TTCTCACTTG	TTTCAGAGGG
2761	TGGGTGAAGC	AGTTGCAAAT	AGATTACAAA	GGCAACGAAA	TAATGACGGC	AGCTGCCTCT
2821	CAAGGGCTGA	CCCGTAAAGG	TGTGTATGCC	GTTCCGTACA	AGGTGAATGA	AAATCCTCTG
2881	TACGCACCCA	CCTCAGAACA	TGTGAACGTC	CTACTGACCC	GCACGGAGGA	CCGCATCGTG
2941	TGGAAAACAC	TAGCCGGCGA	CCCATGGATA	AAAACACTGA	CTGCCAAGTA	CCCTGGGAAT
3001	TTCACTGCCA	CGATAGAGGA	GTGGCAAGCA	GAGCATGATG	CCATCATGAG	GCACATCTTG
3061	GAGAGACCGG	ACCTTACCGA	CGTCTTCCAG	AATAAGGCAA	ACGTGTGTTG	GGCCAAGGCT
3121	TTAGTGCCGG	TGCTGAAGAC	CGCTGGCATA	GACATGACCA	CTGAACAATG	GAACACTGTG
3181	GATTATTTTG	AAACGGACAA	AGCTCACTCA	GCAGAGATAG	TATTGAACCA	ACTATGCGTG
3241	AGGTTCTTTG	GACTCGATCT	GGACTCCGGT	CTATTTTCTG	CACCCACTGT	TCCGTTATCC

3301	ATTAGGAATA	ATCACTGGGA	TAACTCCCCG	TCGCCTAACA	TGTACGGGCT	GAATAAAGAA
3361	GTGGTCCGTC	AGCTCTCTCG	CAGGTACCCA	CAACTGCCTC	GGGCAGTTGC	CACTGGAAGA
3421	GTCTATGACA	TGAACACTGG	TACACTGCGC	AATTATGATC	CGCGCATAAA	CCTAGTACCT
3481	GTAAACAGAA	GACTGCCTCA	TGCTTTAGTC	CTCCACCATA	ATGAACACCC	ACAGAGTGAC
3541	TTTTCTTCAT	TCGTGAGCAA	ATTGAAGGGC	AGAACTGTCC	TGGTGGTCGG	GGAAAAGTTG
3601	TCCGTCCCAG	GCAAAATGGT	TGACTGGTTG	TCAGACCGGC	CTGAGGCTAC	CTTCAGAGCT
3661	CGGCTGGATT	TAGGCATCCC	AGGTGATGTG	CCCAAATATG	ACATAATATT	TGTTAATGTG
3721	AGGACCCCAT	ATAAATACCA	TCACTATCAG	CAGTGTGAAG	ACCATGCCAT	TAAGCTTAGC
3781	ATGTTGACCA	AGAAAGCTTG	TCTGCATCTG	AATCCCGGCG	GAACCTGTGT	CAGCATAGGT
3841	TATGGTTACG	CTGACAGGGC	CAGCGAAAGC	ATCATTTGGT	CTATAGCGCG	GCAGTTCAAG
3901	TTTTCCCGGG	TATGCAAACC	GAAATCCTCA	CTTGAAGAGA	CGGAAGTTCT	GTTTGTATTC
3961	ATTGGGTACG	ATCGCAAGGC	CCGTACGCAC	AATCCTTACA	AGCTTTCATC	AACCTTACC
4021	AACATTTTATA	CAGGTTCCAG	ACTCCACGAA	GCCGGATGTG	CACCCTCATA	TCATGTGGTG
4081	CGAGGGGATA	TTGCCACGGC	CACCGAAGGA	GTGATTATAA	ATGCTGCTAA	CAGCAAAGGA
4141	CAACCTGGCG	GAGGGGTGTG	CGGAGCGGTG	TATAAGAAAT	TCCCGGAAAG	CTTCGATTTA
4201	CAGCCGATCG	AAGTAGGAAA	AGCGCGACTG	GTCAAAGGTG	CAGCTAAACA	TATCATTCAT
4261	GCCGTAGGAC	CAAACCTTCAA	CAAAGTTTCG	GAGGTTGAAG	GTGACAAACA	GTTGGCAGAG
4321	GCTTTATGAGT	CCATCGCTAA	GATTGTCAAC	GATAACAATT	ACAAGTCAGT	AGCGATTCCA
4381	CTGTTGTCCA	CCGGCATCTT	TTCCGGGAAC	AAAGATCGAC	TAACCCAATC	ATTGAACCAT
4441	TTGCTGACAG	CTTTAGACAC	CACTGATGCA	GATGTAGCCA	TATACTGCAG	GGACAAGAAA
4501	TGGGAAATGA	CTCTCAAGGA	AGCAGTGGCT	AGGAGAGAAG	CAGTGGAGGA	GATATGCATA
4561	TCCGACGACT	CTTCAGTGAC	AGAACCTGAT	GCAGAGCTGG	TGAGGGTGCA	TCCGAAGAGT
4621	TCTTTGGCTG	GAAGGAAGGG	CTACAGCACA	AGCGATGGCA	AACTTTTCTC	ATATTTGGAA
4681	GGGACCAAGT	TTCAACAGGC	GGCCAAGGAT	ATAGCAGAAA	TTAATGCCAT	GTGGCCCGTT
4741	GCAACGGAGG	CCAATGAGCA	GGTATGCATG	TATATCCTCG	GAGAAAGCAT	GAGCAGTATT
4801	AGGTCGAAAT	GCCCCGTGCA	AGAGTCGGAA	GCCTCCACAC	CACCTAGCAC	GCTGCCTTGC
4861	TTGTGTCATCC	ATGCCATGAC	TCCAGAAAGA	GTACAGCGCC	TAAAAGCCTC	ACGTCCAGAA
4921	CAAACTACTG	TGTGCTCATC	CTTTCCATTG	CCGAAGTATA	GAATCACTGG	TGTGCAGAAG
4981	ATCCAATGCT	CCCAGCCTAT	ATTGTTCTCA	CCGAAAGTGC	CTGCGTATAT	TCATCCAAGG
5041	AAGTATCTCG	TGGAAACACC	ACCGGTAGAC	GAGACTCCGG	AGCCATCGGC	AGAGAACCAG
5101	TCCACAGAGG	GGACACCTGA	ACAACCACCA	CTTATAACCG	AGGATGAGAC	CAGGACTAGA
5161	ACGCCTGAGC	CGATCATCAT	CGAAGAGGAA	GAAGAGGATA	GCATAAGTTT	GCTGTCAGAT
5221	GGCCCGACCC	ACCAGGTGCT	GCAAGTCGAG	GCAGACATTG	ACGGGCCGCC	CTCTGTATCT
5281	AGCTCATCCT	GGTCCATTCC	TCATGCATCC	GACTTTGATG	TGGACAGTTT	ATCCATACTT
5341	GACACCCTGG	AGGGAGCTAG	CGTGACCAGC	GGGGCAACGT	CAGCCGAGAC	TAACTCTTAC
5401	TTCGCAAAAGA	GTATGGAGTT	TCTGGCGCGA	CCGGTGCCCTG	CGCCTCGAAC	AGTATTCAAG
5461	AACCCTCCAC	ATCCCGCTCC	GCGCACAGA	ACACCGTCAC	TTGCACCCAG	CAGGGCCTGC
5521	TCGAGAACCA	GCCTAGTTTC	CACCCCGCCA	GGCGTGAATA	GGGTGATCAC	TAGAGAGGAG
5581	CTCGAGGCGC	TTACCCCGTC	ACGCACTCCT	AGCAGGTCGG	TCTCGAGAAC	CAGCCTGGTC
5641	TCCAACCCGC	CAGGCGTAAA	TAGGGTGATT	ACAAGAGAGG	AGTTTGAGGC	GTTCTGTAGCA
5701	CAACAACAAT	GACGGTTTGA	TGCGGGTGCA	TACATCTTTT	CCTCCGACAC	CGGTCAAGGG
5761	CATTTACAAC	AAAAATCAGT	AAGGCAACCG	GTGCTATCCG	AAGTGGTGTT	GGAGAGGACC
5821	GAATTGGAGA	TTTCGTATGC	CCCGCGCCTC	GACCAAGAAA	AAGAAGAATT	ACTACGCAAG
5881	AAATTACAGT	TAAATCCCAC	ACCTGCTAAC	AGAAGCAGAT	ACCAGTCCAG	GAAGGTGGAG
5941	AACATGAAAG	CCATAACAGC	TAGACGTATT	CTGCAAGGCC	TAGGGCATTG	TTTGAAGGCA
6001	GAAGGAAAAA	TGGAGTGCTA	CCGAACCCCTG	CATCCTGTTC	CTTTGTATTC	ATCTAGTGTG
6061	AACCGTGCCT	TTTCAAGCCC	CAAGGTCGCA	GTGGAAGCCT	GTAACGCCAT	GTTGAAAGAG
6121	AACTTTCCGA	CTGTGGCTTC	TTACTGTATT	ATTCCAGAGT	ACGATGCCTA	TTTGGACATG
6181	GTTGACGGAG	CTTCATGCTG	CTTAGACACT	GCCAGTTTTT	GCCCTGCAAA	GCTGCGCAGC
6241	TTTCCAAAGA	AACACTCCTA	TTTGGAACCC	ACAATACGAT	CGGCAGTGCC	TTAGCGGATC
6301	CAGAACACGC	TCCAGAACGT	CCTGGCAGCT	GCCACAAAAA	GAAATTGCAA	TGTCACGCAA
6361	ATGAGAGAAT	TGCCCCGTATT	GGATTCGGCG	GCCTTTAATG	TGGAATGCTT	CAAGAAATAT
6421	CCGTGTAATA	ATGAATATTG	GGAAACGTTT	AAAGAAAACC	CCATCAGGCT	TACTGAAGAA
6481	AACGTGGTAA	ATTACATTAC	CAAATTAATA	GGACCAAAAG	CTGCTGCTCT	TTTTGCGAAG
6541	ACACATAATT	TGAATATGTT	GCAGGACATA	CCAATGGACA	GGTTTGTAAT	GGACTTAAAG
6601	AGAGACGTGA	AAGTGACTCC	AGGAACAAAA	CATACTGAAG	AACGGCCCAA	GGTACAGGTG
6661	ATCCAGGCTG	CCGATCCGCT	AGCAACAGCG	TATCTGTGCG	GAATCCACCG	AGAGCTGGTT
6721	AGGAGATTAA	ATGCGGTCCT	GCTTCCGAAC	ATTATACAC	TGTTTGATAT	GTCGGCTGAA
6781	GACTTTGACG	CTATTATAGC	CGAGCACTTC	CAGCCTGGGG	ATTGTGTTCT	GGAAACTGAC

