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(54) **Titre : CONSTRUCTIONS VIRALES DESTINEES A ETRE UTILISEES DANS L'AMELIORATION DE L'AMORCAGE DE LYMPHOCYTES T PENDANT LA VACCINATION**
(54) **Title: VIRAL CONSTRUCTS FOR USE IN ENHANCING T-CELL PRIMING DURING VACCINATION**

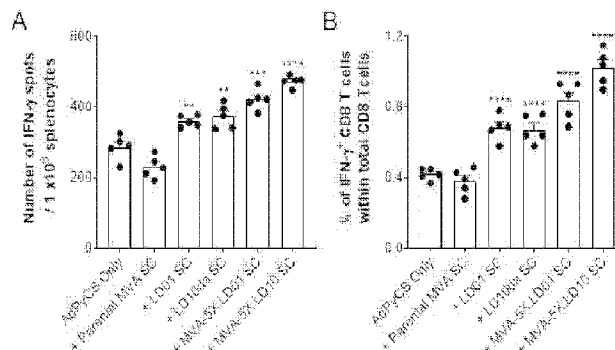


FIG. 9

(57) **Abbrégé/Abstract:**

The invention provides virus-based expression vectors comprising immune-checkpoint inhibitor inserts for use as effective adjuvants in enhancing T-cell priming to an antigen in a host during a vaccination regimen. In particular, the compositions described herein are novel recombinant modified vaccinia Ankara (MVA) viral constructs encoding one or more peptides which, upon administration, are expressed in a multimer conformation and subsequently cleaved and secreted from the cell. Such peptides are capable of downregulating an immune checkpoint pathway, for example, by inhibiting the activation of programmed-cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or another immune checkpoint regulator, or a combination thereof. When used in concert with the administration of an antigen during a vaccination strategy, the immune checkpoint expressing MV A viral construct provides significantly improved antigen-specific CD8⁺ T cell expansion, increased antigenic responses, and improved vaccination efficacy.



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Abstract:

The invention provides virus-based expression vectors comprising immune-checkpoint inhibitor inserts for use as effective adjuvants in enhancing T-cell priming to an antigen in a host during a vaccination regimen. In particular, the compositions described herein are novel recombinant modified vaccinia Ankara (MVA) viral constructs encoding one or more peptides which, upon administration, are expressed in a multimer conformation and subsequently cleaved and secreted from the cell. Such peptides are capable of downregulating an immune checkpoint pathway, for example, by inhibiting the activation of programmed-cell death protein 1 (PD-1), programmed cell death ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), or another immune checkpoint regulator, or a combination thereof. When used in concert with the administration of an antigen during a vaccination strategy, the immune checkpoint expressing MVA viral construct provides significantly improved antigen-specific CD8⁺ T cell expansion, increased antigenic responses, and improved vaccination efficacy.

VIRAL CONSTRUCTS FOR USE IN ENHANCING T-CELL PRIMING DURING VACCINATION

Cross Reference to Related Applications

5 This application claims priority to U.S. Provisional Application No. 63/144,834, filed February 2, 2021. The entirety of this application is hereby incorporated by reference herein for all purposes.

Field of the Invention

10 The invention provides virus-based expression vectors comprising immune-checkpoint inhibitor encoding nucleic acid inserts for use as effective adjuvants in enhancing T-cell priming to an antigen in a host during a vaccination regimen. In particular, the compositions described herein are novel recombinant modified vaccinia Ankara (MVA) viral constructs encoding immune checkpoint inhibitor peptides which, upon administration, are expressed in a multimer
15 conformation and subsequently cleaved and secreted from the cell.

Incorporation by Reference

 The contents of the text file named "19101-014WO1_SEQ_TXT" which was created on February 1, 2022 and is 564 KB in size, are hereby incorporated by reference in their entirety.

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Background of the Invention

 Vaccines are considered one of the most important advances in modern medicine and have greatly improved quality of life by reducing or eliminating many serious infectious diseases. Vaccines have been developed against a wide assortment of human pathogens, including, for
25 example, bacterial toxins (e.g., tetanus and diphtheria toxins), acute viral pathogens (e.g., measles, mumps, rubella), latent or chronic viral pathogens (e.g., varicella zoster virus [VZV] and human papilloma virus [HPV], respectively), respiratory pathogens (e.g., influenza, Bordetella pertussis), and enteric pathogens (e.g., poliovirus, Salmonella typhi). Most approved vaccines can be categorized as live, attenuated vaccines, non-replicating whole-particle vaccines (including virus-
30 like particles, or VLPs), and subunit vaccines.

In order to develop a successful vaccine, however, a powerful and long-lasting protective immunity that consists of humoral and cellular immune responses is needed. Both elements of immunity are essential for effectively eliminating pathogens. While advances have been made in developing vaccines against a number of pathogens, the inability to elicit potent, durable, and protective T cell immunity, particularly CD8⁺ T cell responses, has been a major obstacle and is the primary reason that many vaccine development efforts fail, particularly for intracellular pathogens (see, e.g., Seder et al., Vaccines against intracellular infections requiring cellular immunity. *Nature*. 2000 Aug 17;406(6797):793-8).

One strategy to overcome these inherent obstacles has been the identification and use of adjuvants that augment immunogenicity, and considerable work has gone into evaluating the impact of putative adjuvants on innate immune activation and on adaptive immune responses to model antigens and potential vaccines (see, e.g., Halbroth et al., Development of a Molecular Adjuvant to Enhance Antigen-Specific CD8⁺T Cell Responses. *Sci Rep*. 2018 Oct 9;8(1):15020; Counoupas et al., Delta inulin-based adjuvants promote the generation of polyfunctional CD4⁺T cell responses and protection against *Mycobacterium tuberculosis* infection. *Sci Rep*. 2017 Aug 17;7(1):8582; Thakur et al., Intracellular Pathogens: Host Immunity and Microbial Persistence Strategies. *Immunol Res*. 2019 Apr 14;2019:1356540).

For example, alhydrogel is a well-characterized aluminum hydroxide adjuvant, which is currently contained in several FDA-approved vaccines. Alhydrogel provides a depot effect whereby antigen is released more slowly *in vivo*, resulting in prolonged antigen exposure, which may or may not contribute to adjuvantcy (Hutchison et al., Antigen depot is not required for alum adjuvantcy. *FASEB J*. 2012;26:1272–1279). Additionally, alhydrogel has been shown to activate the inflammasome, which may contribute to the immunogenicity of alhydrogel-based vaccines (Guyen et al., Aluminum hydroxide adjuvant differentially activates the three complement pathways with major involvement of the alternative pathway. *PLoS One*. 2013;8:e74445).

PolyICLC is a double-strand RNA stabilized by poly-L-lysine in carboxymethylcellulose (Levy et al., A modified polyriboinosinic-polyribocytidylic acid complex that induces interferon in primates. *J. Infect. Dis*. 1975;132:434–439). It signals through toll-like receptor-3 (TLR3) and potentially melanoma differentiation-associated protein 5 (MDA5) receptors, eliciting a strong

type I IFN response, and it skews the immune response toward a Th1 profile response (Wang et al., Cutting edge: polyinosinic:polycytidylic acid boosts the generation of memory CD8 T cells through melanoma differentiation-associated protein 5 expressed in stromal cells. *J. Immunol.* 2010;184:2751–2755). PolyI:CLC has been in multiple clinical trials for both therapeutic and vaccine purposes (Martins et al., Vaccine adjuvant uses of poly-ic and derivatives. *Expert Rev. Vaccines.* 2015;14:447–459).

CpG oligodeoxynucleotides (or CpG ODN) are short single-stranded synthetic DNA molecules that contain a cytosine triphosphate deoxynucleotide ("C") followed by a guanine triphosphate deoxynucleotide ("G"). The "p" refers to the phosphodiester link between consecutive nucleotides, although some ODN have a modified phosphorothioate (PS) backbone instead. When these CpG motifs are unmethylated, they act as immunostimulants, and have also been examined as adjuvants (Marshall et al., Identification of a novel cpg DNA class and motif that optimally stimulate B cell and plasmacytoid dendritic cell functions. *J. Leukoc. Biol.* 2003;73:781–792).

MPL is a TLR4 agonist, and the active component of the GSK adjuvant AS04 (Einstein et al., Comparative humoral and cellular immunogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine and HPV-6/11/16/18 vaccine in healthy women aged 18–45 years: follow-up through month 48 in a Phase III randomized study. *Hum. Vaccines Immunother.* 2014;10:3455–3465). MPL has been shown to be highly effective as an adjuvant, particularly in combination with an aluminum-based adjuvant like alhydrogel or a nanoparticle formulation (Bohannon et al., The immunobiology of Toll-Like receptor 4 agonists: from endotoxin tolerance to immunoadjuvants. *Shock.* 2013;40:451–462).

Other well-known adjuvants include alum-based adjuvants, oil based adjuvants, Freund's adjuvant, specol, Ribi adjuvant, myobacterium vaccae, immune stimulating complexes (ISCOMS), MF-59, SBAS-2, SBAS-4, detox B SE (Enhanzyn®), lipid-A mimetic RC-529, amino-alkyl glucosaminide 4-phosphates (AGPs), CRX-527, monophosphoryl lipid A (e.g., MPL-SE), detoxified saponin derivatives (e.g., QS-21, QS7), escin, gigitonin, gypsophila, and Chenopodium quinoa saponins (see, e.g., Alving et al., Adjuvants for Human Vaccines. *Curr Opin Immunol.* 2012 Jun; 24(3): 310–315).

Despite significant advances in the formulation of and use of adjuvants, the majority of adjuvants are designed to generate innate inflammatory danger signals. While these danger signals

are essential for innate immune activation, including antigen presentation and cytokine production, there is limited effect directly on T-cell priming (Powell et al. Polyionic vaccine adjuvants: another look at aluminum salts and polyelectrolytes. *Clin Exp Vaccine Res.* 2015 Jan;4(1):23-45; Petrovsky N. Comparative Safety of Vaccine Adjuvants: A Summary of Current Evidence and Future Needs. *Drug Saf.* 2015 Nov;38(11):1059-74), with most vaccination strategies using common adjuvants failing to elicit long-term memory CD8⁺ T cells (Kamphorst et al., Beyond Adjuvants: Immunomodulation strategies to enhance T cell immunity. *Vaccine.* 2015 Jun 8; 33(02): B21–B28). This is especially true during vaccinations targeting chronic infections and cancer, which require immunomodulation strategies to enhance T-cell responses necessary to overcome the immunosuppressive microenvironment.

One such strategy has been to downregulate immune checkpoint inhibitory receptors such as programmed-cell death protein 1 (PD-1) or programmed cell death ligand 1 (PD-L1). For example, PD-1 functions in regulating the threshold, strength, and duration of T-cell responses to antigen presentation (Okazaki et al., A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. *Nat Immunol.* 2013 Dec;14(12):1212-8). PD-1 is rapidly upregulated upon naïve T-cell activation, which is required to minimize damage to the host from uncontrolled inflammation during infection and after the infection (Ahn et al., Role of PD-1 during effector CD8 T cell differentiation. *PNAS* 2018 May 1;115(18):4749-4754). In non-human primates, immunization with a SIVgag adenovirus-based vaccine in combination with an anti-PD1 mAb significantly elevated peak Gag-specific T-cell responses (Finnefrock et al., PD-1 blockade in rhesus macaques: impact on chronic infection and prophylactic vaccination. *J Immunol.* 2009 Jan 15;182(2):980-7).

While monoclonal antibody (mAb)-based checkpoint inhibitors developed to treat cancer can effectively restore immune function, they do not, however, readily lend themselves to the field of infectious disease vaccinology. Due to their long serum half-life, anti-PD1 mAbs can trigger severe immune-related adverse events (irAEs) and precipitate autoimmune disease (Brahmer et al., Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol.* 2010 Jul 1;28(19):3167-75; Topalian et al., Safety, activity, and immune correlates of anti-PD-1

antibody in cancer. N Engl J Med. 2012 Jun 28;366(26):2443-54), making their use as prophylactic vaccine adjuvants unacceptable.

Accordingly, improved methods of using immune checkpoint inhibitors in vaccination strategy that provide safe and effective immunization is needed.

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Summary of the Invention

Provided herein are compositions comprising a recombinant modified vaccinia Ankara (rMVA) viral vector for use as an adjuvant or vaccine during an immunization protocol in a host such as a human. The rMVA are constructed to express high concentrations of peptides capable of inhibiting one or more immune checkpoint pathways (immune checkpoint inhibitor peptide). In some embodiments, the immune checkpoint inhibitor peptides are expressed from a polycistronic, multimeric nucleic acid insert and secreted from the cell.

It has previously been shown that the use of a PD-1 inhibitor peptide (LD01-SEQ ID NO.: 1), when administered in combination with an adenovirus-based or irradiated sporozoite-based prophylactic malaria vaccine, enhances antigen-specific CD8⁺ T-cell expansion in immune-competent mice (see Phares et al. A peptide-based PD1 antagonist enhances T-cell priming and efficacy of a prophylactic malaria vaccine and promotes survival in a lethal malaria model. Front. Immunol. 11, 1377 (2020), incorporated herein by reference). As shown herein, it has now been found that expressing immune checkpoint inhibitors using MVA as a delivery vehicle provides significant advantages during vaccination strategies, as the natural tropism of the MVA viral vector includes professional antigen presenting cells such as dendritic cells, which are capable of migrating to draining lymph nodes and spread systemically. It is believed that by expressing sufficient and high quantities of therapeutic levels of an immune checkpoint inhibitor, for example in a polycistronic, multimeric conformation, in the lymph node environment during host exposure to an antigen, CD8⁺ T-cell priming is significantly enhanced. As shown in the Examples below, when used in concert with the administration of an antigen during a vaccination strategy, the immune checkpoint expressing rMVA viral construct provides significantly improved antigen-specific CD8⁺ T cell expansion, increased antigenic responses, and improved vaccination efficacy compared to, for example, the naked administration of such immune checkpoint inhibitor peptides, and provides a significant improvement over prior art adjuvant strategies.

In one aspect, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding one or more chimeric polypeptides, for example 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more chimeric polypeptides, each chimeric polypeptide comprising a secretion signal peptide and an immune checkpoint inhibitor peptide. In some embodiments, the rMVA viral vector comprises a heterologous nucleic acid insert encoding two or more chimeric polypeptides, wherein the two or more chimeric polypeptides are expressed from a single heterologous polycistronic nucleic acid insert, wherein each of the nucleic acid sequences encoding the two or more chimeric polypeptides are operably linked in the polycistronic nucleic acid sequence. In some embodiments, the rMVA comprises two or more heterologous polycistronic inserts, for example, 2, 3, or 4, or more polycistronic inserts. In some embodiments, the population of chimeric polypeptides expressed from the rMVA are comprised of two or more different immune checkpoint inhibitor peptides. In some embodiments, the rMVA further encodes one or more antigenic peptides, which when expressed by the rMVA, are capable of inducing sufficient immunogenicity to provide or enhance protective immunity to an infectious agent. In some embodiments, the rMVA further encodes one or more antigenic peptides, which when expressed by the rMVA, are capable of inducing an immune response in the host which ameliorates one or more symptoms or conditions of a disorder, e.g., an infectious disease or cancer.

In some aspects, each of the chimeric polypeptides comprising a secretion signal peptide and an immune checkpoint inhibitor peptide encoded by the polycistronic nucleic acid insert includes a peptide sequence capable of being cleaved during or following translation linked to the C-terminus of the immune checkpoint inhibitor peptide. Where the secretable immune checkpoint inhibitor peptides are inserted in a multimeric conformation, inclusion of a cleavable peptide allows each chimeric polypeptide of the multimer to be expressed as a monomer during translation (e.g., through a translational nascent chain separation event) or, in an alternative embodiment, cleaved into monomers following translation, or a combination of both. In some embodiments, the chimeric polypeptide encoded by the most 3' nucleic acid lacks a cleavable peptide sequence.

In some embodiments, provided herein is an rMVA viral vector comprising a heterologous nucleic acid insert encoding a polypeptide wherein the polypeptide comprises a sequence (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)_x, wherein x = 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M=methionine.

In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises a tandem repeat sequence (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x, wherein x = 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine (see, e.g., FIGs. 1A-1B). In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding two or more polypeptides in a tandem repeat sequence and an additional polypeptide fused to the C-terminus of the last polypeptide in the tandem repeat sequence ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M-methionine (see, e.g., FIGs. 2A-2B).

In some embodiments, the rMVA viral vector comprises a polycistronic nucleic acid insert encoding two or more polypeptides, wherein the polypeptides comprise tandem repeat sequences as described herein, for example a first polypeptide tandem repeat sequence comprising ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, wherein M= methionine, wherein the first polypeptide encoding sequence is oriented in a 5' → 3' direction, and a second polypeptide tandem repeat sequence comprising ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, wherein M= methionine, wherein the second polypeptide encoding sequence is oriented in a 3' → 5' direction, wherein each cistron includes a poxvirus promoter capable of initiating transcription. In some embodiments, x = 3, 4, 5, or 6.

As provided herein, the rMVA is used as an adjuvant to increase the immunogenicity of one or more co-administered antigens during a vaccination protocol. By expressing localized, high quantities of one or more immune checkpoint inhibitor peptides capable of downregulating one or more checkpoint inhibitor pathways, immune modulating activities which typically hinder the development of sufficient antigenicity to induce immunity can be downregulated. In certain aspects, the immune checkpoint inhibitor peptide is capable of inhibiting the activity of an immune checkpoint pathway mediated by a receptor protein select from, but not limited to, programmed

cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain-3 (TIM-3), V-domain Ig suppressor of T-cell activation (VISTA), a B7 homolog protein (B7), B7 homolog 3 protein (B7-H3), B7 homolog 4 protein (B7-H4), B7 homolog 5 protein (B7-H5), OX-40 (OX-40), OX-40 ligand (OX-40L), glucocorticoid-induced TNFR-related protein (GITR), CD137, CD40, B and T lymphocyte attenuator (BTLA), Herpes Virus Entry Mediator (HVEM), galactin-9 (GAL9), killer cell immunoglobulin-like receptor (KIR), Natural Killer Cell Receptor 2B4 (2B4), CD160, checkpoint kinase 1 (CHK1), checkpoint kinase 2 (CHK2), adenosine A2a receptor (A2aR), T cell immunoreceptor with Ig and ITIM domains (TIGIT), inducible T cell co-stimulator (ICOS), inducible T cell co-stimulator ligand (ICOS-L), or combinations thereof. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-1. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-L1. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting CTLA-4. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-1, PD-L1, or CTLA-4, or a combination thereof. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting both PD-1 and CTLA-4.

In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide described in Table 1, or a homolog, derivative, or fragment thereof. In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO: 1-56, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide having an amino acid sequence selected from the group consisting of SEQ ID NO:1-5, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide having an amino acid sequence of SEQ ID NO: 1 (CRRTSTGQISTLRVNITAPLSQ), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide having an amino acid sequence of SEQ ID NO: 5 (STGQISTLRVNITAPLSQ), or an amino acid having an amino acid sequence at least 85%, 90%,

95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide is selected from a peptide having an amino acid sequence of SEQ ID NO: 6 (STGQISTLAVNITAPLSQ), or an amino acid having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

5 In some aspects as provided herein, each of the immune checkpoint inhibitor peptides expressed by the rMVA is fused to a secretion signal peptide on its N-terminus and, wherein the rMVA expresses two or more immune checkpoint inhibitor peptides, to one or more cleavable peptides on its C-terminus. The secretion signal peptide allows the immune checkpoint inhibitor peptide to be translocated into the endoplasmic reticulum (ER). Following co-translational
10 insertion of the growing peptide chain into the ER lumen, a signal peptidase cleaves the signal peptide from the immune checkpoint inhibitor peptide, and the immune checkpoint inhibitor is secreted (see, e.g., Fig. 3A, Fig. 3B, and 3C). The secretion signal peptides for use herein can be any suitable signal peptide that allows for the secretion of the immune checkpoint inhibitor peptide. Secretion signal peptide for use in the present invention are known in the art (see, e.g., Kober et
15 al., Optimized signal peptides for the development of high expressing CHO cell lines. *Biotechnol Bioengin.* 2013;110:1164–1173, incorporated herein by reference). In some embodiments, the secretion signal peptide is a short peptide having a length of between about 15–30 amino acids derived from a natural human excretory protein. In some embodiments, the secretion signal is a secretion signal selected from those of Table 2 (SEQ ID NO: 57-90), or a homolog, derivative, or
20 fragment thereof. In some embodiments, the secretion signal peptide is, or is derived from, for example, but not limited to a human growth factor, a human cytokine, interleukin-1, interleukin-2, human immunoglobulin kappa light chain, trypsinogen, serum albumin, prolactin, tissue plasminogen activator, alkaline phosphatase, or other appropriate secretion signal sequence as described herein. In some embodiments, the secretion signal peptide is derived from human tissue
25 plasminogen activator. In some embodiments, the secretion signal peptide is derived from human tissue plasminogen activator comprising an amino acid sequence DAMKRGLCCVLLLCGAVFVSPSQ (SEQ ID NO: 65), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the secretion signal peptide is derived from human tissue plasminogen activator comprising an amino
30 acid sequence DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGAR (SEQ ID NO: 66), or

peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the Secretion Signal Peptide of the first polypeptide encoded by the polycistronic nucleic acid insert further comprises the initiation amino acid methionine (M).

In some embodiments, one or more of the immune checkpoint inhibitor chimeric polypeptides includes one or more peptide sequences fused to the C-terminus of the immune checkpoint inhibitor peptide which is capable of being cleaved during or following, or a combination thereof, the translation of the polycistronic nucleic acid (see, e.g., Fig. 3A, 3B, and 3C). In some embodiments, the most C-terminus immune checkpoint inhibitor chimeric polypeptide does not include a cleavable peptide. In some embodiments, the cleavable peptide is capable of being cleaved by a proprotein convertase enzyme including, for example, but not limited to furin or a furin-like proprotein convertase. In some embodiments, the cleavable peptide sequence comprises a basic amino acid target sequence (canonically, RX(R/K)R), wherein X = any amino acid (SEQ ID NO: 91). In some embodiments, the cleavable peptide sequence comprises a basic amino acid target sequence (canonically, RX(R/K)R), wherein X = R, K, or H (SEQ ID NO: 92). In some embodiments, the cleavable peptide sequence is RAKR (SEQ ID NO: 93). In some embodiments, the cleavable peptide sequence is RRRR (SEQ ID NO: 94). In some embodiments, the cleavable peptide is RKRR (SEQ ID NO: 95). In some embodiments, the cleavable peptide is RRKR (SEQ ID NO: 96). In some embodiments, the cleavable peptide is RKKR (SEQ ID NO: 97). By including a cleavable peptide sequence on each of the covalently linked chimeric polypeptides, the multimeric polypeptide expressed during translation of the polycistronic nucleic acid insert can be processed through a cleaving mechanism into monomeric chimeric polypeptides following translation. This allows each chimeric polypeptide comprising the immune checkpoint inhibitor peptide to be secreted from the cell and function to downregulate an undesirable immune checkpoint pathway (see, e.g., Fig. 3A).

In some embodiments, each chimeric polypeptide includes one or more peptide sequences fused to the C-terminus of the immune checkpoint inhibitor peptide which is capable of inducing ribozyme skipping during translation of the polycistronic nucleic acid. Ribosomal "skipping" is an alternate mechanism of translation in which a specific peptide sequence prevents the ribosome from covalently linking a new inserted amino acid, but nonetheless continues translation. This results in a "cleavage" of the polyprotein through the induced ribosomal skipping. In some

embodiments, the peptide capable of inducing ribosomal skipping is a cis-acting hydrolase element peptide (CHYSEL). In some embodiments, the CHYSEL sequence comprises a non-conserved sequence of amino-acids with a strong alpha-helical propensity followed by the consensus sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98), wherein the ribosomal skipping cleavage occurs between the G and P sequence. In some embodiments, the CHYSEL sequence comprises DVEENPGP (SEQ ID NO: 99). In some embodiments, the CHYSEL peptide sequence is a sequence selected from those in Table 4, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the CHYSEL peptide sequence is an amino acid sequence selected from SEQ ID NOS: 100-122, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the CHYSEL peptide sequence is an amino acid sequence selected from SEQ ID NOS: 118-122, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the CHYSEL sequence comprises GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 120), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. By including a peptide sequence which induces ribosomal skipping, multiple chimeric polypeptides encoded by the polycistronic nucleic acid insert are expressed as monomers, which are then secreted from the cell and function to downregulate an undesirable immune checkpoint pathway (see, e.g., Fig. 3B).

In some embodiments, the cleavable peptide sequence comprises two or more sequences which are capable of being cleaved by different mechanism, for example a cleavable peptide sequence which is capable of being cleaved following the translation of the polycistronic nucleic acid and a peptide sequence capable of inducing ribozyme skipping during translation of the polycistronic nucleic acid. By providing cleavable peptide sequences subject to multiple modes of cleaving, the efficiency of monomeric formation from the polycistronic nucleic acid can be improved. In some embodiments, the immune checkpoint inhibitor peptide has fused to its C-terminus a furin-cleavable peptide sequence, for example the peptide sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91), and fused to the C-terminus of the furin-cleavable peptide sequence is a CHYSEL peptide sequence comprising, for example D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). For example, by including a furin-cleavable peptide sequence, such as RAKR (SEQ ID NO: 93), fused to the N-terminus of a CHYSEL peptide

sequence between each chimeric polypeptide, the transcribed polycistronic nucleic acid undergoes ribozyme skipping during translation, resulting in the production of monomeric chimeric polypeptides, and following post translational processing and the cleavage of the furin-peptide, all but the arginine (R) and alanine (A) residues of the furin cleavage sequence remains at the C-terminus of immune checkpoint inhibitor peptide, limiting the potential interference of the extra amino acid sequences on the function of the immune checkpoint inhibitor peptide (see e.g., Fig. 3C). In alternative embodiments, the use of the furin-cleavable peptide RRRR (SEQ ID NO: 94), RKRR (SEQ ID NO: 95), or RRKR (SEQ ID NO: 96) results in the complete furin cleavage sequence being removed from the C-terminus of the immune checkpoint inhibitor peptide, with no residual amino acids remaining. In some embodiments, the hybrid cleavage sequence is RAKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 123), or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavage sequence is RRRRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 124), or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavage sequence is RKRRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 125), or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavage sequence is RRKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 126), or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavage sequence is RKKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 127), or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

In some embodiments, the rMVA viral vector comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence selected from SEQ ID NOS: 309-340, or SEQ ID NOS: 341-348. In some embodiments, the rMVA viral vector comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence of SEQ ID NOS: 325-340, or SEQ ID NOS: 345-348. In some embodiments, the rMVA viral vector comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence of SEQ ID NO: 325. In some embodiments, the rMVA viral vector comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence of SEQ ID NO: 329. In some embodiments, the rMVA viral vector

comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence of SEQ ID NO: 333. In some embodiments, the rMVA viral vector comprises a heterologous polycistronic nucleic acid insert encoding a polypeptide having an amino acid sequence of SEQ ID NO: 337.