6841	ATCGCGTCGT	TTGATAAAAAG	TGAGGACGAC	GCCATGGCTC	TGACCGCGTT	AATGATTCTG
6901	GAAGACTTAG	GTGTGGACGC	AGAGCTGTTG	ACGCTGATTG	AGGCGGCTTT	CGGCGAAATT
6961	TCATCAATAC	ATTTGCCCAC	TAAAACTAAA	TTTAAATTCTG	GAGCCATGAT	GAAATCTGGA
7021	ATGTTCTCTCA	CACTGTTTTGT	GAACACAGTC	ATTAACATTG	TAATCGCAAG	CAGAGTGTTG
7081	AGAGAACGGC	TAACCGGATC	ACCATGTGCA	GCATTTCATTG	GAGATGACAA	TATCGTGAAA
7141	GGAGTCAAAT	CGGACAAATT	AATGGCAGAC	AGGTGCGCCA	CCTGGTTGAA	TATGGAAGTC
7201	AAGATTATAG	ATGCTGTGGT	GGGCGAGAAA	GCGCCTTATT	TCTGTGGAGG	GTTTATTTTG
7261	TGTGACTCCG	TGACCGGCAC	AGCGTGCCGT	GTGGCAGACC	CCCTAAAAAG	GCTGTTTAAG
7321	CTTGGCAAAC	CTCTGGCAGC	AGACGATGAA	CATGATGATG	ACAGGAGAAG	GGCATTGCAT
7381	GAAGAGTCAA	CACGCTGGAA	CCGAGTGGGT	ATTCTTTCAG	AGCTGTGCAA	GGCAGTAGAA
7441	TCAAGGTATG	AAACCGTAGG	AACTTCCATC	ATAGTTATGG	CCATGACTAC	TCTAGCTAGC
7501	TGTTGTTAAAT	CATTACAGTA	CCTGAGAGGG	GCCCCATAAA	CTCTCTACGG	CTAACCTGAA
7561	TGGACTACGA	CATAGTCTAG	TCCGCCAAGG	CCACCatgga	cagcaacacg	gtgtcctcct
7621	tccagggtgga	ctgcttcctc	tggcacgtgc	gcaagcgctt	cggcgaccag	gagctgggag
7681	acgccccctt	cctggaccgc	cttcgcccgg	accagaagtc	cctgcccggg	cggggagcga
7741	cgcttgccct	ggacatccgc	acggccaccc	gggaggggaa	gcacatcggt	gagcggtacc
7801	tggaggagga	gtcggacgag	gccctgaaga	tgacgatcgc	gagcgtgccc	gcgccccggt
7861	acctaaccga	gatgacgctg	gaggagatga	gcagggactg	gctgatgctc	atccccaaag
7921	agaagggtgac	cggttccttc	tgcatatgca	tggaccaggc	catcatggac	aaggacatca
7981	tcctgaaggc	caacttcagc	gtcatcttta	accggctgga	ggccctcatc	ctgctccgag
8041	ccttcaccga	cgagggggcc	attgtggggg	agatcagccc	cctccccagc	ctgcccggcc
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8161	acaacaccgt	ccgctgtgag	gagacctcc	agcgggtcac	gtggcgagc	tctgacgaga
8221	acggccggag	ccccctccc	cccaagcaga	agcgggaagat	ggagcgagc	atcgagcccc
8281	aggtgGCTAC	TAACTTCAGC	CTGCTGAAGC	AGGCTGGCGA	CGTGGAGGAG	AACCTGGAC
8341	CTatggtgag	caagggcgag	gagctgttca	ccgggggtggt	gccccctctg	gtcgagctgg
8401	acggcgacgt	aaacggccac	aagttcagcg	tgtccggcga	ggcgagggg	gatgccacct
8461	acggcaagct	gacctgaag	ttcatctgca	ccaccggcaa	gctgcccgtg	ccctggccca
8521	ccctcgtgac	cacctgacc	tacggcgtgc	agtgtctcag	ccgtacccc	gaccacatga
8581	agcagcacga	cttcttcaag	tccgccatgc	ccgaaggcta	cgtccaggag	cgcacatct
8641	tcttcaagga	cgacggcaac	tacaagaccc	gcgcccaggt	gaagtctcag	ggcgacaccc
8701	tggatgaaccg	catcgagctg	aagggcacgc	acttcaagga	ggacggcaac	atcctggggc
8761	acaagctgga	gtacaactac	aacagccaca	acgtctatat	catggccgac	aagcagaaga
8821	acggcatcaa	ggtgaacttc	aagatccgcc	acaacatcga	ggacggcagc	gtgcagctcg
8881	ccgaccacta	ccagcagaac	acccccatcg	gcgacggccc	cgtgctgctg	cccgaacaac
8941	actacctgag	caccagctcc	gccctgagca	aagaccccaa	cgagaagcgc	gatcacatgg
9001	tcctgctgga	gttcgtgacc	gccgcccggg	tcactctcgg	catggacgag	ctgtacaagt
9061	aaTGATAATA	TGTTACGTGC	AAAGGTGATT	GTCACCCCC	GAAAGACCAT	ATTGTGACAC
9121	ACCTCAGTA	TCACGCCCAA	ACATTTACAG	CCGCGGTGTC	AAAAACCGCG	TGGACGTGGT
9181	TAACATCCCT	GCTGGGAGGA	TCAGCCGTAA	TTATTATAAT	TGGCTTGGTG	CTGGCTACTA
9241	TTGTGGCCAT	GTACGTGCTG	ACCAACCAGA	AACATAATTG	AATACAGCAG	CAATTGGCAA
9301	GCTGCTTACA	TAGAACTCGC	GGCGATTGGC	ATGCCGCCCT	AAAATTTTTA	TTTTATTTTT
9361	CTTTTCTTTT	CCGAATCGGA	TTTTGTTTTT	AATATTTCAA	AAAAAAAAAA	AAAAAAAAAA
9421	AAAAAAAAAA	AAAAAAAAAA	aagaagagcG	CAGCTCTGGC	CCGTGTCTCA	AAATCTCTGA
9481	TGTTACATTG	CACAAGATAA	AAATATATCA	TCATGAACAA	TAAAACTGTC	TGCTTACATA
9541	AACAGTAATA	CAAGGGGTGT	TATGAGCCAT	ATTCAACGGG	AAACGTGCGA	GCCGCGATTA
9601	AATTCACA	TGGATGCTGA	TTTATATGGG	TATAAATGGG	CTCGCGATAA	TGTCGGGCAA
9661	TCAGGTGCGA	CAATCTATCG	CTTGTATGGG	AAGCCCGATG	CGCCAGAGTT	GTTTCTGAAA
9721	CATGGCAAAG	GTAGCGTTGC	CAATGATGTT	ACAGATGAGA	TGGTCAGACT	AAACTGGCTG
9781	ACGGAATTTA	TGCCCTCTTC	GACCATCAAG	CATTTTATCC	GTACTCCTGA	TGATGCATGG
9841	TTACTCACCA	CTGCGATCCC	CGGAAAAACA	GCATTCCAGG	TATTAGAAGA	ATATCCTGAT
9901	TCAGGTGAAA	ATATTGTTGA	TGCGCTGGCA	GTGTTCTCTG	GCCGGTTGCA	TTCGATTCTT
9961	GTTTGTAAAT	GTCTTTTTAA	CAGCGATCGC	GTATTTCTGC	TCGCTCAGGC	GCAATCACGA
10021	ATGAATAACG	GTTTGGTTGA	TGCGAGTGAT	TTTGATGACG	AGCGTAATGG	CTGGCCTGTT
10081	GAACAAGTCT	GGAAAGAAAT	GCATAAACTT	TTGCCATTCT	CACCGGATTC	AGTCGTCAC
10141	CATGGTGATT	TCTCACTTGA	TAACCTTATT	TTTGACGAGG	GGAAATTAAT	AGGTTGTATT
10201	GATGTTGGAC	GAGTCGGAAT	CGCAGACCGA	TACCAGGATC	TTGCCATCCT	ATGGAACCTG
10261	CTCGGTGAGT	TTTCTCCTTC	ATTACAGAAA	CGGCTTTTTT	AAAAATATGG	TATTGATAAT
10321	CCTGATATGA	ATAAATTGCA	GTTTCATTTG	ATGCTCGATG	AGTTTTTCTA	ATCAGAATTG