5 Transcription of the nucleic acid insert can be initiated by one or more promoters compatible with the MVA viral vector located 5' of, and operably linked to, the initial start codon of the first coding sequence contained within the nucleic acid. Suitable promoters compatible with a poxviral expression vector are known in the art and include, but are not limited to, pmH5, p11, pSyn, pHyb, or any other suitable MVA promoter sequence. In some embodiments, the promoter
10 is a natural promoter for an MVA ORF. In some embodiments, the promoter is selected from a promoter in Table 7, or a nucleic acid having a sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the promoter sequence is selected from SEQ ID NOS: 128-308, or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the promoter sequence is selected from SEQ ID NOS: 130-132, or a nucleic
15 acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the promoter sequence is SEQ ID NO: 130, or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

In some embodiments wherein multiple immune checkpoint inhibitor peptides are expressed, because the chimeric polypeptides are transcribed as a single transcript, the
20 polycistronic nucleic acid insert includes one or more termination signals (for example, a stop codon such as TAA, TAG, or TGA or a combination or multiples thereof) only following the ORF sequence of the last chimeric polypeptide. When transcribed, the multiple chimeric polypeptides result in a single transcript which is then translated. Following post-translational processing, the multiple monomeric chimeric polypeptides are produced.

25 The provided rMVA viral constructs of the present invention can be used as an adjuvant for treating or preventing an infectious disease or cancer, or inducing an immune response against an infectious disease or cancer, in a subject. In some embodiments, the rMVA viral construct is administered to a subject in need thereof, for example a human, in a prophylactic vaccination protocol to prevent an infectious disease, for example at a priming stage, a boosting stage, or both
30 a priming stage and boosting stage. In an alternative embodiment, the rMVA viral construct is

administered to a subject in need thereof, for example a human, in a treatment modality incorporating a vaccination protocol, for example, to treat a cancer. Accordingly, the rMVA viral construct can be administered in concert with one or more antigens intended to induce an immune response against an antigenic target in order to induce partial or complete immunization in a subject in need thereof.

Thus, the rMVA of the present invention can be administered with one or more antigens targeting an infectious disease or cancer. Examples of antigens and antigen delivery vehicles that the rMVA can be used with as an adjuvant include: an antigenic protein, polypeptide, or peptide, or fragment thereof; a nucleic acid, for example mRNA or DNA, encoding one or more antigens; a polysaccharide or a conjugate of a polysaccharide to a protein; glycolipids, for example gangliosides; a toxoid; a subunit (e.g., of a virus, bacterium, fungi, amoeba, parasite, etc.); a virus like particle; a live virus; a split virus; an attenuated virus; an inactivated virus; an enveloped virus; a viral vector expressing one or more antigens; a tumor associated antigen; or any combination thereof.

In particular aspects, the present invention provides a method of preventing or treating, or inducing an immune response against, an infectious disease in a subject in need thereof, said method comprising administering an effective amount of the rMVA of the present invention in combination, alternation, or coordination with a prophylactically effective or therapeutically effective amount of one or more antigens, or antigen expressing vectors, wherein the rMVA enhances immunity directed against the targeted infectious diseases.

In some embodiments, the targeted infection is a viral infection, including but not limited to: a double-stranded DNA virus, including but not limited to Adenoviruses, Herpesviruses, and Poxviruses; a single stranded DNA, including but not limited to Parvoviruses; a double stranded RNA virus, including but not limited to Reoviruses; a positive-single stranded RNA virus, including but not limited to Coronaviruses, for example SARS-CoV2, Picornaviruses, and Togaviruses; a negative-single stranded RNA virus, including but not limited to Orthomyxoviruses, and Rhabdoviruses; a single-stranded RNA-Retrovirus, including but not limited to Retroviruses; or a double-stranded DNA-Retrovirus, including but not limited to Hepadnaviruses. In some embodiments, the targeted virus is adenovirus, avian influenza, coxsackievirus, cytomegalovirus, dengue fever virus, ebola virus, Epstein-Barr virus, equine

encephalitis virus, flavivirus, hepadnavirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus, human immunodeficiency virus, human papillomavirus, influenza virus, Japanese encephalitis virus, JC virus, measles morbillivirus, marburg virus, Middle Eastern respiratory syndrome-coronavirus, mumps rubulavirus, orthomyxovirus, papillomavirus, parainfluenza virus, parvovirus, picornavirus, poliovirus, pox virus, rabies virus, reovirus, respiratory syncytial virus, retrovirus, rhabdovirus, rhinovirus, Rift Valley fever virus, rotavirus, rubella virus, rubeola virus, severe acute respiratory syndrome-coronavirus 1, severe acute respiratory syndrome coronavirus 2, smallpox virus, togavirus, swine influenza virus, varicella-zoster virus, variola major, variola minor, and yellow fever virus.

In some embodiments, the targeted infection is a bacterium, including but not limited to a *Borrelia* species, *Bacillus anthracis*, *Borrelia burgdorferi*, *Bordetella pertussis*, *Camphylobacter jejuni*, *Chlamydia* species, *Chlamydial psittaci*, *Chlamydial trachomatis*, *Clostridium* species, *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, *Corynebacterium diphtheriae*, *Coxiella* species, an *Enterococcus* species, *Erlchia* species, *Escherichia coli*, *Francisella tularensis*, *Haemophilus* species, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Lactobacillus* species, a *Legionella* species, *Legionella pneumophila*, *Leptospiriosis interrogans*, *Listeria* species, *Listeria monocytogenes*, *Mycobacterium* species, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycoplasma* species, *Mycoplasma pneumoniae*, *Neisseria* species, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Pneumococcus* species, *Pseudomonas* species, *Pseudomonas aeruginosa*, *Salmonella* species, *Salmonella typhi*, *Salmonella enterica*, *Streptococcus* species, *Rickettsia* species, *Rickettsia rickettsii*, *Rickettsia typhi*, *Shigella* species, *Staphylococcus* species, *Staphylococcus aureus*, *Streptococcus* species, *Streptococcus pneumoniae*, *Streptococcus pyrogenes*, *Streptococcus mutans*, *Treponema* species, *Treponema pallidum*, a *Vibrio* species, *Vibrio cholerae* and *Yersinia pestis*.

In some embodiments, the targeted infection is a fungal infection, including but not limited to a fungus from an *Aspergillus* species, *Candida* species, *Candida albicans*, *Candida tropicalis*, *Cryptococcus* species, *Cryptococcus neoformans*, *Entamoeba histolytica*, *Histoplasma capsulatum*, *Leishmania* species, *Nocardia asteroides*, *Plasmodium falciparum*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Toxoplasma* species, *Trypanosoma brucei*, *Schistosoma mansoni*, *Fusarium* species and *Trichophyton* species.

In some embodiments, the targeted infection is a parasite, including but not limited to a parasite from *Plasmodium* species, *Toxoplasma* species, *Entamoeba* species, *Babesia* species, *Trypanosoma* species, *Leshmania* species, *Pneumocystis* species, *Trichomonas* species, *Giardia* species and *Schistosoma* species.

5 In some embodiments, a method of preventing or treating, or inducing an immune response to, a cancer in a subject in need thereof, said method comprising administering an effective amount of the rMVA of the present invention in combination, alternation, or coordination with a prophylactically effective or therapeutically effective amount of one or more tumor associated antigens, or tumor associated antigen expressing vectors, wherein the rMVA enhances immunity
10 directed against the cancer. In some embodiments, the tumor associated antigen (TAA) is, but is not limited to: an oncofetal TAA, which is typically only expressed in fetal tissues and in cancerous somatic cells; an oncoviral TAA, which is typically encoded by tumorigenic transforming viruses; an overexpressed/accumulated TAA, which is typically expressed by both normal and neoplastic tissue, with the level of expression highly elevated in neoplasia; a cancer-testis TAA, which is
15 typically expressed only by cancer cells and adult reproductive tissues such as testis and placenta; a lineage-restricted TAA, which is typically expressed largely by a single cancer histotype; a mutated TAA, which is typically only expressed by cancer as a result of genetic mutation or alteration in transcription; a post-translationally altered TAA, which typically has tumor-associated alterations in glycosylation, etc.; and an idiotypic TAA, which is typically highly
20 polymorphic genes where a tumor cell expresses a specific “clonotype”, i.e., as in B cell, T cell lymphoma/leukemia resulting from clonal aberrancies. In some embodiments, the TAA is selected from: Wilm’s tumor protein (WT1); melanoma antigen preferentially expressed in tumors (PRAME); survivin; cancer/testis antigen 1 (NY-ESO-1); melanoma-associated antigen 3 (MAGE-A3); melanoma-associated antigen 4 (MAGE-A4); proteinase 3 (Pr3); Cyclin A1; highly
25 homologous synovial sarcoma X 2 (SSX2); Neutrophil Elastase (NE); mucin 1 (MUC1); alphafetoprotein (AFP); carcinoembryonic antigen (CEA); cancer antigen 125 (CA-125); epithelial tumor antigen (ETA); tyrosinase; abnormal products of ras; abnormal products of p53; Epstein Bar Virus early antigen (EA), latent membrane protein 1(LMP1), and latent membrane protein 2 (LMP2); a gangliosides for example, GM1b, GD1c, GM3, GM2, GM1a, GD1a, GT1a,
30 GD3, GD2, GD1b, GT1b, GQ1b, GT3, GT2, GT1c, GQ1c, and GP1c; and a ganglioside derivative

for example, 9-O-Ac-GD3, 9-O-Ac-GD2, 5-N-de-GM3, N-glycolyl GM3, NeuGcGM3, and fucosyl-GM1; or a combination thereof.

In some embodiments, the antigen is derived from an amino acid sequence of SEQ ID NOS:349-394.

5 In alternative embodiments, the rMVA viral vectors of the present invention, in addition to the ability to express multiple immune checkpoint inhibitor peptides, may further be constructed to encode and express one or more antigenic peptides. The one or more antigenic peptides can be encoded on one or more separate nucleic acid inserts, or in an alternative embodiment, the one or more antigenic peptides are encoded on the same polycistronic nucleic acid insert as the multiple
10 immune checkpoint inhibitor peptides. In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Antigenic Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and M = methionine. In some embodiments, the antigenic peptide is contained in a chimeric
15 polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10 and M= methionine (see, e.g., FIGs. 4A-4B). In some embodiments, the antigenic peptide is also provided so that 2 or more antigenic peptides are encoded in the polycistronic
20 nucleic acid insert, with each chimeric polypeptide separated by a cleavable peptide described herein. In some embodiments, the antigenic peptide is contained in a chimeric polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the antigenic peptide, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal
25 Peptide-Antigenic Peptide-Cleavable Peptide)_y), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and M = methionine. In some embodiments, the antigen containing chimeric polypeptide fused to the C-terminus of the last antigen containing chimeric polypeptide does not include a cleavable sequence, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable
30 Peptide)_x(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_x(Secretion Signal

Peptide-Antigenic Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and $M =$ methionine. In some embodiments, the antigenic peptide contained in the chimeric polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the antigenic peptide can be oriented in the polycistronic nucleic acid insert so that the antigen containing chimeric polypeptide encoding nucleic acid is located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example $((M)(\text{Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide})_y(\text{Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide})_x)$ or, alternatively $((M)(\text{Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide})_y(\text{Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide})_x(\text{Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide}))$, wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $M =$ methionine. In some embodiments, the antigenic peptide includes its natural secretion signal peptide. In alternative peptides, the Secretion Signal Peptide is not derived from the antigen, but rather derived from a different protein, synthetic secretion signal, or a consensus secretion signal peptide. In some embodiments, the antigenic peptide is selected from SEQ ID NOS. 349-394.

In some embodiments, the antigenic peptide encoded by the polycistronic nucleic acid insert in the rMVA is contained in a chimeric polypeptide that includes a viral glycoprotein signal sequence fused to the N-terminus of the antigenic peptide, and a viral glycoprotein transmembrane domain fused to the C-terminus of the antigenic peptide, and the rMVA is further constructed to encode a viral matrix protein, wherein upon translational cleavage of the antigenic containing chimeric peptide, the viral matrix protein and antigen-viral glycoprotein chimeric polypeptide are capable of forming a non-infectious virus-like particle (VLP). In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises $((M)(\text{Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide})_x(\text{Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain}))$, wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $M =$ methionine (see, e.g., Fig. 5A & 5B). In some embodiments, the antigenic peptide is contained in a chimeric polypeptide comprising a viral glycoprotein signal sequence fused to the N-terminus of the antigenic peptide, and a viral glycoprotein transmembrane

domain fused to the C-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the viral glycoprotein transmembrane domain, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine. In some embodiments, the antigen containing chimeric polypeptide fused to the C-terminus of the last antigen containing chimeric polypeptide does not include a cleavable sequence, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, wherein y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine. In some embodiments, the (Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y, wherein y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, can be oriented in the polycistronic nucleic acid insert so that the antigen containing chimeric polypeptide encoding nucleic acid is located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x) or, alternatively ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine. In yet a further embodiment, the polycistronic nucleic acid insert of the rMVA further encodes the viral matrix protein, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)(Viral Matrix Protein)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine (see, e.g., Fig. 6A & 6B). In alternative embodiments, the coding sequences for both the antigen containing chimeric polypeptide and the viral matrix protein are contained in the polycistronic nucleic acid in one or more copies, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-

Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and $M =$ methionine. In some embodiments, the most C-terminus viral matrix protein lacks a cleavable peptide, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x(Viral Matrix Protein-Cleavable Peptide)_y(Viral Matrix Protein)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $M =$ methionine. In some embodiments, the ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y), wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $M =$ methionine, can be oriented in the polycistronic nucleic acid insert so that the sequences are located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x) or, alternatively ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein $M =$ methionine. In some embodiments, the natural secretion signal from the antigen is replaced with a viral Glycoprotein Signal Peptide. In some embodiments, the antigenic peptide is selected from SEQ ID NOS: 349-394.

The production of virus-like particles containing a target antigen are particularly suitable for use in vaccine strategies against enveloped viruses, as they are capable of inducing both strong and durable humoral and cellular immune responses. See, e.g., Salvato et al., A Single Dose of Modified Vaccinia Ankara Expressing Lassa Virus-like Particles Protects Mice from Lethal Intracerebral Virus Challenge. *Pathogens* (2019) 8:133. Suitable glycoproteins and matrix proteins for use to produce the antigen containing VLPs include, but are not limited to, those derived from: a *Filoviridae*, for example Marburg virus, Ebola virus, or Sudan virus; a *Retroviridae*, for example

human immunodeficiency virus type 1 (HIV-1); an *Arenaviridae*, for example Lassa virus; a *Flaviviridae*, for example Dengue virus and Zika virus. In particular embodiments, the glycoprotein and matrix proteins are derived from Marburg virus (MARV). In particular embodiments, the glycoprotein is derived from the MARV GP protein (Genbank accession number AFV31202.1). The amino acid sequence of the MARV GP protein is provided as SEQ ID NO: 395 in Table 10 below. In particular embodiments, the MARV GPS domain comprises amino acids 2 to 19 of the glycoprotein (WTTCFFISLILIQGIKTL) (SEQ ID NO: 396, which can be encoded by, for example the MVA optimized nucleic acid sequence of SEQ ID NO: 397), the GPTM domain comprises amino acid sequences 644-673 of the glycoprotein (WWTSDWGVLTNLGILLLSIAVLIALSCICRIFTKYIG) (SEQ ID NO: 398, which can be encoded by, for example the MVA optimized nucleic acid sequence of SEQ ID NO: 399). In some embodiments, the MARV GPS signal further comprises a methionine as the first amino acid.

The MARV VP40 amino acid sequence is available at GenBank accession number JX458834, and provided below in Table 10 as SEQ ID NO: 400, or a nucleic acid sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some embodiments, the MARV VP40 signal further comprises a methionine as the first amino acid.

In some embodiments, the rMVA antigenic peptide encoded by the polycistronic nucleic acid insert in the rMVA is contained in a chimeric polypeptide that includes a viral glycoprotein signal sequence fused to the N-terminus of the antigenic peptide, and a viral glycoprotein transmembrane domain fused to the C-terminus of the antigenic peptide, and the rMVA is further constructed to encode a viral matrix protein, wherein upon translational cleavage of the antigenic containing chimeric peptide, the viral matrix protein and antigen-viral glycoprotein chimeric polypeptide are capable of forming a non-infectious virus-like particle (VLP).

In alternative embodiments, the rMVA viral vectors of the present invention, in addition to the ability to express multiple immune checkpoint inhibitor peptides, are further constructed to encode and express one or more antigenic peptides, wherein the one or more antigenic peptides are encoded on one or more separate nucleic acid inserts.

In some aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising one or more heterologous nucleic acid inserts encoding one or more chimeric polypeptides, each chimeric polypeptide comprising ((M)(Immune Checkpoint Inhibitor

Peptide)x), wherein $x = 1-10$, and M is methionine, wherein the heterologous nucleic acid inserts are under the control of a vaccinia virus promoter. In particular aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising one or more heterologous nucleic acid inserts encoding one or more chimeric polypeptides, each chimeric polypeptide comprising ((M)(Immune Checkpoint Inhibitor Peptide)x), wherein $x = 1-10$, the Immune Checkpoint Inhibitor comprises SEQ ID NO:1, and M is methionine, wherein the heterologous nucleic acid inserts are under the control of a vaccinia virus promoter. In particular aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising one or more heterologous nucleic acid inserts encoding one or more chimeric polypeptides, each chimeric polypeptide comprising ((M)(Immune Checkpoint Inhibitor Peptide)x), wherein $x = 1-10$, the Immune Checkpoint Inhibitor comprises SEQ ID NO:5, and M is methionine, wherein the heterologous nucleic acid inserts are under the control of a vaccinia virus promoter.

In some aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising i) a first nucleic acid sequence encoding a chimeric amino acid sequence comprising (a) an extracellular fragment of MUC-1, (b) a transmembrane domain of a glycoprotein (GP) of Marburg virus (MARV), and (c) an intracellular fragment of MUC-1; ii) a second nucleic acid sequence encoding a MARV VP40 matrix protein; iii) a third nucleic acid sequence encoding one or more immune checkpoint inhibitor peptides; and wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs). In particular aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising i) a first nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 402; ii) a second nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 404; iii) a third nucleic acid sequence encoding one or more immune checkpoint inhibitor peptides; and wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs). In particular aspects, provided herein is a recombinant modified vaccinia ankara (rMVA) viral vector comprising i) a first nucleic acid sequence encoding a

chimeric amino acid sequence comprising the amino acid sequence of SEQ ID NO: 403; ii) a second nucleic acid sequence encoding a MARV VP40 matrix protein comprising the amino acid sequence of SEQ ID NO: 405; iii) a third nucleic acid sequence encoding one or more immune checkpoint inhibitor peptides; and wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

In one embodiment, the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into one or more deletion sites of the MVA selected from I, II, III, IV, V or VI.

In another embodiment, the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into the MVA in a natural deletion site, a modified natural deletion site, or between essential or non-essential MVA genes.

In another embodiment, the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into the same natural deletion site, a modified natural deletion site, or between the same essential or non-essential MVA genes.

In another embodiment, the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into different natural deletion sites, different modified deletion sites, or between different essential or non-essential MVA genes.

In another embodiment, wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted between two essential and highly conserved MVA genes; and the matrix protein sequence is inserted into a restructured and modified deletion III.

In another embodiment, wherein the first nucleic acid sequence is inserted between MVA genes I8R and G1L, the second nucleic acid sequence is inserted between MVA genes A50R and B1R in the restructured and modified deletion site III, and the third nucleic acid sequence is inserted between the two essential MVA genes A5R and A6L.

In another embodiment, wherein the vaccinia virus promoter is a nucleic acid sequence selected from SEQ ID NOS: 128-308.

In another embodiment, wherein the vaccinia virus promoter is SEQ ID NO:130, or a nucleic acid sequence 95% identical thereto.

In some embodiments, the MUC-1 nucleic acid sequence is provided as SEQ ID NO:403, or a nucleic acid sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some
5 embodiments, the Marburg VP40 nucleic acid sequence is provided as SEQ ID NO:404, or a nucleic acid sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some
embodiments, the 5xLD01 nucleic acid sequence is provided as SEQ ID NO:408, or a nucleic acid
sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some embodiments, the
5xLD10 nucleic acid sequence is provided as SEQ ID NO:409, or a nucleic acid sequence 70%,
10 75%, 80%, 85%, 90%, 95% or more identical thereto.

Also provided herein are shuttle vectors comprising the polycistronic nucleic acid sequences to be inserted into the MVA as described herein, as well as isolated nucleic acid sequences comprising the polycistronic nucleic acid sequence inserts described herein. Further
provided herein are cells comprising the rMVA viral vectors described herein.

Brief Description of the Drawings

FIG. 1A provides an exemplary linear schematic of an exemplary recombinant MVA viral vector polycistronic nucleic acid insert open reading frame (ORF) encoding multiple chimeric polypeptides, wherein each chimeric polypeptide comprises a secretion signal peptide, an immune
20 checkpoint inhibitor peptide fused to the C-terminus of the signal peptide, and a cleavable peptide fused to the C-terminus of the immune checkpoint inhibitor peptide. The polycistronic nucleic acid insert can encode from 2 to 10 or more chimeric polypeptides, and includes a methionine as its first amino acid.

FIG. 1B provides an exemplary linear schematic of an exemplary recombinant MVA viral
25 vector comprising a polycistronic nucleic acid insert encoding multiple chimeric polypeptides, wherein each chimeric polypeptide comprises a secretion signal peptide (SP), an immune checkpoint inhibitor peptide (ICIP) fused to the C-terminus of the secretion signal peptide, and a cleavable peptide (cleavage sequence) fused to the C-terminus of the immune checkpoint inhibitor peptide. As exemplified, a promoter capable of initiating transcription of an MVA ORF (e.g.,
30 mH5 promoter (pmH5)) is operably linked to a nucleic acid encoding multiple chimeric

polypeptides. The insert may include a translation initiation sequence, for example a Kozak sequence, prior to the start codon of the most 5' chimeric polypeptide ORF. As exemplified, a stop codon is present 3' of the last chimeric polypeptide ORF.

FIG. 2A provides an exemplary linear schematic of an exemplary recombinant MVA viral vector polycistronic nucleic acid insert open reading frame (ORF) encoding multiple chimeric polypeptides, wherein all of the chimeric polypeptides comprise a secretion signal peptide (SP), an immune checkpoint inhibitor peptide fused to the C-terminus of the signal peptide, and a cleavable peptide fused to the C-terminus of the immune checkpoint inhibitor peptide, except for the most C-terminus chimeric polypeptide, which lacks a cleavable peptide. The polycistronic nucleic acid insert can encode from 2 to 10 or more chimeric polypeptides, and includes a methionine as its first amino acid.

FIG. 2B provides an exemplary linear schematic of an exemplary recombinant MVA viral vector comprising a polycistronic nucleic acid insert encoding multiple chimeric polypeptides, wherein each chimeric polypeptide comprises a secretion signal peptide (SP), an immune checkpoint inhibitor peptide (ICIP) fused to the C-terminus of the secretion signal peptide, and a cleavable peptide (cleavage sequence) fused to the C-terminus of the immune checkpoint inhibitor peptide, except for the most C-terminus chimeric polypeptide, which lacks a cleavable peptide. As exemplified, a promoter capable of initiating transcription of an MVA ORF (e.g., mH5 promoter (pmH5)) is operably linked to a nucleic acid encoding multiple chimeric polypeptides. The insert may include a translation initiation sequence, for example a Kozak sequence, prior to the start codon of the most 5' chimeric polypeptide ORF. As exemplified, a stop codon is present 3' of the last chimeric polypeptide ORF.

FIGS. 3A, 3B, and 3C provide exemplary schematics of the translational processing of the various expressed chimeric polypeptides encoded by the polycistronic nucleic acid inserts of the present invention. In Fig. 3A, the chimeric polypeptides encode a cleavable peptide sequence, for example a furin or furin-like cleavage sequence, which is cleaved following translation of the polycistronic nucleic acid transcript. In addition, during or following translation, the secretion signal peptide fused to the immune checkpoint inhibitor peptide is also cleaved, and the resultant monomeric immune checkpoint inhibitor peptides are subsequently secreted from the cell. In Fig. 3B, the chimeric polypeptides encode a cleavable peptide sequence, for example a CHYSEL

cleavage sequence, that induces ribosomal skipping, wherein the polyprotein undergoes a co-translational cleavage, resulting in the production of monomeric immune checkpoint inhibitor peptides during translation. Following or during translation, the chimeric polypeptide undergoes further cleavage of the secreted signal peptide, and the resultant monomeric immune checkpoint inhibitor peptides are subsequently secreted from the cell. In Fig. 3C, the chimeric polypeptides encode multiple cleavable peptide sequences, for example both a furin or furin-like cleavage sequence and a CHYSEL sequence, for example, RAKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 123). During translation, induces ribosomal skipping at glycine (G) and proline (P) amino acids at the C-terminus of the CHYSEL sequence, wherein the polyprotein undergoes a co-translational cleavage, resulting in the production of monomeric immune checkpoint inhibitor peptides during translation. The monomeric immune checkpoint inhibitor peptides undergo further processing during or after translation, wherein the secreted signal peptide is cleaved. In addition, following translation, the furin or furin-like peptide sequence is cleaved, resulting in monomeric immune checkpoint inhibitor peptides containing only the arginine (R) and alanine (A) residues of the furin or furin like cleavage sequence, reducing the potential for interference with the immune checkpoint inhibitor peptides.

FIG. 4A provides an exemplary linear schematic of an exemplary recombinant MVA viral vector polycistronic nucleic acid insert open reading frame (ORF) encoding multiple chimeric polypeptides comprising tandem repeats of a secretion signal peptide, an immune checkpoint inhibitor peptide fused to the C-terminus of the signal peptide, and a cleavable peptide fused to the C-terminus of the immune checkpoint inhibitor peptide, and a chimeric polypeptide comprising a signal peptide fused to an antigenic peptide, the antigenic containing chimeric polypeptide fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide. The polycistronic nucleic acid insert can encode from 1 to 10 or more immune checkpoint inhibitor containing chimeric peptides, and includes a methionine as its first amino acid. This same general concept described above is applicable to any of the constructs provided herein which include cleavable sequences.