10381	GTTAATTGGT	TGTAACACTG	GCAGAGCATT	ACGCTGACTT	GACGGGACGG	CGCAAGCTCA
10441	TGACCAAAAT	CCCTTAACGT	GAGTTACGCG	TCGTTCCACT	GAGCGTCAGA	CCCCGTAGAA
10501	AAGATCAAAG	GATCTTCTTG	AGATCCTTTT	TTTCTGCGCG	TAATCTGCTG	CTTGCAAACA
10561	AAAAAACCAC	CGCTACCAGC	GGTGGTTTGT	TTGCCGGATC	AAGAGCTACC	AACTCTTTTT
10621	CCGAAGGTAA	CTGGCTTCAG	CAGAGCGCAG	ATACCAAATA	CTGTTCTTCT	AGTGTAGCCG
10681	TAGTTAGGCC	ACCACTTCAA	GAACCTGTGA	GCACCGCCTA	CATACCTCGC	TCTGCTAATC
10741	CTGTTACCAG	TGGCTGCTGC	CAGTGGCGAT	AAGTCGTGTC	TTACCGGGTT	GGACTCAAGA
10801	CGATAGTTAC	CGGATAAGGC	GCAGCGGTGC	GGCTGAACGG	GGGGTTCGTG	CACACAGCCC
10861	AGCTTGGAGC	GAACGACCTA	CACCGAAGCTG	AGATACCTAC	AGCGTGAGCT	ATGAGAAAGC
10921	GCCACGCTTC	CCGAAGGGAG	AAAGGCGGAC	AGGTATCCGG	TAAGCGGCAG	GGTCGGAACA
10981	GGAGAGCGCA	CGAGGGAGCT	TCCAGGGGGA	AACGCCTGGT	ATCTTTATAG	TCCTGTCGGG
11041	TTTCGCCACC	TCTGACTTGA	GCGTCGATTT	TTGTGATGCT	CGTCAGGGGG	GCGGAGCCTA
11101	TGGA AAAACG	CCAGCAACGC	GGCCTTTTTA	CGGTTCTCTG	CCTTTTGCTG	GCCTTTTGCT
11161	CACAT					
Strand NS1-mCherry (SEQ ID NO: 7)						
1	ACAAAGGCAA	CGAAATAATG	ACGGCAGCTG	CCTCTCAAGG	GCTGACCCGT	AAAGGTGTGT
61	ATGCCGTTTC	GTACAAGGTG	AATGAAAATC	CTCTGTACGC	ACCCACCTCA	GAACATGTGA
121	ACGTCTTACT	GACCCGCACG	GAGGACCGCA	TCGTGTGGAA	AACACTAGCC	GGCGACCCAT
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241	AAGCAGAGCA	TGATGCCATC	ATGAGGCACA	TCTTGGAGAG	ACCGGACCCT	ACCGACGTCT
301	TCCAGAAATA	GGCAAACGTG	TGTTGGGCCA	AGGCTTTAGT	GCCGGTGCTG	AAGACCGCTG
361	GCATAGACAT	GACCACTGAA	CAATGGAACA	CTGTGGATTA	TTTTGAAACG	GACAAAGCTC
421	ACTCAGCAGA	GATAGTATTG	AACCAACTAT	GCGTGAGGTT	CTTTGGACTC	GATCTGGACT
481	CCGGTCTATT	TTCTGCACCC	ACTGTTCCGT	TATCCATTAG	GAATAATCAC	TGGGATAACT
541	CCCCGTCGCC	TAACATGTAC	GGGCTGAATA	AAGAAGTGGT	CCGTCAGCTC	TCTCGCAGGT
601	ACCCACAAC	GCCTCGGGCA	GTTGCCACTG	GAAGAGTCTA	TGACATGAAC	ACTGGTACAC
661	TGCGCAATTA	TGATCCGCGC	ATAAACCTAG	TACCTGTAAA	CAGAAGACTG	CCTCATGCTT
721	TAGTCCTCCA	CCATAATGAA	CACCCACAGA	GTGACTTTTC	TTCATTCTGC	AGCAAATTGA
781	AGGGCAGAAC	TGTCCTGGTG	GTCGGGGAAA	AGTTGTCCGT	CCCAGGCAAA	ATGGTTGACT
841	GGTTGTCAGA	CCGGCCTGAG	GCTACCTTCA	GAGCTCGGCT	GGATTTAGGC	ATCCCAGGTG
901	ATGTGCCCAA	ATATGACATA	ATATTTGTGA	ATGTGAGGAC	CCCATATAAA	TACCATCACT
961	ATCAGCAGTG	TGAAGACCAT	GCCATTAAGC	TTAGCATGTT	GACCAAGAAA	GCTTGTCTGC
1021	ATCTGAATCC	CGGCGGAACC	TGTGTCAGCA	TAGGTTATAG	TTACGCTGAC	AGGGCCAGCG
1081	AAAGCATCAT	TGGTGTCTATA	GCGCGGCAGT	TCAAGTTTTC	CCGGGTATGC	AAACCGAAAT
1141	CCTCACTTGA	AGAGACGGAA	GTTCTGTTTG	TATTCATTGG	GTACGATCGC	AAGGCCCGTA
1201	CGCACAATCC	TTACAAGCTT	TCATCAACCT	TGACCAACAT	TTATACAGGT	TCCAGACTCC
1261	ACGAAGCCGG	ATGTGCACCC	TCATATCATG	TGGTGCAGAG	GGATATTGCC	ACGGCCACCG
1321	AAGGAGTGAT	TATAAATGCT	GCTAACAGCA	AAGGACAACC	TGGCGGAGGG	GTGTGCGGAG
1381	CGCTGTATAA	GAAATTCCCG	GAAAGCTTCG	ATTTACAGCC	GATCGAAGTA	GGAAAAGCGC
1441	GACTGGTCAA	AGGTGCAGCT	AAACATATCA	TTCATGCCGT	AGGACCAAAC	TTCAACAAAG
1501	TTTCGGAGGT	TGAAGGTGAC	AAACAGTTGG	CAGAGGCTTA	TGAGTCCATC	GCTAAGATTG
1561	TCAACGATAA	CAATTACAAG	TCAGTAGCGA	TTCCACTGTT	GTCCACCGGC	ATCTTTTCCG
1621	GGAACAAAGA	TCGACTAACC	CAATCATTGA	ACCATTTGCT	GACAGCTTTA	GACACCACTG
1681	ATGCAGATGT	AGCCATATAC	TGCAGGGACA	AGAAATGGGA	AATGACTCTC	AAGGAAGCAG
1741	TGGCTAGGAG	AGAAGCAGTG	GAGGAGATAT	GCATATCCGA	CGACTCTTCA	GTGACAGAAC
1801	CTGATGCAGA	GCTGGTGAGG	GTGCATCCGA	AGAGTTCTTT	GGCTGGAAGG	AAGGGCTACA
1861	GCACAAGCGA	TGGCAAAACT	TTCTCATATT	TGGAAGGGAC	CAAGTTTTCAC	CAGGCGGCCA
1921	AGGATATAGC	AGAAATTAAT	GCCATGTGGC	CCGTTGCAAC	GGAGGCCAAT	GAGCAGGTAT
1981	GCATGTATAT	CCTCGGAGAA	AGCATGAGCA	GTATTAGGTC	GAAATGCCCC	GTGCAAGAGT
2041	CGGAAGCCTC	CACACCACCT	AGCACGCTGC	CTTGCTTGTC	CATCCATGCC	ATGACTCCAG
2101	AAAGAGTACA	GCGCCTAAAA	GCCTCACGTC	CAGAACAAAT	TACTGTGTGC	TCATCTTTTC
2161	CATTGCCGAA	GTATAGAATC	ACTGGTGTGC	AGAAGATCCA	ATGCTCCAG	CCTATATTGT
2221	TCTCACCGAA	AGTGCCTGCG	TATATTTCATC	CAAGGAAGTA	TCTCGTGGAA	ACACCACCGG
2281	TAGACGAGAC	TCCGGAGCCA	TCCGCAGAGA	ACCAATCCAC	AGAGGGGACA	CCTGAACAAC
2341	CACCACTTAT	AACCGAGGAT	GAGACCAAGG	CTAGAACGCC	TGAGCCGATC	ATCATCGAAG
2401	AGGAAGAAGA	GGATAGCATA	AGTTTGCTGT	CAGATGGCCC	GACCCACGAC	GTGCTGCAAG
2461	TCGAGGCAGA	CATTACCGGG	CCGCCCTCTG	TATCTAGCTC	ATCCTGGTCC	ATTCTCATG
2521	CATCCGACTT	TGATGTGGAC	AGTTTATCCA	TACTTGACAC	CCTGGAGGGA	GCTAGCGTGA
2581	CCAGCGGGGC	AACGTCAGCC	GAGACTAACT	CTTACTTCGC	AAAGAGTATG	GAGTTTCTGG