FIG. 4B provides an exemplary linear schematic of an exemplary recombinant MVA viral vector comprising a polycistronic nucleic acid insert encoding multiple chimeric polypeptides comprising tandem repeats of a secretion signal peptide (SP), an immune checkpoint inhibitor

peptide (ICIP) fused to the C-terminus of the signal peptide, and a cleavable peptide (cleavage sequence) fused to the C-terminus of the immune checkpoint inhibitor peptide, and an antigen containing chimeric polypeptide comprising a secretion signal peptide (SP) fused to an antigenic peptide (Antigen), the antigen containing chimeric polypeptide fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide. As exemplified, a promoter capable of initiating transcription of an MVA ORF (e.g., mH5 promoter (pmH5)) is operably linked to a nucleic acid encoding the multiple chimeric polypeptides. The insert may include a translation initiation sequence, for example a Kozak sequence, prior to the start codon of the most 5' chimeric polypeptide ORF. As exemplified, a stop codon is present 3' of the last chimeric polypeptide ORF.

FIG. 5A provides an exemplary linear schematic of an exemplary recombinant MVA viral vector polycistronic nucleic acid insert open reading frame (ORF) encoding multiple chimeric polypeptides comprising tandem repeats of a secretion signal peptide, an immune checkpoint inhibitor peptide fused to the C-terminus of the signal peptide, and a cleavable peptide fused to the C-terminus of the immune checkpoint inhibitor peptide, and an antigen containing chimeric polypeptide comprising a viral glycoprotein signal peptide fused to an antigenic peptide, which is fused to the transmembrane domain of a viral glycoprotein, wherein the antigen containing chimeric polypeptide is fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide. The polycistronic nucleic acid insert can encode from 1 to 10 or more immune checkpoint inhibitor containing chimeric polypeptides, and includes a methionine as its first amino acid.

FIG. 5B provides an exemplary linear schematic of an exemplary recombinant MVA viral vector comprising a polycistronic nucleic acid insert encoding multiple chimeric polypeptides comprising tandem repeats of a secretion signal peptide (SP), an immune checkpoint inhibitor peptide (ICIP) fused to the C-terminus of the signal peptide, and a cleavable peptide (Cleavage sequence) fused to the C-terminus of the immune checkpoint inhibitor peptide, and an antigen containing chimeric polypeptide comprising a viral glycoprotein signal peptide (GPSP) fused to an antigenic peptide (Antigen), which is fused to the transmembrane domain of a viral glycoprotein transmembrane domain (GPTM), fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide. As exemplified, a promoter capable of initiating transcription of an

MVA ORF (e.g., mH5 promoter (pmH5)) is operably linked to the polycistronic nucleic acid encoding the multiple chimeric polypeptides. The insert may include a translation initiation sequence, for example a Kozak sequence, prior to the start codon of the most 5' chimeric polypeptide ORF. As exemplified, a stop codon is present 3' of the last polypeptide ORF.

FIG. 6A provides an exemplary linear schematic of an exemplary recombinant MVA viral vector polycistronic nucleic acid insert open reading frame (ORF) encoding multiple chimeric polypeptides comprising tandem repeats of a secretion signal peptide, an immune checkpoint inhibitor peptide fused to the C-terminus of the signal peptide, and a cleavable peptide fused to the C-terminus of the immune checkpoint inhibitor peptide, and an antigen containing chimeric polypeptide comprising a viral glycoprotein signal peptide fused to an antigenic peptide, which is fused to the transmembrane domain of a viral glycoprotein and further fused to a cleavable peptide, wherein the antigen containing chimeric polypeptide is fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide, and further comprising a viral matrix protein, wherein the viral matrix protein is fused to the C-terminus of the cleavable peptide of the antigen containing chimeric polypeptide. The polycistronic nucleic acid insert can encode from 1 to 10 or more immune checkpoint inhibitor containing chimeric polypeptides, and includes a methionine as its first amino acid.

FIG. 6B provides an exemplary linear schematic of an exemplary recombinant MVA viral vector comprising a polycistronic nucleic acid insert encoding multiple chimeric polypeptides comprising a secretion signal peptide (SP), an immune checkpoint inhibitor peptide (ICIP) fused to the C-terminus of the signal peptide, and a cleavable peptide (Cleavage sequence) fused to the C-terminus of the immune checkpoint inhibitor peptide, and an antigen containing chimeric polypeptide comprising a viral glycoprotein signal peptide (GPSP) fused to an antigenic peptide (Antigen), which is fused to the transmembrane domain of a viral glycoprotein transmembrane domain (GPTM) fused to a cleavable peptide, wherein the antigen containing chimeric polypeptide is fused to the most C-terminus immune checkpoint inhibitor containing chimeric peptide, and further comprising a viral matrix protein, wherein the viral matrix protein is fused to the C-terminus of the cleavable peptide of the antigen containing chimeric polypeptide. As exemplified, a promoter capable of initiating transcription of an MVA ORF (e.g., mH5 promoter (pmH5)) is operably linked to the polycistronic nucleic acid encoding the multiple chimeric polypeptides. The

insert may include a translation initiation sequence, for example a Kozak sequence, prior to the start codon of the most 5' chimeric polypeptide ORF. As exemplified, a stop codon is present 3' of the viral matrix protein ORF.

FIG. 7 is a schematic of MVA-5X.LD01 and MVA-5X.LD10 vectors illustrating the design of peptide sequences inserted into the MVA genome between two essential genes under control of an MVA specific promoter. LD01 and LD10 sequences are preceded by a signal sequence routing peptide for secretion and followed by a cleavage site to separate duplicated peptides. The secretion signal, peptide sequence and cleavage site are repeated 5 times and then transcription is terminated with a stop codon.

FIG. 8 shows the production of LD01 and LD10 by MVA-infected cells. (FIG. 8A) DF-1 cells were infected with MVA-5X.LD01, MVA-5X.LD10 or parental MVA. Two days following infection cells were fixed, permeabilized and stained with an antibody specific for LD01 and LD10. Results show the peptides are detected intracellularly. LD01- and LD10-positive cells were stained as shown. Photomicrographs are presented at a magnification of 20x. (FIG. 8B) DF-1 cells were infected with MVA-5X.LD01, MVA-5X.LD10 or parental MVA. Two days following infection, supernatant was harvested, concentrated and dotted onto membrane along with chemically synthesized peptide (LD01) and probed with an antibody specific for LD01 and LD10. Results indicate that the peptides are secreted from the infected cells.

FIG. 9 shows the delivery of LD01 or LD10 via a viral vector enhances expansion of vaccine-induced, antigen-specific CD8⁺ T cells. (FIG. 9A and FIG. 9B). At day 12 post-AdPyCS immunization, immunogenicity was assessed by measuring the number of splenic PyCS-specific, IFN- γ -secreting CD8⁺ T cells using the ELISpot assay (FIG. 9A) and flow cytometry (FIG. 9B) after stimulation with the H-2kd restricted CD8 epitope SYVPSAEQI (SEQ ID NO: 406). A 100 μ g dose of LD01 or LD10da was given SC immediately following vaccination. For viral vectors, 10⁷ TCID₅₀ of MVA-5X.LD01, MVA-5X.LD10 or parental MVA was injected SC subsequent to vaccination. Data are expressed as the mean $\pm$ SEM. Data from one of two independent experiments are shown. Significant differences between AdPyCS alone and treated mice were determined using a two-tailed Unpaired t-test and denoted by ** (p < 0.001), *** (p < 0.0005) and **** (p < 0.0001). For FIG. 9A, the x axis is AdPyCS alone and treated mice and the y axis is number of IFN- γ spots per 1x10⁶ splenocytes measured in counts. For FIG. 9B, the x axis is

AdPyCS alone and treated mice and the y axis is number of IFN- γ CB8 T cells within total CB8 T cells measured in percentage.

FIG. 10 shows a PCR gel of LD10, MUC-1, and VP40 inserts amplified from MVA-VLP-MUC-1-LD10 virus infected DF-1 cell DNA samples. DF1 cells infected with parental MVA (negative control), plasmids carrying LD10, MUC-1, or VP40 inserts (positive controls), or MVA-VLP-MUC-1-LD10 recombinant virus were harvested for viral DNA. PCR analysis confirmed insert integrity.

FIG. 11 shows the expected PCR fragment sizes of LD10, MUC-1, and VP40 insert sizes collected from DF-1 cells infected with MVA-VLP-MUC-1-LD10 virus. The expected fragment sizes matched the band sizes of the PCR gel.

FIG. 12 shows the expression of recombinant MUC-1 protein in DF-1 cells infected with MVA-VLP-MUC-1-LD10. DF1 cells were infected with parental modified vaccinia Ankara (pMVA) or MVA encoding VLP-MUC-1-LD10. Uninfected cells were included as negative controls. Cellular lysate and supernatant were harvested for protein and analyzed by immunoblotting. Membranes were probed with MUC-1 antibody (mouse monoclonal VU4H5, Santa Cruz #sc-7313, 1:200), labeling a protein band of approximately 63 kDa in the MVS-VLP-MUC-1-LD10 lysate sample.

FIG. 13 shows the expression of recombinant VP40 protein in DF-1 cells infected with MVA-VLP-MUC-1-LD10. DF1 cells were infected with parental modified vaccinia Ankara (pMVA) or MVA encoding VLP-MUC-1-LD10. Uninfected cells were included as negative controls. Cellular lysate and supernatant were harvested for protein and analyzed by immunoblotting. Membranes were probed with VP40 antibody, labeling a protein band of approximately 32 kDa in the MVS-VLP-MUC-1-LD10 supernatant and lysate samples.

FIG. 14 shows the expression of recombinant LD10 protein in DF-1 cells infected with MVA-VLP-MUC-1-LD10. DF1 cells were transfected with parental modified vaccinia Ankara (pMVA) or MVA encoding VLP-MUC-1-LD10. Uninfected cells were included as negative controls. Cellular lysates were harvested for protein and applied to nitrocellulose membrane using a dot blot apparatus. Twenty micrograms of LD10 peptide was also loaded onto the membrane as a positive control of the LD10 antibody. The membrane was probed with LD10 antibody, demonstrating signal in the MVA-VLP-MUC-1-LD10 and LD10 peptide samples.

FIG. 15 shows the percentages of MUC-1-positive plaques following infection of DF-1 cells with different amounts of recombinant MVA-VLP-MUC-1-LD10 virus. DF1 cells were infected in 3 wells each of 30 plaque forming units (PFU) and 60 PFU of MVA-VLP-MUC-1-LD10 virus in a 6 well plate. All wells were probed with MUC-1 antibody and the number of MUC-1-positive plaques were counted. The wells were then washed before being probed again with MVA antibody and the number of MVA-positive plaques were counted. To calculate the purity of the vaccine, the percentage of MUC-1-positive plaques versus the number of MVA-positive plaques is shown. The number of positive plaques for each individual replicate are shown at the bottom of the figure.

FIG. 16 shows the percentages of VP40-positive plaques following infection of DF-1 cells with different amounts of recombinant MVA-VLP-MUC-1-LD10 virus. DF1 cells were infected in 3 wells each of 30 plaque forming units (PFU) and 60 PFU of MVA-VLP-MUC-1-LD10 virus in a 6 well plate. All wells were probed with MUC-1 antibody and the number of VP40-positive plaques were counted. The wells were then washed before being probed again with MVA antibody and the number of MVA-positive plaques were counted. To calculate the purity of the vaccine, the percentage of VP40-positive plaques versus the number of MVA-positive plaques is shown. The number of positive plaques for each individual replicate are shown at the bottom of the figure.

Detailed Description of the Invention

Definitions

Where a term is provided in the singular, the inventors also contemplate aspects of the invention described by the plural of that term. As used in this specification and in the appended claims, the singular forms "a", "an", and "the" include plural references unless the context clearly dictates otherwise, e.g., "a peptide" or a "chimeric polypeptide" includes a plurality of peptides or chimeric polypeptides. Thus, for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein, and/or which will become apparent to those persons skilled in the art upon reading this disclosure.

The term "adjuvant" as used herein means the use of the rMVA as described herein to enhance the immunogenicity of one or more antigens.

The term "antigen" refers to a substance or molecule, such as a protein, or fragment thereof, e.g., a peptide, that is capable of inducing an immune response.

"Chimeric" or "fused" as used herein indicates the covalent joining of peptides or proteins that do not naturally exist, resulting in a hybrid polypeptide. Translation of the chimeric or fused polypeptides described herein provide functional properties derived from each of the respective fused peptides or proteins.

"Coding sequence" or "encoding nucleic acid" or "nucleic acid sequence encoding" or the like, as used herein means the nucleic acids (RNA or DNA molecule) that comprise a nucleotide sequence which encodes an amino acid sequence, for example, a polypeptide, protein, peptide, or fragment thereof. The coding sequence can further include initiation and termination signals operably linked to regulatory elements including a promoter and polyadenylation signal capable of directing expression in the cells of human or mammal to which the nucleic acid is administered.

The term "conservative amino acid substitution" refers to substitution of a native amino acid residue with a non-native residue such that there is little or no effect on the size, polarity, charge, hydrophobicity, or hydrophilicity of the amino acid residue at that position, and without resulting in substantially altered immunogenicity. For example, these may be substitutions within the following groups: valine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. Conservative amino acid modifications to the sequence of a polypeptide (and the corresponding modifications to the encoding nucleotides) may produce polypeptides having functional and chemical characteristics similar to those of a parental polypeptide.

The term "deletion" in the context of a polypeptide or protein refers to removal of codons for one or more amino acid residues from the polypeptide or protein sequence, wherein the regions on either side are joined together. The term deletion in the context of a nucleic acid refers to removal of one or more bases from a nucleic acid sequence, wherein the regions on either side are joined together.

The term "fragment" in the context of a proteinaceous agent refers to a peptide or polypeptide comprising an amino acid sequence of at least 2 contiguous amino acid residues, at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15

contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino acid residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100
5 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues of the amino acid sequence of a peptide, polypeptide, or protein. In one embodiment, the fragment constitutes at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference
10 polypeptide. In one embodiment, a fragment of a full-length protein retains activity of the full-length protein. In another embodiment, the fragment of the full-length protein does not retain the activity of the full-length protein.