2641	CGCGACCGGT	GCCTGCGCCT	CGAACAGTAT	TCAGGAACCC	TCCACATCCC	GCTCCGCGCA
2701	CAAGAACACC	GTCACCTGCA	CCCAGCAGGG	CCTGCTCGAG	AACCAGCCTA	GTTTCCACCC
2761	CGCCAGGCGT	GAATAGGGTG	ATCACTAGAG	AGGAGCTCGA	GGCGCTTACC	CCGTCACGCA
2821	CTCCTAGCAG	GTCGGTCTCG	AGAACCAGCC	TGGTCTCCAA	CCCGCCAGGC	GTAAATAGGG
2881	TGATTACAAG	AGAGGAGTTT	GAGGCGTTTC	TAGCACAACA	ACAATGACGG	TTTGATGCGG
2941	GTGCATACAT	CTTTTCTCTC	GACACCGGTC	AAGGGCATT	ACAACAAAA	TCAGTAAGGC
3001	AAACGGTGCT	ATCCGAAGTG	GTGTTGGAGA	GGACCGAATT	GGAGATTTTC	TATGCCCCGC
3061	GCCTCGACCA	AGAAAAAGAA	GAATTACTAC	GCAAGAAATT	ACAGTTAAAT	CCCACACCTG
3121	CTAACAGAAG	CAGATACCAG	TCCAGGAAGG	TGGAGAACAT	GAAAGCCATA	ACAGCTAGAC
3181	GTATTCTGCA	AGGCCTAGGG	CATTATTTGA	AGGCAGAAGG	AAAAGTGGAG	TGCTACCGAA
3241	CCCTGCATCC	TGTTCTTTTG	TATTCATCTA	GTGTGAACCG	TGCCTTTTTC	AGCCCCAAGG
3301	TCGCAGTGGA	AGCCTGTAAC	GCCATGTTGA	AAGAGAACTT	TCCGAGTTTC	GCTTCTTACT
3361	GTATTATTCC	AGAGTACGAT	GCCTATTTGG	ACATGGTTGA	CGGAGCTTCA	TGCTGCTTAG
3421	ACACTGCCAG	TTTTTGCCCT	GCAAAGCTGC	GCAGCTTTCC	AAAGAAACAC	TCCTATTTGG
3481	AACCCACAAT	ACGATCGGCA	GTGCCTTCAG	CGATCCAGAA	CACGCTCCAG	AACGTCTTGG
3541	CAGCTGCCAC	AAAAAGAAAT	TGCAATGTCA	CGCAAATGAG	AGAATTGCC	GTATTGGATT
3601	CGGCGGCCTT	TAATGTGGAA	TGCTTCAAGA	AATATGCGTG	TAATAATGAA	TATTGGGAAA
3661	CGTTTAAAGA	AAACCCATC	AGGCTTACTG	AAGAAAACGT	GGTAAATTAC	ATTACCAAAT
3721	TAAAAGGACC	AAAAGCTGCT	GCTCTTTTTG	CGAAGACACA	TAATTTGAAT	ATGTTGCAGG
3781	ACATACCAAT	GGACAGGTTT	GTAATGGACT	TAAAGAGAGA	CGTGAAAGTG	ACTCCAGGAA
3841	CAAAACATAC	TGAAGAACGG	CCCAGGTAC	AGGTGATCCA	GGCTGCCGAT	CCGCTAGCAA
3901	CAGCGTATCT	GTGCGGAATC	CACCGAGAGC	TGGTTAGGAG	ATTAAATGCG	GTCTGCTTC
3961	CGAACATTCA	TACACTGTTT	GATATGTCGG	CTGAAGACTT	TGACGCTATT	ATAGCCGAGC
4021	ACTTCCAGCC	TGGGGATTGT	GTTCTGGAAA	CTGACATCGC	GTCGTTTTGAT	AAAAGTGAGG
4081	ACGACGCCAT	GGCTCTGACC	GCCTTAATGA	TTCTGGAAGA	CTTAGGTGTG	GACGCAGAGC
4141	TGTTGACGCT	GATTGAGGCG	GCTTTCGGCG	AAATTTTCATC	AATACATTTG	CCCACTAAAA
4201	CTAAATTTAA	ATTTCGAGCC	ATGATGAAAT	CTGGAATGTT	CCTCACACTG	TTTGTGAACA
4261	CAGTCATTAA	CATTGTAATC	GCAAGCAGAG	TGTTGAGAGA	ACGGCTAACC	GGATCACCAT
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4441	AGAAAGCGCC	TTATTTCTGT	GGAGGGTTTA	TTTTGTGTGA	CTCCGTGACC	GGCACAGCGT
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4561	ATGAACATGA	TGATGACAGG	AGAAGGGCAT	TGCATGAAGA	GTCAACACGC	TGGAACCGAG
4621	TGGGTATTCT	TTCAGAGCTG	TGCAAGGCAG	TAGAATCAAG	GTATGAAACC	GTAGGAACTT
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4741	GAGGGGCCCC	TATAACTCTC	TACGGCTAAC	CTGAATGGAC	TACGACATAG	TCTAGTCCGC
4801	CAAGGCCACC	atggacagca	acacgggtgtc	ctccttccag	gtggactgct	tcctctggca
4861	cgtgcgcaag	cgcttcgccc	accaggagct	ggcgacgccc	cccttcctgg	accgcttcg
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5521	GAAGCAGGCT	GGCGACGTGG	AGGAGAACCC	TGGACCTATG	GTGAGCAAGG	GCGAGGAGGA
5581	TAACATGGCC	ATCATCAAGG	AGTTCATGCG	CTTCAAGGTG	CACATGGAGG	GCTCCGTGAA
5641	CGGCCACGAG	TTCGAGATCG	AGGGCGAGGG	CGAGGGCCGC	CCCTACGAGG	GCACCCAGAC
5701	CGCCAAGCTG	AAGGTGACCA	AGGGTGGtCC	CCTGCCCTTC	GCCTGGGACA	TCCTGTCCCC
5761	TCAGTTTCATG	TACGGCTCCA	AGGCCTACGT	GAAGCACCCC	GCCGACATCC	CCGACTACTT
5821	GAAGCTGTCC	TTCCCCGAGG	GCTTCAAGTG	GGAGCGCGTG	ATGAACCTTC	AGGACGCGCG
5881	CGTGGTGACC	GTGACCCAGG	ACTCCTCCCT	GCAGGACGGC	GAGTTCATCT	ACAAGGTGAA
5941	GCTGCGCGGC	ACCAACTTCC	CCTCCGACGG	CCCCGTAATG	CAGAAGAAGA	CCATGGGCTG
6001	GGAGGCCTCC	TCCGAGCGGA	TGTACCCCGA	GGACGGCGCC	CTGAAGGGCG	AGATCAAGCA
6061	GAGGCTGAAG	CTGAAGGACG	GCGGCCACTA	CGACGCTGAG	GTCAAGACCA	CCTACAAGGC
6121	CAAGAAGCCC	GTGCAGCTGC	CCGGCGCCTA	CAACGTCAAC	ATCAAGTTGG	ACATCACCTC

6181	CCACAACGAG	GACTACACCA	TCGTGGAACA	GTACGAACGC	GCCGAGGGCC	GCCACTCCAC
6241	CGGCGGCATG	GACGAGCTGT	ACAAGTGATA	ATATGTTACG	TGCAAAGGTG	ATTGTCACCC
6301	CCCGAAAGAC	CATATTGTGA	CACACCCTCA	GTATCACGCC	CAAACATTTA	CAGCCGCGGT
6361	GTCAAAAACC	GCGTGGACGT	GGTTAACATC	CCTGCTGGGA	GGATCAGCCG	TAATTATTAT
6421	AATTGGCTTG	GTGCTGGCTA	CTATTGTGGC	CATGTACGTG	CTGACCAACC	AGAAACATAA
6481	TTGAATACAG	CAGCAATTGG	CAAGCTGCTT	ACATAGAACT	CGCGGCGATT	GGCATGCCGC
6541	CTTAAAAATT	TTATTTTATT	TTTCTTTTCT	TTTCCGAATC	GGATTTTGTG	TTTAATATTT
6601	CAAAAAAATA	AAAAAATAA	AAAAAATAA	AAAAAATAA	ATAGGGATAA	CAGGGTAATT
6661	GAGCAAAAGG	CCAGCAAAAG	GCCAGGAACC	GTAAAAAGGC	CGCGTTGCTG	GCGTTTTTCC
6721	ATAGGCTCCG	CCCCCTTGAC	GAGCATCACA	AAAATCGACG	CTCAAGTCAG	AGGTGGCGAA
6781	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG	AAGCTCCCTC	GTGCGCTCTC
6841	CTGTTCCGAC	CCTGCCGCTT	ACCGGATATC	TGTCGCGCTT	TCTCCCTTCG	GGAAGCGTGG
6901	CGTTTTCTCA	TAGCTCACGC	TGTAGGTATC	TCAGTTCGGT	GTAGGTCGTT	CGCTCCAAGC
6961	TGGGCTGTGT	GCACGAACCC	CCCGTTCAGC	CCGACCGCTG	CGCCTTATCC	GGTAACTATC
7021	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT	GGCAGCAGCC	ACTGGTAAAC
7081	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT	CTTGAAGTGG	TGGCCTAACT
7141	ACGGCTACAC	TAGAAGAACA	GTATTTGGTA	TCTGCGCTCT	GCTGAAGCCA	GTTACCTTCG
7201	GAAAAAGAGT	TGGTAGCTCT	TGATCCGGCA	AACAAACCAC	CGCTGGTATT	GGTGGTTTTC
7261	TTGTTTGCAA	GCAGCAGATT	ACGCGCAGAA	AAAAAGGATC	TCAAGAAGAT	CCTTTGATCT
7321	TTTCTACGGG	GTCTGACGCT	CAGTGAACG	AAAACCTCAC	TTAAGGGATT	TTGGTCATGA
7381	GATTATCAAA	AAGGATCTTC	ACCTAGATCC	TTTAAATTA	AAAATGAAGT	TTTAAATCAA
7441	TCTAAAGTAT	ATATGAGTAA	ACTTGGTCTG	ACAGTTACCA	ATGCTTAATC	AGTGAGGCAC
7501	CTATCTCAGC	GATCTGTCTA	TTTCGTTTCAT	CCATAGTTGC	CTGACTCCCC	GTCTGTAGTA
7561	TAACCTACGAT	ACGGGAGGGC	TTACCATCTC	GCCCCAGTGC	TGCAATGATA	CCGCGAGACC
7621	CACGCTCACC	GGCTCCAGAT	TTATCAGCAA	TAAACCAGCC	AGCCGGAAGG	GCCGAGCGCA
7681	GAAGTGGTCC	TGCAACTTTA	TCCGCCTCCA	TCCAGTCTAT	TAATTGTTGC	CGGGAAGCTA
7741	GAGTAAGTAG	TTCCGCCAGT	AATAGTTTGC	GCAACGTTGT	TGCCATTGCT	ACAGGCATCG
7801	TGGTGTACAG	CTCGTCGTTT	GGTATGGCTT	CATTCAGCTC	CGGTTCCTCA	CGATCAAGGC
7861	GAGTTACATG	ATCCCCCATG	TTGTGCAAAA	AAGCGGTTAG	CTCCTTCGGT	CCTCCGATCG
7921	TAGTTCAGAA	TAAGTTGGCC	GCAGTGTTAT	CACCTCATGG	TATGGCAGCA	CTGCATAATT
7981	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	TGGTGAGTAC	TCAACCAAGT
8041	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	GTTGCTCTTG	CCCGGCGTCA	ATACGGGATA
8101	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	TGGAACACGT	TCTTCGGGGC
8161	GAAACTCTC	AAGGATCTTA	CCGCTGTTGA	GATCCAGTTC	GATGTAACCC	ACTCGTGCAC
8221	CCAACGTATC	TTCAGCATCT	TTTACTTTCA	CCAGCGTTTC	TGGGTGAGCA	AAAACAGGAA
8281	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	CGACACGGAA	ATGTTGAATA	CTCATACTCT
8341	TCCTTTTTCA	ATATTATTGA	AGCATTTATC	AGGGTTATTG	TCTCATGAGC	GGATACATAT
8401	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	GGGTTCCGCG	CACATTTCCC	CGAAAAGTGC
8461	CACCTGACGT	TAGGGATAAC	AGGGTAATTA	ATACGACTCA	CTATAATGGG	CGGCGCATGA
8521	GAGAAGCCCA	GACCAATTAC	CTACCCAAAA	TGGAGAAAGT	TCACGTTGAC	ATCGAGGAAG
8581	ACAGCCCATT	CCTCAGAGCT	TTGCAGCGGA	GCTTCCCGCA	GTTTGAGGTA	GAAGCCAAGC
8641	AGGTCACTGA	TAATGACCAT	GCTAATGCCA	GAGCGTTTTTC	GCATCTGGCT	TCAAAACTGA
8701	TCGAAACGGA	GGTGGACCCA	TCCGACACGA	TCCTTGACAT	TGGAAGTGCG	CCCGCCCGCA
8761	GAATGTATTC	TAAGCACAAG	TATCATTGTA	TCTGTCCGAT	GAGATGTGCG	GAAGATCCGG
8821	ACAGATTGTA	TAAGTATGCA	ACTAAGCTGA	AGAAAAACTG	TAAGGAAATA	ACTGATAAGG
8881	AATTGGACAA	GAAAAATGAG	GAGCTCGCCG	CCGTCTAGAG	CGACCCTGAC	CTGGAAACTG
8941	AGACTATGTG	CCTCCACGAC	GACGAGTCGT	GTCGCTACGA	AGGGCAAGTC	GCTGTTTACC
9001	AGGATGTATA	CGCGGTTGAC	GGACCGACAA	GTCTCTATCA	CCAAGCCAAT	AAGGGAGTTA
9061	GAGTCGCCCTA	CTGGATAGGC	TTTGACACCA	CCCCTTTTAT	GTTTAAAGAAC	TTGGCTGGAG
9121	CATATCCATC	ATACTCTACC	AACTGGGCCG	ACGAAACCGT	GTTAACGGCT	CGTAACATAG
9181	GCCTATGCAG	CTCTGACGTT	ATGGAGCGGT	CACGTAGAGG	GATGTCCATT	CTTAGAAAGA
9241	AGTATTTGAA	ACCATCCAAC	AATGTTCTAT	TCTCTGTTGG	CTCGACCATC	TACCACGAGA
9301	AGAGGGACTT	ACTGAGGAGC	TGGCACCTGC	CGTCTGTATT	TCACCTTACG	GCGAAGCAAA
9361	ATTACACATG	TCGGTGTGAG	ACTATAGTTA	GTTGCGACGG	GTACGTCGTT	AAAAGAATAG
9421	CTATCAGTCC	AGGCCTGTAT	GGGAAGCCTT	CAGGCTATGC	TGCTACGATG	CACCGCGAGG
9481	GATTCTTGTG	CTGCAAAGTG	ACAGACACAT	TGAACGGGGA	GAGGGTCTCT	TTTCCCGTGT
9541	GCACGTATGT	GCCAGCTACA	TTGTGTGACC	AAATGACTGG	CATACTGGCA	ACAGATGTCA
9601	GTGCGGACGA	CGCGCAAAAA	CTGCTGGTTG	GGCTCAACCA	GCGTATAGTC	GTCAACGGTC
9661	GCACCCAGAG	AAACACCAAT	ACCATGAAAA	ATTACCTTTT	GCCCGTAGTG	GCCCAGGCAT

9721	TTGCTAGGTG	GGCAAAGGAA	TATAAGGAAG	ATCAAGAAGA	TGAAAGGCCA	CTAGGACTAC
9781	GAGATAGACA	GTTAGTCATG	GGGTGTTGTT	GGGCTTTTAG	AAGGCACAAG	ATAACATCTA
9841	TTTATAAGCG	CCCGGATACC	CAAACCATCA	TCAAAGTGAA	CAGCGATTTC	CACTCATTCG
9901	TGCTGCCCAG	GATAGGCAGT	AACACATTGG	AGATCGGGCT	GAGAACAAGA	ATCAGGAAAA
9961	TGTTAGAGGA	GCACAAGGAG	CCGTACCTC	TCATTACCGC	CGAGGACGTA	CAAGAAGCTA
10021	AGTGCGCAGC	CGATGAGGCT	AAGGAGGTGC	GTGAAGCCGA	GGAGTTGCGC	GCAGCTCTAC
10081	CACCTTTGGC	AGCTGATGTT	GAGGAGCCCA	CTCTGGAAGC	CGATGTCGAC	TTGATGTTAC
10141	AAGAGGCTGG	GGCCGGCTCA	GTGGAGACAC	CTCGTGGCTT	GATAAAGGTT	ACCAGCTACG
10201	ATGGCGAGGA	CAAGATCGGC	TCTTACGCTG	TGCTTTCTCC	GCAGGCTGTA	CTCAAGAGTG
10261	AAAAATTATC	TTGCATCCAC	CCTCTCGCTG	AACAAGTCAT	AGTGATAACA	CACTCTGGCC
10321	GAAAAGGGCG	TTATGCCGTG	GAACCATACC	ATGGTAAAGT	AGTGGTGCCA	GAGGGACATG
10381	CAATACCCGT	CCAGGACTTT	CAAGCTCTGA	GTGAAAGTGC	CACCATTTGT	TACAACGAAC
10441	GTGAGTTCGT	AAACAGGTAC	CTGCACCATA	TTGCCACACA	TGGAGGAGCG	CTGAACACTG
10501	ATGAAGAATA	TTACAAAAC	GTCAAGCCCA	GCGAGCACGA	CGGCGAATAC	CTGTACGACA
10561	TCGACAGGAA	ACAGTGCCTC	AAGAAAGAAC	TAGTCACTGG	GCTAGGGGTC	ACAGGCGAGC
10621	TGGTGGATCC	TCCCTTCCAT	GAATTGCGCT	ACGAGAGTCT	GAGAACACGA	CCAGCCGCTC
10681	CTTACCAAGT	ACCAACCATA	GGGGTGTATG	GCGTGCCAGG	ATCAGGCAAG	TCTGGCATCA
10741	TTAAAAGCGC	AGTCACCAAA	AAAGATCTAG	TGGTGAGCGC	CAAGAAAAGAA	AACCTGTCAG
10801	AAATTATAAG	GGACGTCAAG	AAAATGAAAG	GGCTGGACGT	CAATGCCAGA	ACTGTGGACT
10861	CAGTGCTCTT	GAATGGATGC	AAACACCCCG	TAGAGACCCT	GTATATTGAC	GAAGCTTTTG
10921	CTTGTCATGC	AGGTACTCTC	AGAGCGCTCA	TAGCCATTAT	AAGACCTAAA	AAGGCAGTGC
10981	TCTGCGGGGA	TCCCAAACAG	TGCGGTTTTT	TTAACATGAT	GTGCCTGAAA	GTGCATTTTA
11041	ACCACGAGAT	TTGCACACAA	GTCTTCCACA	AAAGCATCTC	TCGCCGTTGC	ACTAAATCTG
11101	TGACTTCGGT	CGTCTCAACC	TTGTTTTACG	ACAAAAAAAT	GAGAACGACG	AATCCGAAAG
11161	AGACTAAGAT	TGTGATTGAC	ACTACCGGCA	GTACCAAACC	TAAGCAGGAC	GATCTCATTC
11221	TCACTTGTTT	CAGAGGGTGG	GTGAAGCAGT	TGCAAATAGA	TT	
Non-cytopathic-NS1-EGFP (SEQ ID NO: 8)						
1	ATGTGCACCC	TCATATCATG	TGGTGCAGAG	GGATATTGCC	ACGGCCACCG	AAGGAGTGAT
61	TATAAATGCT	GCTAACAGCA	AAGGACAACC	TGGCGGAGGG	GTGTGCGGAG	CGCTGTATAA
121	GAAATTCCTCG	GAAAGCTTCG	ATTTACAGCC	GATCGAAGTA	GGAAAAGCGC	GA CTGGTCAA
181	AGGTGCAGCT	AAACATATCA	TTTCATGCCGT	AGGACCAAAC	TTCAACAAAG	TTTCGGAGGT
241	TGAAGGTGAC	AAACAGTTGG	CAGAGGCTTA	TGAGTCCATC	GCTAAGATTG	TCAACGATAA
301	CAATTACAAG	TCAGTAGCGA	TTCCACTGTT	GTCCACCGGC	ATCTTTTCCG	GGAACAAAAG
361	TGCACTAACC	CAATCATTTGA	ACCATTTGCT	GACAGCTTTA	GACACCACTG	ATGCAGATGT
421	AGCCATATAC	TGCAGGGACA	AGAAATGGGA	AATGACTCTC	AAGGAAGCAG	TGGCTAGGAG
481	AGAAGCAGTG	GAGGAGATAT	GCATATCCGA	CGACTCTTCA	GTGACAGAAC	CTGATGCAGA
541	GCTGGTGAGG	GTGCATCCGA	AGAGTTCTTT	GGCTGGAAGG	AAGGGCTACA	GCACAAGCGA
601	TGGCAAAACT	TTCTCATATT	TGGAAGGGAC	CAAGTTTCAC	CAGGCGGCCA	AGGATATAGC
661	AGAAATTAAT	GCCATGTGGC	CCGTTGCAAC	GGAGGCCAAT	GAGCAGGTAT	GCATGTATAT
721	CCTCGGAGAA	AGCATGAGCA	GTATTAGGTC	GAAATGCCCC	GTCGAAGAGT	CGGAAGCCTC
781	CACACCACCT	AGCACGCTGC	CTTGCTTGTTG	CATCCATGCC	ATGACTCCAG	AAAGAGTACA
841	GCGCCTAAAA	GCCTCACGTC	CAGAACAAAT	TACTGTGTGC	TCATCCTTTC	CATTGCCGAA
901	GTATAGAATC	ACTGGTGTGC	AGAAGATCCA	ATGCTCCCAG	CCTATATTGT	TCTCACCGAA
961	AGTGCCCTGCG	TATATTCATC	CAAGGAAGTA	TCTCGTGGA	ACACCACCGG	TAGACGAGAC
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1081	AACCGAGGAT	GAGACCAGGA	CTAGAACGCC	TGAGCCGATC	ATCATCGAAG	AGGAAGAAGA
1141	GGATAGCATA	AGTTTGCTGT	CAGATGGCCC	GACCCACCAG	GTGCTGCAAG	TCGAGGCAGA
1201	CATTACGGG	CCGCCCTCTG	TATCTAGCTC	ATCCTGGTCC	ATTCTCTCATG	CATCCGACTT
1261	TGATGTGAGC	AGTTTATCCA	TACTTGACAC	CCTGGAGGGA	GCTAGCGTGA	CCAGCGGGGC
1321	AACGTCAGCC	GAGACTAACT	CTTACTTCGC	AAAGAGTATG	GAGTTTCTGG	CGCGACCGGT
1381	GCCTGCGCCT	CGAACAGTAT	TCAGGAACCC	TCCACATCCC	GCTCCGCGCA	CAAGAACACC
1441	GTCACTTGCA	CCCAGCAGGG	CCTGCTCGAG	AACCAGCCTA	GTTTCCACCC	CGCCAGGCGT
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1561	GTCGGTCTCG	AGAACCAGCC	TGGTCTCCAA	CCCGCCAGGC	GTAAATAGGG	TGATTACAAG
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1681	CTTTTCTCTC	GACACCGGTC	AAGGGCATTT	ACAACAAAAA	TCAGTAAGGC	AAACGGTGCT
1741	ATCCGAAGTG	GTGTTGGAGA	GGACCGAATT	GGAGATTTTC	TATGCCCCGC	GCCTCGACCA