The term "fragment" in the context of a nucleic acid refers to a nucleic acid comprising an nucleic acid sequence of at least 2 contiguous nucleotides, at least 5 contiguous nucleotides, at
15 least 10 contiguous nucleotides, at least 15 contiguous nucleotides, at least 20 contiguous nucleotides, at least 25 contiguous nucleotides, at least 30 contiguous nucleotides, at least 35 contiguous nucleotides, at least 40 contiguous nucleotides, at least 50 contiguous nucleotides, at least 60 contiguous nucleotides, at least 70 contiguous nucleotides, at least contiguous 80 nucleotides, at least 90 contiguous nucleotides, at least 100 contiguous nucleotides, at least 125
20 contiguous nucleotides, at least 150 contiguous nucleotides, at least 175 contiguous nucleotides, at least 200 contiguous nucleotides, at least 250 contiguous nucleotides, at least 300 contiguous nucleotides, at least 350 contiguous nucleotides, or at least 380 contiguous nucleotides of the nucleic acid sequence encoding a peptide, polypeptide or protein. In one embodiment the fragment constitutes at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of
25 the reference nucleic acid sequence. In a preferred embodiment, a fragment of a nucleic acid encodes a peptide or polypeptide that retains activity of the full-length protein. In another embodiment, the fragment encodes a peptide or polypeptide that of the full-length protein does not retain the activity of the full-length protein.

As used herein, the phrase "heterologous sequence" refers to any nucleic acid, protein, polypeptide, or peptide sequence which is not normally associated in nature with another nucleic acid or protein, polypeptide, or peptide sequence of interest.

As used herein, the phrase "heterologous nucleic acid insert" refers to any nucleic acid sequence that has been, or is to be inserted into the recombinant vectors described herein. The heterologous nucleic acid insert may refer to only the gene product encoding sequence or may refer to a sequence comprising a promoter, a gene product encoding sequence (for example secretion signal peptide-immune checkpoint inhibitor peptide chimeric polypeptides) and any regulatory sequences associated or operably linked therewith.

The term "homopolymer stretch" refers to a sequence comprising at least four of the same nucleotides uninterrupted by any other nucleotide, e.g., GGGG or TTTTTT.

The terms "percent identical," "percent homologous," or "percent similarity", and the like, when used in the context of nucleic acid sequences refers to the residues in the two sequences being compared which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length of the sequence, or, or alternatively a fragment of at least about 50 to 2500 nucleotides. Similarly, the terms "percent identical," "percent homologous," or "percent similarity", may be readily determined for amino acid sequences, over the full-length of a protein, or a fragment thereof. Suitably, a fragment is at least about 8 amino acids in length and may be up to about 7500 amino acids. Examples of suitable fragments are described herein. Generally, "identity", "homology" or "similarity" is determined in reference to "aligned" sequences. "Aligned" sequences or "alignments" refer to multiple nucleic acid sequences or protein (amino acids) sequences, often containing corrections for missing or additional bases or amino acids as compared to a reference sequence. Alignments can be performed using any of a variety of publicly or commercially available Multiple Sequence Alignment Programs. Examples of such programs include, "Clustal Omega", "Clustal W", "CAP Sequence Assembly", "MAP", and "MEME", which are accessible through Web Servers on the internet. Other sources for such programs are known to those of skill in the art. Alternatively, Vector NTI utilities are also used. There are also a number of algorithms known in the art that can be used to measure nucleotide sequence identity, including those contained in the programs described above. As another example, polynucleotide sequences can be compared using Fasta™,

a program in GCG Version 6.1. Fasta™ provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences. For instance, percent sequence identity between nucleic acid sequences can be determined using Fasta™ with its default parameters (a word size of 6 and the NOPAM factor for the scoring matrix) as provided in GCG Version 6.1, herein incorporated by reference. Multiple sequence alignment programs are also available for amino acid sequences, e.g., the “Clustal Omega”, “Clustal X”, “MAP”, “PIMA”, “MSA”, “BLOCKMAKER”, “MEME”, and “Match-Box” programs. Generally, any of these programs are used at default settings, although one of skill in the art can alter these settings as needed. Alternatively, one of skill in the art can utilize another algorithm or computer program which provides at least the level of identity or alignment as that provided by the referenced algorithms and programs. See, e.g., J. D. Thomson et al, Nucl. Acids. Res., “A comprehensive comparison of multiple sequence alignments”, 27(13):2682-2690 (1999).

The term “inducing an immune response” means eliciting a humoral response (e.g., the production of antibodies) or a cellular response (e.g., the activation of T cells), or both a humoral and a cellular response, directed against one or more antigenic proteins or fragments thereof expressed by the rMVA in a subject to which the rMVA has been administered.

The term “modified vaccinia Ankara,” “modified vaccinia ankara,” “Modified Vaccinia Ankara,” or “MVA” generally refers to a highly attenuated strain of vaccinia virus developed by Dr. Anton Mayr by serial passage on chick embryo fibroblast cells; or variants or derivatives thereof. MVA is reviewed in Mayr, A. et al. 1975 Infection 3:6-14. The genomic sequence of MVA and various variants is described, for example, at GenBank Accession Numbers AY603355, U94848, and DQ983238. In some embodiments, the MVA as provided herein can be derived synthetically, for example, through chemically synthesized plasmids and reconstituted to the full length genomic MVA sequence in a host cell, for example, as described in US2018/0251736, US2021/0230560, and WO2021/158565, each incorporated herein by reference.

“Nucleic acid” or “oligonucleotide” or “polynucleotide” as used herein means at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid can be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and

complements thereof. A single strand provides a probe that can hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

Nucleic acids can be single stranded or double stranded, or can contain portions of both double stranded and single stranded sequence. The nucleic acid can be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid can contain combinations of deoxyribo- and ribonucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids can be obtained by chemical synthesis methods or by recombinant methods.

"Operably linked" as used herein means that expression of a gene is under the control of a promoter with which it is spatially connected. A promoter can be positioned 5' (upstream) or 3' (downstream) of a gene under its control. The distance between the promoter and a gene can be approximately the same as the distance between that promoter and the gene it controls in the gene from which the promoter is derived. As is known in the art, variation in this distance can be accommodated without loss of promoter function.

A "peptide," "protein," "polypeptide," or "polyprotein" as used herein can mean a linked sequence of amino acids and can be natural, synthetic, or a modification or combination of natural and synthetic.

"Promoter" as used herein means a synthetic or naturally-derived molecule which is capable of conferring, activating, or enhancing the transcription of a nucleic acid in a cell. A promoter can comprise one or more specific transcriptional regulatory sequences to further enhance expression and/or to alter the spatial expression and/or temporal expression of same. A promoter can also comprise distal enhancer or repressor elements, which can be located as much as several thousand base pairs from the start site of transcription.

The term "prevent," "preventing," and "prevention" refers to the inhibition of the development or onset of a condition (e.g., an infection), or the prevention of the recurrence, onset, or development of one or more symptoms of a condition in a subject resulting from the administration of a therapy or the administration of a combination of therapies.

The term "prophylactically effective amount" refers to the amount of a composition (e.g., the target antigenic composition and/or rMVA described herein) which is sufficient to result in the

prevention of the development, recurrence, or onset of a condition or a symptom thereof (e.g., a viral infection) or symptom associated therewith or to enhance or improve the prophylactic effect(s) of another therapy.

5 The term "recombinant," with respect to a viral vector, means a vector (e.g., a viral genome) that has been manipulated *in vitro*, e.g., using recombinant nucleic acid techniques to express heterologous viral nucleic acid sequences.

10 The term "regulatory sequence" and "regulatory sequences" refers collectively to promoter sequences, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, and the like, which collectively provide for the transcription and translation of a coding sequence. Not all of these control sequences need always be present so long as the selected gene is capable of being transcribed and translated.

15 The term "shuttle vector" refers to a genetic vector (e.g., a DNA plasmid) that is useful for transferring genetic material from one host system into another. A shuttle vector can replicate alone (without the presence of any other vector) in at least one host (e.g., *E. coli*). In the context of MVA vector construction, shuttle vectors are usually DNA plasmids that can be manipulated in *E. coli* and then introduced into cultured cells infected with MVA vectors, resulting in the generation of new recombinant MVA vectors via, for example, homologous recombination.

20 The term "silent mutation" means a change in a nucleotide sequence that does not cause a change in the primary structure of the protein encoded by the nucleotide sequence, e.g., a change from AAA (encoding lysine) to AAG (also encoding lysine).

25 The "host," "patient," or "subject" treated is typically a human patient, although it is to be understood the methods described herein are effective with respect to other animals, such as mammals. More particularly, the term patient can include animals used in assays such as those used in preclinical testing including but not limited to mice, rats, monkeys, dogs, pigs and rabbits; as well as domesticated swine (pigs and hogs), ruminants, equine, poultry, felines, bovines, murines, canines, and the like. Determination of those subjects "at risk" can be made by any objective or subjective determination by a diagnostic test or opinion of a subject or health care provider (e.g., genetic test, enzyme or protein marker, marker history, and the like).

The term "synonymous codon" refers to the use of a codon with a different nucleic acid sequence to encode the same amino acid, e.g., AAA and AAG (both of which encode lysine). Codon optimization changes the codons for a protein to the synonymous codons that are most frequently used by a vector or a host cell.

5 The term "therapeutically effective amount" means the amount of the composition (e.g., the antigenic composition and/or recombinant MVA vector or pharmaceutical composition) that, when administered to a subject for treating or preventing a disorder, e.g., an infection or cancer, is sufficient to affect such treatment or prevention for the disorder.

10 The term "treating" or "treat" refer to the eradication or control of a disorder, the reduction or amelioration of the progression, severity, and/or duration of a disorder or one or more symptoms caused by the disorder resulting from the administration of one or more therapies.

15 The term "vaccine" means material used to provoke an immune response and confer immunity after administration of the material to a subject. Such immunity may include a cellular or humoral immune response that occurs when the subject is exposed to the immunogen after vaccine administration.

 The term "virus-like particles" or "VLP" refers to a structure which resembles a virus but is not infectious because it does not contain viral genetic material.

20 For the recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the number 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, and 7.0 are explicitly contemplated.

Modified Vaccinia Ankara (MVA) Viral Vectors

25 Modified vaccinia Ankara (MVA) in particular has been employed as a safe and potent viral vector vaccine against infectious diseases. MVA is a highly attenuated strain of vaccinia virus derived by extensive serial passages in chicken embryo fibroblasts (CEF) (Sutter G, Staib C. Vaccinia vectors as candidate vaccines: the development of modified vaccinia virus Ankara for antigen delivery. Current Drug Targets-Infectious Disorders. 2003;3:263-71). MVA is distinguished by its great attenuation, as demonstrated by diminished virulence and reduced ability
30 to replicate in primate cells, while maintaining good immunogenicity. The MVA virus has been

analyzed to determine alterations in the genome relative to the parental strain chorioallantois vaccinia virus Ankara (CVA) strain. Six major deletions of genomic DNA (deletion I, II, III, IV, V, and VI) totaling 31,000 base pairs have been identified (Meyer, H. et al. 1991 J Gen Virol 72: 1031 -1038). The resulting MVA virus is host cell restricted to avian cells. Accordingly, MVA vaccines can be produced in large scale in chicken cell lines.

The viral vector compositions provided herein comprise the vaccinia virus strain modified vaccinia Ankara (MVA). Modified vaccinia Ankara (MVA) has been generated by long-term serial passages of the Ankara strain of vaccinia virus (CVA) on chicken embryo fibroblasts (for review see Mayr A, et al. Abstammung, eigenschafter und verwendung des attenuierten vaccinia-stammes. Infection 3: 6-14, 1975; Swiss Patent No. 568,392). The MVA virus is publicly available from American Type Culture Collection as ATCC No. VR-1508. MVA is distinguished by its great attenuation, as demonstrated by diminished virulence and reduced ability to replicate in primate cells, while maintaining good immunogenicity. The MVA virus has been analyzed to determine alterations in the genome relative to the parental CVA strain. Six major deletions of genomic DNA (deletion I, II, III, IV, V, and VI) totaling 31,000 base pairs have been identified (Meyer, H. et al. 1991 J Gen Virol 72: 1031 -1038). The resulting MVA virus is host cell replication restricted to avian cells.

In particular embodiments, the MVA for use is the MVA available as ATCC VR-1566, a virus isolated by serial passage of CVA (Ankara) strain in chick embryo fibroblasts (CEF) in the laboratory of Professor Anton Mayr, then given to the National Institutes of Health, where it was plaque purified three times in CEF cells. VR-1566 was derived by limited further passage of stock received from the NIH in the SL-29 chicken embryo fibroblast cell line [ATCC CRL-1590].

In alternative embodiments, the MVA is derived from an MVA having the genomic sequence as described in at GenBank Accession Numbers AY603355, U94848, and DQ983238. In some embodiments, the MVA as provided herein can be derived synthetically, for example, through chemically synthesized plasmids and reconstituted to the full length genomic MVA sequence in a host cell, for example, as described in US2018/0251736, US2021/0230560, and WO2021/158565, each incorporated herein by reference.