1801	AGAAAAAGAA	GAATTACTAC	GCAAGAAATT	ACAGTTAAAT	CCCACACCTG	CTAACAGAAG
1861	CAGATACCAG	TCCAGGAAGG	TGGAGAACAT	GAAAGCCATA	ACAGCTAGAC	GTATTCTGCA
1921	AGGCCTAGGG	CATTATTTGA	AGGCAGAAGG	AAAAGTGGAG	TGCTACCGAA	CCCTGCATCC
1981	TGTTCCCTTG	TATTCATCTA	GTGTGAACCG	TGCCTTTTCA	AGCCCCAAGG	TCGCAGTGGA
2041	AGCCTGTAAC	GCCATGTTGA	AAGAGAACTT	TCCGACTGTG	GCTTCTTACT	GTATTATTCC
2101	AGAGTACGAT	GCCTATTTGG	ACATGGTTGA	CGGAGCTTCA	TGCTGCTTAG	ACACTGCCAG
2161	TTTTTGGCCT	GCAAAGCTGC	GCAGCTTTCC	AAAGAAACAC	TCCTATTTGG	AACCCACAAT
2221	ACGATCGGCA	GTGCCTTCAG	CGATCCAGAA	CACGCTCCAG	AACGTCTCGG	CAGCTGCCAC
2281	AAAAAGAAAT	TGCAATGTCA	CGCAAATGAG	AGAATTGCCC	GTATTGGATT	CGGCGGCCTT
2341	TAATGTGGAA	TGCTTCAAGA	AATATGCGTG	TAATAATGAA	TATTGGGAAA	CGTTTAAAGA
2401	AAACCCCATC	AGGCCTTACTG	AAGAAAACGT	GGTAAATTAC	ATTACCAAAT	TAAAAGGACC
2461	AAAAGCTGCT	GCTCTTTTGG	CGAAGACACA	TAATTTGAAT	ATGTTGCAGG	ACATACCAAT
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2581	TGAAGAACGG	CCCAAGGTAC	AGGTGATCCA	GGCTGCCGAT	CCGCTAGCAA	CAGCGTATCT
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2761	TGGGGATTGT	GTTCTGGAAG	CTGACATCGC	GTCGTTTGAT	AAAAGTGAGG	ACGACGCCAT
2821	GGCTCTGACC	GCGTTAATGA	TTCTGGAAGA	CTTAGGTGTG	GACGCAGAGT	TGTTGAGCCT
2881	GATTGAGGCG	GCTTTCGGCG	AAATTTTCATC	AATACATTTG	CCCACTAAAA	CTAAATTTAA
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3181	TTATTTCTGT	GGAGGGTTTA	TTTTGTGTGA	CTCCGTGACC	GGCACAGCGT	GCCGTGTGGC
3241	AGACCCCTTA	AAAAGGCTGT	TTAAGCTTGG	CAAACCTCTG	GCAGCAGACG	ATGAACATGA
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3361	TTCAGAGCTG	TGCAAGGCAG	TAGAATCAAG	GTATGAAACC	GTAGGAACTT	CCATCATAGT
3421	TATGGCCATG	ACTACTCTAG	CTAGCAGTGT	TAAATCATTC	AGCTACCTGA	GAGGGGCCCC
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4261	GGCGACGTGG	AGGAGAACCC	TGGACCTatg	gtgagcaagg	gcgaggagct	gttcaccggg
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4921	cccaacgaga	agcgcgatca	catggtctct	ctggagttcg	tgaccgcgcg	cgggatcact
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5461	AACAATAAAA	CTGTCTGCTT	ACATAAACAG	TAATACAAGG	GGTGTATATGA	GCCATATTCA
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5641	CGATGCGCCA	GAGTTGTTTC	TGAAACATGG	CAAAGGTAGC	GTTGCCAATG	ATGTTACAGA
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6601	AAATACTGTT	CTTCTAGTGT	AGCCGTAGTT	AGGCCACCAC	TTCAAGAACT	CTGTAGCACC
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6721	GTGTCTTACC	GGGTTGGACT	CAAGACGATA	GTTACCGGAT	AAGGCGCAGC	GGTCGGGCTG
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7321	GCTTCAAAAC	TGATCGAAAC	GGAGGTGGAC	CCATCCGACA	CGATCCTTGA	CATTGGGAAGT
7381	GCGCCCCCCC	GCAGAAATGTA	TTCTAAGCAC	AAGTATCATT	GTATCTGTCC	GATGAGATGT
7441	GCGGAAGATC	CGGACAGATT	GTATAAGTAT	GCAACTAAGC	TGAAGAAAAA	CTGTAAGGAA
7501	ATAACTGATA	AGGAATTGGA	CAAGAAAATG	AAGGAGCTCG	CCGCCGTCAT	GAGCGACCCT
7561	GACCTGGAAA	CTGAGACTAT	GTGCCTCCAC	GACGACGAGT	CGTGTCTGTA	CGAAGGGCAA
7621	GTCGCTGTTT	ACCAGGATGT	ATACGCGGTT	GACGGACCGA	CAAGTCTCTA	TCACCAAGCC
7681	AATAAGGGAG	TTAGAGTCGC	CTACTGGATA	GGCTTTGACA	CCACCCCTTT	TATGTTTAAG
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7861	ATTCTTAGAA	AGAAGTATTT	GAAACCATCC	AACAATGTTT	TATTCTCTGT	TGGCTCGACC
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7981	CGTGGCAAGC	AAAATTACAC	ATGTCGGTGT	GAGACTATAG	TTAGTTGCGA	CGGGTACGTC
8041	GTTAAAAGAA	TAGCTATCAG	TCCAGGCCTG	TATGGGAAGC	CTTCAGGCTA	TGCTGCTACG
8101	ATGCACCGCG	AGGGATTCTT	GTGCTGCAAA	GTGACAGACA	CATTGAACGG	GGAGAGGGTC
8161	TCTTTTCCCG	TGTGCACGTA	TGTGCCAGCT	ACATTGTGTG	ACCAAATGAC	TGGCATACTG
8221	GCAACAGATG	TCAGTGCGGA	CGACGCGCAA	AAACTGCTGG	TTGGGCTCAA	CCAGCGTATA
8281	GTCGTCAACG	GTGCGACCCA	GAGAAACACC	AATACCATGA	AAAATTACCT	TTTGCCCGTA
8341	GTGGCCCAAG	CATTTGCTAG	GTGGGCAAG	GAATATAAGG	AAGATCAAGA	AGATGAAAGG
8401	CCACTAGGAC	TACGAGATAG	ACAGTTAGTC	ATGGGGTGT	GTTGGGCTTT	TAGAAGGCAC
8461	AAGATAACAT	CTATTTATAA	GCGCCCGGAT	ACCCAAACCA	TCATCAAAGT	GAACAGCGAT
8521	TTCCACTCAT	TCGTGCTGCC	CAGGATAGGC	AGTAACACAT	TGGAGATCGG	GCTGAGAACAC
8581	AGAATCAGGA	AAATGTTAGA	GGAGCACAAG	GAGCCGTCAC	CTCTCATTAC	CGCCGAGGAC
8641	GTACAAGAAG	CTAAGTGCGC	AGCCGATGAG	GCTAAGGAGG	TGCGTGAAGC	CGAGGAGTTG
8701	CGCGCAGCTC	TACCACCTTT	GGCAGCTGAT	GTTGAGGAGC	CCACTCTGGA	AGCCGATGTC
8761	GACTTGATGT	TACAAGAGGC	TGGGGCCGGC	TCAGTGGAGA	CACCTCGTGG	CTTGATAAAG
8821	GTTACCAGCT	ACGATGGCGA	GGACAAGATC	GGCTCTTACG	CTGTGCTTTC	TCCGCAGGCT

8881	GTACTCAAGA	GTGAAAAATT	ATCTTGCATC	CACCTCTCTG	CTGAACAAGT	CATAGTGATA
8941	ACACACTCTG	GCCGAAAAGG	GCGTTATGCC	GTGGAACCAT	ACCATGGTAA	AGTAGTGGTG
9001	CCAGAGGGAC	ATGCAATACC	CGTCCAGGAC	TTTCAAGCTC	TGAGTGAAAG	TGCCACCATT
9061	GTGTACAACG	AACGTGAGTT	CGTAAACAGG	TACCTGCACC	ATATTGCCAC	ACATGGAGGA
9121	GCGCTGAACA	CTGATGAAGA	ATATTACAAA	ACTGTCAAGC	CCAGCGAGCA	CGACGGCGAA
9181	TACCTGTACG	ACATCGACAG	GAAACAGTGC	GTCAAGAAAG	AACTAGTCAC	TGGGCTAGGG
9241	CTCACAGGCG	AGCTGGTGGA	TCCTCCCTTC	CATGAATTCT	CCTACGAGAG	TCTGAGAACA
9301	CGACCAGCCG	CTCCTTACCA	AGTACCAACC	ATAGGGGTGT	ATGGCGTGCC	AGGATCAGGC
9361	AAGTCTGGCA	TCATTAAAAG	CGCAGTCACC	AAAAAAGATC	TAGTGGTGAG	CGCCAAGAAA
9421	GAAAACTGTG	CAGAAATTAT	AAGGGACGTC	AAGAAAATGA	AAGGGCTGGA	CGTCAATGCC
9481	AGAAGTGTGG	ACTCAGTGCT	CTTGAATGGA	TGCAAAACACC	CCGTAGAGAC	CCTGTATATT
9541	GACGAAGCTT	TTGCTTGTC	TGCAGGTACT	CTCAGAGCGC	TCATAGCCAT	TATAAGACCT
9601	AAAAAGGCAG	TGCTCTGCGG	GGATCCCAAA	CAGTGCAGTT	TTTTTAACAT	GATGTGCTCG
9661	AAAGTGCATT	TTAACCACGA	GATTTGCACA	CAAGTCTTCC	ACAAAAGCAT	CTCTCGCCGT
9721	TGCACTAAAT	CTGTGACTTC	GGTCGTCTCA	ACCTTGTTTT	ACGACAAAAA	AATGAGAACG
9781	ACGAATCCGA	AAGAGACTAA	GATTGTGATT	GACACTACCG	GCAGTACCAA	ACCTAAGCAG
9841	GACGATCTCA	TTCTCACTTG	TTTCAGAGGG	TGGGTGAAGC	AGTTGCAAAAT	AGATTACAAA
9901	GGCAACGAAA	TAATGACGGC	AGCTGCCTCT	CAAGGGCTGA	CCCGTAAAGT	GATGTATGCC
9961	GTTTCGGTACA	AGGTGAATGA	AAATCCTCTG	TACGCACCCA	CCTCAGAACA	TGTGAACGTC
10021	CTACTGACCC	GCACGGAGGA	CCGCATCGTG	TGGAACACAC	TAGCCGGCGA	CCCATGGATA
10081	AAAACACTGA	CTGCCAAGTA	CCCTGGGAAT	TTCACTGCCA	CGATAGAGGA	GTGGCAAGCA
10141	GAGCATGATG	CCATCATGAG	GCACATCTTG	GAGAGACCGG	ACCCTACCGA	CGTCTTCCAG
10201	AATAAGGCAA	ACGTGTGTTG	GGCCAAGGCT	TTAGTGCCGG	TGCTGAAGAC	CGCTGGCATA
10261	GACATGACCA	CTGAACAATG	GAACACTGTG	GATTATTTTG	AAACGGACAA	AGCTCACTCA
10321	GCAGAGATAG	TATTGAACCA	ACTATGCGTG	AGGTTCTTTG	GACTCGATCT	GGACTCCGGT
10381	CTATTTTCTG	CACCCACTGT	TCCGTTATCC	ATTAGGAATA	ATCACTGGGA	TAACCTCCCG
10441	TCGCCTAACA	TGTACGGGCT	GAATAAAGAA	GTGGTCCGTC	AGCTCTCTCG	CAGGTACCCA
10501	CAACTGCCTC	GGGCAGTTGC	CACTGGAAGA	GTCTATGACA	TGAACACTGG	TACACTGCGC
10561	AATTATGATC	CGCGCATAAA	CCTAGTACCT	GTAAACAGAA	GACTGCCTCA	TGCTTTAGTC
10621	CTCCACCATA	ATGAACACCC	ACAGAGTGAC	TTTTCTTCAT	TCGTCAAGCA	ATTGAAGGGC
10681	AGAAGTGTCC	TGGTGGTCGG	GGAAAAGTTG	TCCGTCCCAG	GCAAAATGGT	TGACTGGTTG
10741	TCAGACCGGC	CTGAGGCTAC	CTTCAGAGCT	CGGCTGGATT	TAGGCATCCC	AGGTGATGTG
10801	CCCAAATATG	ACATAATATT	TGTTAATGTG	AGGACCCCAT	ATAAATACCA	TCACTATCAG
10861	CAGTGTGAAG	ACCATGCCAT	TAAGCTTAGC	ATGTTGACCA	AGAAAGCTTG	TCTGCATCTG
10921	AATCCCAGGC	GAACCTGTGT	CAGCATAGGT	TATGGTTACG	CTGACAGGGC	CAGCGAAAGC
10981	ATCATTGGTG	CTATAGCGCG	GCTGTTCAAG	TTTTCCCGGG	TATGCAAAAC	GAAATCCTCA
11041	CTTGAAGAGA	CGGAAGTTCT	GTTTGTATTTC	ATTGGGTACG	ATCGCAAGGC	CCGTACGCAC
11101	AATCCTTACA	AGCTTTCATC	AACCTTGACC	AACATTTATA	CAGGTTCCAG	ACTCCACGAA
11161	GCCGG					

What is claimed is:

1. A polynucleotide or a set of polynucleotides comprising a first nucleic acid molecule encoding an influenza non-structural (NS1) protein and a second nucleic acid molecule encoding a heterologous target mRNA.
2. The polynucleotide or set of polynucleotides of claim 1, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are present in a first vector.
3. The polynucleotide or set of polynucleotides of claim 1, wherein the first nucleic acid molecule encoding the influenza NS1 protein is present in a first vector, and wherein the second nucleic acid molecule encoding the target mRNA is present in a second vector.
4. The polynucleotide or set of polynucleotides of any one of claims 1 to 3, wherein the first nucleic acid molecule encoding the influenza NS1 protein is expressed under the control of a first promoter.
5. The polynucleotide or set of polynucleotides of any one of claims 1 to 4, wherein the second nucleic acid molecule encoding the target mRNA is expressed under the control of a second promoter.
6. The polynucleotide or set of polynucleotides of claim 5, wherein the first promoter and the second promoter are the same.
7. The polynucleotide or set of polynucleotides of claim 5, wherein the first promoter and the second promoter are the different.
8. The polynucleotide or set of polynucleotides of claim 1 or 2, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are expressed under the control of a first promoter, wherein the first promoter drives expression of both the influenza NS1 protein and the target mRNA.
9. The polynucleotide or set of polynucleotides of claim 8, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are linked by an IRES sequence.

10. The polynucleotide or set of polynucleotides of any one of claims 2 to 9, wherein the first vector, the second vector, or both comprise one or more regulatory elements.
11. The polynucleotide or set of polynucleotides of any one of claims 1 to 10, wherein the expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.
12. The polynucleotide or set of polynucleotides of claim 11, wherein the expression of the target mRNA is increased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, or at least about 300% relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.
13. The polynucleotide or set of polynucleotides of claim 11 or 12, wherein the increase in the expression of the target mRNA persists for at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 24 hours, at least about 30 hours, at least about 36 hours, at least about 42 hours, or at least about 48 hours.
14. The polynucleotide or set of polynucleotides of any one of claims 1 to 13, wherein the target mRNA encodes a biologically active polypeptide.
15. The polynucleotide or set of polynucleotides of claim 14, wherein the biologically active polypeptide comprises a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof.
16. The polynucleotide or set of polynucleotides of claim 15, wherein the cytokine is IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, IFN- α , IFN- β , IFN- γ , CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL, TWEAK, TRANCE, TGF- β , TGF- β 1, TGF- β 2, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, MSP, a fragment thereof, a variant thereof, or any combination thereof.

17. The polynucleotide or set of polynucleotides of any one of claims 1 to 15, wherein the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof.
18. The polynucleotide or set of polynucleotides of claim 17, wherein the target mRNA encodes a p35 subunit of IL-12 and a p40 subunit of IL-12.
19. The polynucleotide or set of polynucleotides of claim 18, wherein the p35 subunit and the p40 subunit are expressed from a single promoter.
20. The polynucleotide or set of polynucleotides of claim 18 or 19, wherein the p35 subunit and the p40 subunit are expressed as a single contiguous polypeptide.
21. The polynucleotide or set of polynucleotides of any one of claims 18 to 20, wherein the p35 subunit and the p40 subunit are linked by one or more covalent bonds.
22. The polynucleotide or set of polynucleotides of any one of claims 18 to 21, wherein the p35 subunit and the p40 subunit are linked by one or more peptide bonds.
23. The polynucleotide or set of polynucleotides of claim 18 or 19, wherein a portion of the mRNA that encodes the p35 subunit is separated from a portion of the mRNA that encodes the p40 subunit by an IRES.
24. The polynucleotide or set of polynucleotides of any one of claims 1 to 13, wherein the target mRNA encodes a miRNA, siRNA, shRNA, a dsRNA, antisense oligonucleotide, a guide RNA, or any combination thereof.
25. The polynucleotide or set of polynucleotides of any one of claims 4 to 24, wherein the first promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.
26. The polynucleotide or set of polynucleotides of any one of claims 5 to 25, wherein the second promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.
27. The polynucleotide or set of polynucleotides of any one of claims 1 to 26, wherein the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof.

28. The polynucleotide or set of polynucleotides of any one of claims 1 to 26, wherein the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, or any combination thereof.
29. The polynucleotide or set of polynucleotides of any one of claims 1 to 28, wherein the influenza NS1 is H5N1 NS1.
30. The polynucleotide or set of polynucleotides of any one of claims 1 to 28, wherein the influenza NS1 is H1N1 NS1.
31. The polynucleotide or set of polynucleotides of claim 30, wherein the H1N1 NS1 is the H1N1 TX91 variant NS1.
32. The polynucleotide or set of polynucleotides of any one of claims 1 to 31, wherein the influenza NS1 encoded by the first nucleic acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2.
33. The polynucleotide or set of polynucleotides of any one of claims 1 to 32, wherein the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2.
34. The polynucleotide or set of polynucleotides of any one of claims 1 to 33, wherein the first nucleic acid molecule comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein.
35. The polynucleotide or set of polynucleotides of any one of claims 1 to 32, wherein the first nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein.

36. The polynucleotide or set of polynucleotides of any one of claims 1 to 35, comprising one or more modified nucleic acid molecule.
37. The polynucleotide or set of polynucleotides of any one of claims 1 to 36, wherein: (i) the first nucleic acid molecule, (ii) the second nucleic acid molecule, or (iii) both (i) and (ii) are circular RNA.
38. A polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA, wherein the self-replicating target mRNA comprises one or more modified nucleic acid molecule.
39. The polynucleotide or set of polynucleotides of any one of claims 1 to 38, wherein less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, or less than about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules.
40. The polynucleotide or set of polynucleotides of any one of claims 1 to 39, wherein about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules.
41. The polynucleotide or set of polynucleotides of any one of claims 36 to 40, wherein the one or more modified nucleic acid molecule is a modified rNTP.
42. The polynucleotide or the set of polynucleotides of any one of claims 36 to 41, wherein the one or more modified nucleic acid molecule comprises N1-methylpseudo uracil, 5-methyl cytosine, N6-methyladenosine, or combinations thereof.
43. A vector or a set of vectors comprising the polynucleotide or the set of polynucleotides of any one of claims 1 to 42.
44. The vector or the set of vectors of claim 43, which is a replicon.
45. The vector or the set of vectors of claim 43 or 44, which is a Venezuelan equine encephalitis (VEE) replicon or a derivative or portion thereof.

46. The vector or the set of vectors of claim any one of claims 43 to 45, which is a Venezuelan equine encephalitis (VEE) replicon comprising a nucleotide sequence encoding a lysine at residue 739, according to the wild-type amino acid sequence VEE.
47. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof.
48. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; and (vi) a VEE 3'UTR or a derivative thereof.
49. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA; (v) an E1 sequence; and (vi) a VEE 3'UTR or a derivative thereof.
50. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.
51. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

52. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding the influenza NS1 protein; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.
53. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target mRNA; and (v) a VEE 3'UTR or a derivative thereof.
54. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.
55. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.
56. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1 or an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.
57. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H1N1 TX91 variant NS1; (iv) a second

nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

58. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.
59. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 1; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.
60. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule encoding an H5N1 NS1; (iv) a P2A linker; (v) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; (vi) an E1 sequence; and (vii) a VEE 3'UTR or a derivative thereof.
61. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a VEE 3'UTR or a derivative thereof.