The construction of the recombinant MVA (rMVA) viral vectors of the present invention can be prepared by methods known in the art. For example, a DNA-construct which contains the heterologous polycistronic nucleic acid sequence described herein can be flanked by MVA DNA sequences adjacent to a predetermined insertion site (e.g. between two conserved essential MVA genes such as I8R/G1L (see, e.g., U.S. Pat. No. 9133478, incorporated herein by reference in its entirety); in restructured and modified deletion III (see, e.g., U.S. Pat. No. 9,133,480, incorporated herein by reference in its entirety); or at other non-essential sites within the MVA genome) is introduced into cells infected with MVA, to allow homologous recombination. Once the DNA-construct has been introduced into the eukaryotic cell and the foreign DNA has recombined with the viral DNA, it is possible to isolate the desired rMVA in a manner known per se, preferably with the aid of a marker. The DNA-construct to be inserted can be linear or circular. A plasmid or polymerase chain reaction product is preferred. Such methods of making recombinant MVA vectors are described in, e.g., U.S. Pat. No. 9,133,478, incorporated by reference herein. For the expression of a DNA sequence or gene, it is necessary for regulatory sequences, which are required for the transcription of the polycistronic nucleic acid sequence, to be present on the DNA. Such regulatory sequences (called promoters) are known to those skilled in the art, and include for example those described further below. The DNA-construct can be introduced into the MVA infected cells by transfection, for example by means of calcium phosphate precipitation (Graham et al. 1973 Virol 52:456-467; Wigler et al. 1979 Cell 16:777-785), by means of electroporation (Neumann et al. 1982 EMBO J. 1:841-845), by microinjection (Graessmann et al. 1983 Meth Enzymol 101:482-492), by means of liposomes (Straubinger et al. 1983 Meth Enzymol 101:512-527), by means of spheroplasts (Schaffner 1980 PNAS USA 77:2163-2167) or by other methods known to those skilled in the art.

In some embodiments, the rMVA as provided herein can be derived synthetically, for example, through chemically synthesized plasmids and reconstituted to the full length genomic MVA sequence in a host cell, for example, as described in US2018/0251736, US2021/0230560, and WO2021/158565, each incorporated herein by reference.

As described above, the heterologous polycistronic nucleic acid sequence of the present invention can be inserted into any suitable site within the rMVA genomic sequence. In some

embodiments, the polycistronic nucleic acid sequence is inserted into the MVA vector in a natural deletion site, a modified natural deletion site, or between essential or non-essential MVA genes.

Immune Checkpoint Inhibitor Peptides

5 Provided herein are compositions comprising a recombinant modified vaccinia Ankara (rMVA) viral vector for use as an adjuvant or vaccine during an immunization protocol in a host such as a human, the rMVA constructed to express high concentrations of peptides capable of inhibiting one or more immune checkpoint pathways (immune checkpoint inhibitor peptide). In some embodiments, the immune checkpoint inhibitor peptides are expressed from a polycistronic
10 nucleic acid sequence comprising tandem repeats of the immune checkpoint inhibitors capable of being processed into monomers and secreted from the cell to enhance the immunogenicity of a targeted antigen. In some embodiments, the rMVA is used as an adjuvant to increase the immunogenicity of one or more co-administered antigens during a vaccination protocol. In some embodiments, the rMVA further encodes one or more antigenic peptides and is used as an
15 adjuvating vaccine. By expressing localized, high quantities of two or more immune checkpoint inhibitor peptides capable of downregulating one or more checkpoint inhibitor pathways, immune modulating activities which typically hinder the development of sufficient antigenicity to induce immunity can be downregulated.

 In certain aspects, the immune checkpoint inhibitor peptide is capable of inhibiting the
20 activity of an immune checkpoint pathway mediated by a receptor protein select from, but not limited to, programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), programmed death-ligand 2 (PD-L2), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte-activation gene 3 (LAG-3), T-cell immunoglobulin and mucin domain-3 (TIM-3), V-domain Ig suppressor of T-cell activation (VISTA), a B7 homolog protein (B7), B7 homolog 3
25 protein (B7-H3), B7 homolog 4 protein (B7-H4), B7 homolog 5 protein (B7-H5), OX-40 (OX-40), OX-40 ligand (OX-40L), glucocorticoid-induced TNFR-related protein (GITR), CD137, CD40, B and T lymphocyte attenuator (BTLA), Herpes Virus Entry Mediator (HVEM), galactin-9 (GAL9), killer cell immunoglobulin-like receptor (KIR), Natural Killer Cell Receptor 2B4 (2B4), CD160, checkpoint kinase 1 (CHK1), checkpoint kinase 2 (CHK2), adenosine A2a receptor
30 (A2aR), T cell immunoreceptor with Ig and ITIM domains (TIGIT), inducible T cell co-stimulator

(ICOS), inducible T cell co-stimulator ligand (ICOS-L), or combinations thereof. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-1. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-L1. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting CTLA-4. In some
5 embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting PD-1, PD-L1, or CTLA-4, or a combination thereof. In some embodiments, the immune checkpoint inhibitor peptide is capable of inhibiting both PD-1 and CTLA-4.

In some embodiments, the immune checkpoint inhibitor is an inhibitor capable of inhibiting PD-1, PD-L1, CTLA4, LAG-3, TIM3, OX40, or a combination thereof. In some embodiments,
10 the immune checkpoint inhibitor is capable of inhibiting PD-1 and CTLA4.

In some embodiments, the immune checkpoint inhibitor peptide is selected from the peptide sequences disclosed in Table 1, or a fragment, homolog, or derivative thereof. In some embodiments, the immune checkpoint inhibitor peptide is selected from the peptide sequences of SEQ ID Nos: 1-56, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or
15 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide is selected from the peptide sequences of SEQ ID Nos: 1-15, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 1, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some
20 embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 2, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 3, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide
25 sequences of SEQ ID No: 4, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 5, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 6, or peptide having an amino acid
30 sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the

immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 7, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 8, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 9, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 10, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 11, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 12, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 13, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 14, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences of SEQ ID No: 15, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the immune checkpoint inhibitor peptide has the peptide sequences selected from SEQ ID NOS: 16-56, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

Table 1 – Immune Checkpoint Inhibitor Peptides

SEQ ID NO:	Peptide Identifier	Peptide Sequence
1	LD01	CRRTSTGQISTLRVNITAPLSQ
2	LD01r	RTSTGDITSLRVITA
3	LD01 TQ19	TSTGQISTLRVNITAPLSQ
4	LD04	STLRVNITAPLSQRYRVRIR
5	LD10	STGQISTLRVNITAPLSQ

6	LD10 R9A	STGQISTLAVNITAPLSQ
7	LD10 P15A	STGQISTLRVNITAALSQ
8	LD11	CHHTSTGQISTLRVNITAPLSQ
9	LD17m	STGQISTARVNITAPLSQ
10	LD40	QISTLRVNITA
11	QP20	QTRTVPMPKIHPPWQNVVP
12	HD20	HHHQVYQVRSHWTGMHSGHD
13	WQ20	WNLPA SFHNHHIRPHEHEWIQ
14	SQ20	SSYHHFKMPELHFGKNTFHQ
15	LD12	CRRTSTGQISTARVNITAPLSQ
16	C1	YSAYQCWCWRQQGTS
17	C2	YHQYSCWCWRPPGPY
18	C3	YASYHCWCWRDPGRS
19	C4	TAYWNCWCWREKAGQ
20	C5	TYGYSCWCWRQHPWT
21	C6	STAYHCWCWREPYGN
22	C7	TTRYSCWCWRDDAYA
23	CLP001	HYPFRPHANQAS
24	CLP002	WHRSYYTWNLNT
25	CLP003	WHFSYNWRWLPP
26	CLP004	DYHDPSLPTLRK
27	Human PD-L1 Inhibitor I	FNWDYSWKSERLKEAYDL
28	Human PD-L1 Inhibitor II	FNWDYSLEELREKAKYK
29	Human PD-L1 Inhibitor III	TEKDYRHGNIRMKLAYDL
30	Human PD-L1 Inhibitor IV	GNWDYNSQRAQLYNQ
31	Human PD-L1 Inhibitor V	LDYVNRKMYQ
32	PPA-1	NYSKPTDRQYHF
33	PPA-2	KHAHHTHNLRLP
34	PPA-3	AAKMDGHLHGGQ
35	PPA-4	MRNRERYPKPY
36	PPA-5	TYLQRPSTNLER
37	P1	SCFPNWSLRPMNQ
38	P2	MDEKAQKGPAKL VFFACEKG
39	TP	SCFPNWSLRPMNQ
40	AP	MDEKAQKGPAKL VFFACEKG

41	PD6478	MLNWNRLSPSNQTEK
42	PL120131	GADYKRITVKVN
43	C17	CNMHTPMVC
44	C19	CNWMINKEC
45	C25	CVPMTYRAC
46	P29	ADFRVMADMSMY
47	P1309	SHTTTEHAKDKI
48	P26	GLIPLTTHMHIGK
49	P1310	TFLNSVPTYSYW
50	P1307	SHDTRSPFTWGR
51	P24	GSWNTFRAQPTI
52	P22	SFAGVHQFRPAP
53	P1303	ALWPSSSMSSTI
54	P1301	HDAFPRPHLFRN
55	P20	LTPHKHHKHLHA
56	GTX89379	C-KWYSHSKEKIQN

The immune checkpoint inhibitors of Table 1 have previously been described in, for example: SEQ ID NOS: 1-15 in U.S. Pat. Nos. 10,098,950, 10,799,555, and 10,799,581, and U.S. Pat. App. Nos. 2018/0071385, 2018/0185474, 2018/0200328, and 2018/0339044; SEQ ID NOS: 16-22 in Li et al., Peptide Blocking of PD-1/PD-L1 Interaction for Cancer Immunotherapy, Cancer Immunol Res February 1 2018 (6) (2) 178-188; SEQ ID NOS: 23-26 in Liu et al., Discovery of low-molecular weight anti-PD-L1 peptides for cancer immunotherapy. J. Immunotherapy Cancer 7, 270 (2019); SEQ ID NOS: 27-31 in Keir et al. D-1 and its ligands in T-cell immunity. Curr Opin Immunol. 2007;19(3):309-14 and Li et al., Discovery of peptide inhibitors targeting human programmed death 1 (PD-1) receptor. Oncotarget. 2016;7(40):64967-64976; SEQ ID NOS: 32-36 in Wang et al., Journal of Medicinal Chemistry 2019 62 (4), 1715-1730; SEQ ID NOS: 37-40 in Xiao et al., ACS Appl. Mater. Interfaces 2020, 12, 36, 40042-40051; SEQ ID NOS: 41-42 in Boohaker et al., Rational design and development of a peptide inhibitor for the PD-1/PD-L1 interaction, Cancer Letters, 2018, 434, Pages 11-21; SEQ ID NOS: 43-45 in Zhai et al., A novel cyclic peptide targeting LAG-3 for cancer immunotherapy by activating antigen-specific CD8⁺ T cell responses, Acta Pharmaceutica Sinica B, 2020, 10(6), Pages 1047-1060; 6, June 2020; SEQ ID NOS: 46-56 in Zhong et al., The biologically functional identification of a novel TIM3-binding

peptide P26 in vitro and in vivo. Cancer Chemother Pharmacol. 2020;86(6):783-792. All of the references are incorporated herein by reference.

Secretion Signal Peptide

As provided herein, the immune checkpoint inhibitor peptides expressed by the rMVA are secreted from the cell. In some embodiments, secretion may be accomplished by including the natural secretion signal associated with the immune checkpoint inhibitor peptide, if applicable. In alternative embodiments, the immune checkpoint inhibitor peptide expressed by the rMVA may be heterologous to the host or may not have appropriate secretion signaling to ensure secretion from the host cell. Because of this, secretion of the immune checkpoint inhibitor peptide can be accomplished by expressing a chimeric polypeptide that includes a secretion signal peptide fused to the immune checkpoint inhibitor peptide.

During the translation of the chimeric polypeptide comprising the secretion signal peptide and immune checkpoint inhibitor peptide, the signal peptide is recognized as it emerges from the ribosome; it is bound by the signal recognition particle (SRP) and translation is halted. This entire complex is transported to the external face of the Endoplasmic Reticulum (ER) where it binds to the SRP receptor, and the signal sequence is transferred to a translocon. While bound to the translocon, translation is reinitiated and the protein passes through the ER membrane and into the lumen. As it does this, the signal peptide is recognized by a signal peptidase and is cleaved to generate the immune checkpoint inhibitor peptide, which is trafficked through the Golgi network before being secreted from the cell via the classical secretory pathway.

Secretion signals suitable for use in the present invention can be naturally occurring secretion signals, consensus secretion signals (see, e.g., US20100305002, incorporated herein by reference), or a synthetic secretion signal.

In some embodiments, the secretion signal is selected from a peptide sequence of Table 2, or a homolog, derivative, or fragment thereof. In some embodiments, the secretion signal has a peptide sequence selected from SEQ ID NOS: 57-90, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

In some embodiments, the secretion signal is derived from the human tissue plasminogen activator (tPA) secretion signal or a homolog, derivative, or fragment thereof. In some

embodiments, the secretion signal peptide has the peptide sequence of SEQ ID NO: 65, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the secretion signal peptide has the peptide sequence of SEQ ID NO: 66, or a peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. It has been found that the tPA secretion signal is a particularly suitable secretion signal for use in the present invention, as it further enhances expression of the immune checkpoint inhibitor peptides.