62. The vector or the set of vectors of claim any one of claims 43 to 46, wherein the vector comprises (i) a VEE 5'UTR or a derivative thereof; (ii) one or more non-structural protein (nsP); (iii) a first nucleic acid molecule comprising the nucleotide sequence set forth in SEQ ID NO: 2; (iv) a second nucleic acid molecule encoding the target mRNA, wherein the target mRNA encodes a human IL-12 polypeptide; and (v) a 3'UTR from a parent replicon, *e.g.*, a 3'UTR from VEE.
63. The vector of the set of vectors of any one of claims 47 to 62, wherein the one or more nsP comprises a VEE nsP or a derivative thereof.
64. The vector of the set of vectors of claim 63, wherein the VEE nsP is selected from nsP2, nsP3, nsP4, and any combination thereof.
65. A cell comprising the polynucleotide or set of polynucleotides of any one of claims 1 to 42 or the vector or the set of vectors of any one of claims 43 to 64.
66. The cell of claim 65, which is a mammalian cell.
67. The cell of claim 65 or 66, which is a human cell.
68. The cell of any one of claims 65 to 67, which is an immune cell.
69. A pharmaceutical composition comprising the polynucleotide or set of polynucleotides of any one of claims 1 to 42, the vector or the set of vectors of any one of claims 43 to 64, or the cell of any one of claims 65 to 68 and a pharmaceutically acceptable carrier.
70. A method of expressing a target mRNA in a cell, comprising transfecting the cell with the polynucleotide or set of polynucleotides of any one of claims 1 to 42 or the vector or the set of vectors of any one of claims 43 to 64.
71. The method of claim 70, wherein the cell is a human cell.
72. The method of claim 70 or 71, wherein the cell is an ex vivo human cell.
73. The method of any one of claims 70 to 72, wherein the cell is a human immune cell.
74. A method of treating a subject in need thereof, comprising administering to the subject the polynucleotide or set of polynucleotides of any one of claims 1 to 42, the vector or the set

of vectors of any one of claims 43 to 64, the cell of any one of claims 65 to 68, or the pharmaceutical composition of claim 69.

75. A method of expressing a target mRNA in a subject in need thereof, comprising administering to the subject the polynucleotide or set of polynucleotides of any one of claims 1 to 42, the vector or the set of vectors of any one of claims 43 to 64, the cell of any one of claims 66 to 68, or the pharmaceutical composition of claim 69.
76. The method of claim 74 or 75, wherein the subject is afflicted with a cancer.
77. The method of claim 76, wherein the cancer is selected from the group consisting of melanoma, squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine cancer, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, gastric cancer, and various types of head and neck cancer, including squamous cell head and neck cancer. In some aspects, the cancer can be melanoma, lung cancer, colorectal cancer, renal-cell cancer, urothelial carcinoma, Hodgkin's lymphoma, and any combination thereof.
78. A method of expressing a target mRNA in a cell, comprising co-expressing the target mRNA and an influenza NS1 protein in the cell, wherein the target mRNA is not an influenza mRNA.
79. The method of claim 78, wherein the influenza NS1 protein is encoded by a first nucleic acid molecule and the target mRNA is encoded by a second nucleic acid molecule.
80. The method of claim 78 or 79, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are present in a first vector.
81. The method of claim 78 or 79, wherein the first nucleic acid molecule encoding the influenza NS1 protein is present in a first vector, and wherein the second nucleic acid molecule encoding the target mRNA is present in a second vector.

82. The method of any one of claims 78 to 81, wherein the first nucleic acid molecule encoding the influenza NS1 protein is expressed under the control of a first promoter.
83. The method of any one of claims 78 to 82, wherein the second nucleic acid molecule encoding the target mRNA is expressed under the control of a second promoter.
84. The method of claim 83, wherein the first promoter and the second promoter are the same.
85. The method of claim 83, wherein the first promoter and the second promoter are the different.
86. The method of any one of claims 78 to 81, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are expressed under the control of a first promoter, wherein the first promoter drives expression of both the influenza NS1 protein and the target mRNA.
87. The method of claim 86, wherein the first nucleic acid molecule encoding the influenza NS1 protein and the second nucleic acid molecule encoding the target mRNA are linked by an IRES sequence.
88. The method of any one of claims 80 to 87, wherein the first vector, the second vector, or both comprise one or more regulatory elements.
89. The method of any one of claims 78 to 88, wherein expression of the target mRNA is increased relative to the expression of the target mRNA in the absence of the first nucleic acid molecule encoding the influenza NS1 protein.
90. The method of any one of claims 79 to 89, wherein: (i) the first nucleic acid molecule, (ii) the second nucleic acid molecule, or (iii) both (i) and (ii) are circular RNA.
91. A method of expressing a target mRNA in a cell, comprising transfecting the cell with a polynucleotide or a set of polynucleotides comprising a self-replicating target mRNA comprising one or more modified nucleic acid molecules.
92. The of claim 91, wherein less than about 50%, less than about 45%, less than about 40%, less than about 35%, less than about 30%, or less than about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules.

93. The method of claims 91 or 92, wherein about 25% of the nucleic acids in the polynucleotide or the set of polynucleotides are modified nucleic acid molecules.
94. The method any one of claims 91 to 93, wherein the one or more modified nucleic acid molecules is a modified rNTP.
95. The method of any one of claims 91 to 94, wherein the one or more modified nucleic acid molecules comprises N1-methylpseudo uracil, 5-methyl cytosine, N6-methyladenosine, or combinations thereof.
96. The method of any one of claims 91 to 95, wherein expression of the target mRNA is increased relative to the expression of the target mRNA from a self-replicating target mRNA not comprising one or more modified nucleic acid molecules.
97. The method of claim 96, wherein the expression of the target mRNA is increased by at least about 10%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 90%, at least about 100%, at least about 125%, at least about 150%, at least about 175%, at least about 200%, at least about 225%, at least about 250%, at least about 275%, or at least about 300%.
98. The method of claim 96 or 97, wherein the increase in the expression of the target mRNA persists for at least about 6 hours, at least about 12 hours, at least about 18 hours, at least about 24 hours, at least about 30 hours, at least about 36 hours, at least about 42 hours, or at least about 48 hours.
99. The method of any one of claims 70 to 98, wherein the target mRNA encodes a biologically active polypeptide.
100. The method of claim 99, wherein the biologically active polypeptide comprises a cytokine, a chemokine, a growth factor, a clotting factor, an enzyme, or any combination thereof.
101. The method of claim 100, wherein the cytokine is IL-1 α , IL-1 β , IL-1RA, IL-18, IL-2, IL-4, IL-7, IL-9, IL-13, IL-15, IL-3, IL-5, GM-CSF, IL-6, IL-11, G-CSF, IL-12, LIF, OSM, IL-10, IL-20, IL-14, IL-16, IL-17, IFN- α , IFN- β , IFN- γ , CD154, LT- β , TNF- α , TNF- β , 4-1BBL, APRIL, CD70, CD153, CD178, GITRL, LIGHT, OX40L, TALL-1, TRAIL,

TWEAK, TRANCE, TGF- β , TGF- β 1, TGF- β 2, TGF- β 3, Epo, Tpo, Flt-3L, SCF, M-CSF, MSP, a fragment thereof, a variant thereof, or any combination thereof.

102. The method of claim 101, wherein the target mRNA encodes an IL-12 polypeptide or a fragment or variant thereof.
103. The method of claim 102, wherein the target mRNA encodes a p35 subunit of IL-12 and a p40 subunit of IL-12.
104. The method of claim 103, wherein the p35 subunit and the p40 subunit are expressed from a single promoter.
105. The method of claim 103 or 104, wherein the p35 subunit and the p40 subunit are expressed as a single contiguous polypeptide.
106. The method of any one of claims 103 to 105, wherein the p35 subunit and the p40 subunit are linked by one or more covalent bonds.
107. The method of any one of claims 103 to 106, wherein the p35 subunit and the p40 subunit are linked by one or more peptide bonds.
108. The method of claim 103 or 107, wherein a portion of the mRNA that encodes the p35 subunit is separated from a portion of the mRNA that encodes the p40 subunit by an IRES.
109. The method of any one of claims 70 to 98, wherein the target mRNA encodes a miRNA, siRNA, shRNA, a dsRNA, antisense oligonucleotide, a guide RNA, or any combination thereof.
110. The method of any one of claims 82 to 90, wherein the first promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.
111. The method of any one of claims 83 to 90, 109, and 110, wherein the second promoter is an inducible promoter, a tissue specific promoter, or a constitutively active promoter.
112. The method of any one of claims 78 to 90 and 109 to 111, wherein the influenza NS1 is a type A influenza virus NS1, a type B influenza virus NS1, a type C influenza virus NS1, or a variant thereof.

113. The method of any one of claims 78 to 90 and 109 to 112, wherein the influenza NS1 is an H1N1 NS1, H1N2 NS1, H2N2 NS1, H3N2 NS1, H5N1 NS1, H7N9 NS1, H7N7 NS1, H9N2 NS1, H7N2 NS1, H7N3 NS1, H5N2 NS1, H10N7 NS1, or a combination thereof.
114. The method of any one of claims 78 to 90 and 109 to 113, wherein the influenza NS1 is H5N1 NS1.
115. The method of any one of claims 78 to 90 and 109 to 113, wherein the influenza NS1 is H1N1 NS1.
116. The method of claim 115, wherein the H1N1 NS1 is the H1N1 TX91 variant NS1.
117. The method of any one of claims 78 to 90 and 109 to 113, wherein the influenza NS1 encoded by the first nucleic acid molecule comprises an amino acid sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2.
118. The method of any one of claims 78 to 90 and 109 to 113, wherein the influenza NS1 encoded by the first nucleic acid molecule comprises the amino acid sequence encoded by the nucleotide sequence set forth in SEQ ID NO: 1 or 2.
119. The method of any one of claims 78 to 90 and 109 to 113, wherein the first nucleic acid molecule comprises a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein.
120. The method of any one of claims 78 to 90 and 109 to 113, wherein the first nucleic acid molecule comprises the nucleotide sequence set forth in SEQ ID NO: 1 or 2, wherein the nucleotide sequence encodes an influenza NS1 protein.