Table 2 – Secretion Signal Peptides

SEQ ID NO:	Secretion Signal	Peptide Sequence
57	Human OSM	GVLLTQRTLLSLVLALLFPSMASM
58	VSV-G	KCLLYLAFLFIGVNC
59	Mouse Ig Kappa	ETDTLLLWVLLLWVPGSTGD
60	Human IgG2 H	GWSCIILFLVATATGVHS
61	BM40	RAWIFFLLCLAGRALA
62	Secrecon	WWRLWWLLLLLLLLLWPMVWA
63	Human IgKVIII	DMRVPAQLLGLLLLWLRGARC
64	CD33	PLLLLLPLLWAGALA
65	tissue plasminogen activator (tPA)	DAMKRGLCCVLLLCGAVFVSPS
66	tissue plasminogen activator (tPA)	DAMKRGLCCVLLLCGAVFVSPSQEIH ARFRRGAR
67	Human Chymotrypsinogen	AFLWLLSCWALLGTTFG
68	Human trypsinogen-2	NLLLILTFVAAAVA
69	Human IL-2	YRMQLLSICIALSLALVTNS
70	Gaussia luc	GVKVLFFALICIAVAEA
71	Albumin (HSA)	KWVTFISLLFSSAYS
72	Influenza Haemagglutinin	KTIHALSYIFCLVLG
73	Human insulin	ALWMRLLPLLALLALWGPDPAAA
74	Silkworm Fibroin LC	KPIFLVLL
75	Alkaline phosphatase	LGPCMLLLLLLLLLGLRLQLSLG
76	Secron 2	RPTWAWWLFLVLLALWAPARG
77	Human cystatin s	AGPLRAPLLLLAILAVALAVSPAAGSS
78	Lactotransferrin	HKLVFLVLLFLGALGLCLA

79	Erythropoietin	GVHECPAWLWLLLSLLSLPLGLPVLG
80	Human α -1-antichymotrypsin	ERMLPLLALGLLAAGFCPAVLC
81	TNF receptor superfamily - member 6 isoform 4	HLGIWTLLPLVLTSA
82	Human prolactin	NIKGSPPWKGSLLLLLVSNLLLCQSVAP
83	Osteopontin	RLAVVCLCLFGLASC
84	ATC	GRMLPLLALLLLAAGFCPAVLA
85	Consensus	RSLSVLALLLLLLLAPASAA
86	Consensus	KSLSALVLLLLLLLLPGALAA
87	Consensus	RGAALVLLLLLLLLLALALAAPVP
88	Consensus	RGAALVLLLLLLLLLAGVLAAP
89	Consensus	RGAALVLLLLLLLLSPALA
90	Consensus	RSLSVLALLLLLLLAPASAA

In some embodiments, the Secretion Signal Peptide of the first polypeptide encoded by the polycistronic nucleic acid insert further comprises the initiation amino acid methionine (M).

5 Cleavable Sequences

In addition to the secretion signal peptide on the N-terminus of each immune checkpoint inhibitor peptide, the polypeptide may also include a self-cleaving peptide fused to the C-terminus of the immune checkpoint inhibitor peptide. By providing a self-cleaving peptide sequence fused to the C-terminus of the immune checkpoint inhibitor peptide, the multiple immune checkpoint inhibitor peptides can be cleaved into multiple monomers during or following translation. Suitable cleavage sequences are known in the art (see, e.g., Donnelly et al., Analysis of the aphthovirus 2A/2B polyprotein ‘cleavage’ mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal ‘skip’. J. Gen. Virol. 82, 1013–1025 (2001), incorporated by reference in its entirety herein).

In some embodiments, one or more of the immune checkpoint inhibitor chimeric polypeptides includes one or more peptide sequences fused to the C-terminus of the immune checkpoint inhibitor peptide which is capable of being cleaved during or following, or a combination thereof, the translation of the polycistronic nucleic acid (see, e.g., Fig. 3A, 3B, and

3C). In some embodiments, the most C-terminus immune checkpoint inhibitor chimeric polypeptide does not include a cleavable peptide.

In some embodiments, the cleavable peptide is capable of being cleaved by a proprotein convertase enzyme including, for example, but not limited to furin or a furin-like proprotein convertase (Table 3). In some embodiments, the cleavable peptide sequence comprises a basic amino acid target sequence (canonically, RX(R/K)R), wherein X = any amino acid (SEQ ID NO: 91). In some embodiments, the cleavable peptide sequence comprises a basic amino acid target sequence (canonically, RX(R/K)R), wherein X = R, K, or H (SEQ ID NO: 92). In some embodiments, the cleavable peptide sequence is RAKR (SEQ ID NO: 93). In some embodiments, the cleavable peptide sequence is RRRR (SEQ ID NO: 94). In some embodiments, the cleavable peptide is RKRR (SEQ ID NO: 95). In some embodiments, the cleavable peptide is RRKR (SEQ ID NO: 96). In some embodiments, the cleavable peptide is RKKR (SEQ ID NO: 97). By including a cleavable peptide sequence on each of the covalently linked chimeric polypeptides, the multimeric polypeptide expressed during translation of the polycistronic nucleic acid insert can be processed through a cleaving mechanism into monomeric chimeric polypeptides following translation. This allows each chimeric polypeptide comprising the immune checkpoint inhibitor peptide to be secreted from the cell and function to downregulate an undesirable immune checkpoint pathway (see, e.g., Fig. 3A).

Table 3 – Cleavable Peptide Sequences

SEQ ID NO:	Cleavable Peptide Sequence
91	RX(R/K)R
92	RX(R/K)R, X = R, K, or H
93	RAKR
94	RRRR
95	RKRR
96	RRKR
97	RKKR

In some embodiments, each chimeric polypeptide includes one or more peptide sequences fused to the C-terminus of the immune checkpoint inhibitor peptide which is capable of inducing ribozyme skipping during translation of the polycistronic nucleic acid. Ribosomal "skipping" is

an alternate mechanism of translation in which a specific peptide sequence prevents the ribosome from covalently linking a new inserted amino acid, but nonetheless continues translation. This results in a “cleavage” of the polyprotein through the induced ribosomal skipping (see, e.g., Fig. 3B).

5 In some embodiments, the peptide capable of inducing ribosomal skipping is a cis-acting hydrolase element peptide (CHYSEL). In some embodiments, the CHYSEL sequence comprises a non-conserved sequence of amino-acids with a strong alpha-helical propensity followed by the consensus sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98), wherein the ribosomal skipping cleavage occurs between the G and P sequence. In some embodiments, the
10 CHYSEL sequence comprises DVEENPGP (SEQ ID NO: 99).

In some embodiments, the CHYSEL cleavage sequence is derived from one or more 2A self-processing peptides. 2A sequences are oligopeptides located between the P1 and P2 proteins in some members of the viral families, for example the picornavirus family, and can undergo self-cleavage to generate the mature viral proteins P1 and P2 in eukaryotic cells (Ahier et al.,
15 Simultaneous expression of multiple proteins under a single promoter in *Caenorhabditis elegans* via a versatile 2A-based toolkit. *Genetics*. 2014;196:605–613; Luke et al., Occurrence, function and evolutionary origins of '2A-like' sequences in virus genomes. *J Gen Virol*. 2008 Apr;89(Pt 4):1036-42; Doronina et al., Dissection of a co-translational nascent chain separation event. *Biochem Soc Trans*. 2008 Aug;36(Pt 4):712-6; Martin et al., A Model for Nonstoichiometric,
20 Cotranslational Protein Scission in Eukaryotic Ribosomes. *Bioorganic Chemistry*, Volume 27, Issue 1, February 1999, 55-79). The first discovered 2A was F2A (foot-and-mouth disease virus), after which E2A (equine rhinitis A virus), P2A (porcine teschovirus-1 2A), and T2A (thossea asigna virus 2A) were also identified (Ryan et al., Cleavage of foot-and-mouth disease virus polyprotein is mediated by residues located within a 19 amino acid sequence. *The Journal of general virology*.
25 1991;72(Pt 11):2727–2732; Szymczak et al., Development of 2A peptide-based strategies in the design of multicistronic vectors. *Expert opinion on biological therapy*. 2005;5:627–638).

In some embodiments, the CHYSEL cleavage sequence is derived from one or more 2A self-processing peptides provided for in Table 4, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the CHYSEL cleavage
30 sequence is derived from one or more 2A self-processing peptides having an amino acid sequence

selected from SEQ ID NOS: 100-117, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

Table 4 – CHYSEL Sequences

SEQ ID NO:	Origin	Peptide Sequence
98		D(V/I)EXNPGP
99		DVEENPGP
100	Picornaviridae: Aphthovirus: Foot-and-mouth disease virus	PSDARHKQRIVAPAKQLLNFDLLKLAGDVESNP GP
101	Avisivirus: Avisivirua A	ARRTLEWARREVGAIDETTHKDILLGGDIEENP GP
102	Avihepatovirus: Duck hepatitis A virus	RLKTLAFELNLEIESDQIRNKKDLTTEGVEPNPG P
103	Cardiovirus: Encephalomyocarditis: virus	VREENVFGLYRIFNAHYAGYFADLLIHDIENTNP G
104	Cosavirus: Cosavirus A	IMADSVLPRPLTRAERDVARDLLLIIAGDIESNP G
105	Erbovirus: Equine rhinitis B virus	SEPIPEATLSTILSEGATNFSLLKLAGDVELNPGP
106	Erbovirus: Seneca Valley virus	RYKNARAWCPSMLPFRSYKQKMLMQSGDIETN PGP
107	Hunnivirus: Hunnivirus A	CPRPGMAIDPPAQSSGATNFSLLRLAGDVELNP GP
108	Kunsagivirus: Kunsagivirus A	SPRSLHFLIGRPRPRVPPSPSLLSGDVEPNPG/P
109	Mischivirus: Mischivirus A	DSYPASGEEEEDDFHDMEDHSDILLGGDVEENP GP
110	Mosavirus: Mosavirus A2	TNSRAKLMVDEEDYVIQRSABRSVLLDGDVESN PGP
111	Pasivirus: Pasivirus A1	DIPSFQRDFINWLGSKHEELQNMILQCGDVEQNP GP
112	Teschovirus: Porcine teschovirus 1	EGLSSAMTVMAFQGGATNFSLLKQAGDVEEN PGP
113	Iflaviridae: Iflavivirus: Infectious flacherie virus	NYPLVPSIGNVARTLTRAIEIEDELIRAGIESNP GP
114	Tetraviridae: Betatetra virus: Thosea asigna virus	RSRRLRGPRPQNLGVRAEGRSLLTCGDVEENP GP
115	Dicistroviridae: Cripavirus: Cricket paralysis virus	FQQWKLVSNDCEAFRLKRTQLLMSGDVESN PGP
116	Reoviridae: Rotavirus: Human rotavirus C	LKKHNGAGYPLIVANSKFQIDKILISGDIELNPGP
117	Cypovirus: Lymantria: Dispar cypovirus 1	TDFLSMTAFDFQQAQVFRSNYDLLKCGDVESNP GP

In some embodiments, the cleavage sequence is a 2A cleavage sequence derived from foot-and-mouth disease virus (FMDV), for example derived from the amino acid sequence comprising VKQTLNFDLLKLAGDVESNPGP (SEQ ID. No. 118), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the 2A cleavage sequence is a 2A or 2A-like cleavage sequence selected from GSGEGRGSLTTCGDVEENPGP (SEQ ID NO: 119), GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 120), GSGQCTNYALLKLAGDVESNPGP (SEQ ID NO: 121), or GSGVKQTLNFDLLKLAGDVESNPGP (SEQ ID NO: 122), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the 2A-like cleavage sequence is GSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 120), or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

Table 5 – 2A/2A-like Cleavage Sequences

SEQ ID NO:	Peptide Sequence
118	VKQTLNFDLLKLAGDVESNPGP
119	GSGEGRGSLTTCGDVEENPGP
120	GSGATNFSLLKQAGDVEENPGP
121	GSGQCTNYALLKLAGDVESNPGP
122	GSGVKQTLNFDLLKLAGDVESNPGP

In some embodiments, the cleavable peptide sequence comprises two or more sequences which are capable of being cleaved by different mechanism, for example a cleavable peptide sequence which is capable of being cleaved following the translation of the polycistronic nucleic acid and a peptide sequence capable of inducing ribozyme skipping during translation of the polycistronic nucleic acid. By providing cleavable peptide sequences subject to multiple modes of cleaving, the efficiency of monomeric formation from the polycistronic nucleic acid can be improved. In some embodiments, the immune checkpoint inhibitor peptide has fused to its C-terminus a furin-cleavable peptide sequence, for example the peptide sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91), and fused to the C-terminus of the furin-cleavable peptide sequence is a CHYSEL peptide sequence, for example a peptide comprising the amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). By including a

furin-cleavable peptide sequence, such as RAKR (SEQ ID NO: 93), fused to the N-terminus of a CHYSEL peptide sequence between each chimeric polypeptide, the transcribed polycistronic nucleic acid undergoes ribozyme skipping during translation, resulting in the production of monomeric chimeric polypeptides, and all but the arginine (R) and alanine (A) residues of the furin cleavage sequence remains at the C-terminus of immune checkpoint inhibitor peptide, limiting the potential interference of the extra amino acid sequences on the function of the immune checkpoint inhibitor peptide (see e.g., Fig. 3C). In alternative embodiments, including a furin-cleavable peptide sequence, such as RRRR (SEQ ID NO: 94), RKRR (SEQ ID NO: 95), or RRKR (SEQ ID NO: 96), fused to the N-terminus of a CHYSEL peptide sequence between each chimeric polypeptide, the transcribed polycistronic nucleic acid undergoes ribozyme skipping during translation, resulting in the production of monomeric chimeric polypeptides, and the remaining furin cleavage sequence and CHYSEL peptide sequence are removed at the C-terminus of immune checkpoint inhibitor peptide.

In some embodiments, the hybrid cleavable peptide sequence comprises RAKR (SEQ ID NO: 93) fused to a CHYSEL containing amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). In some embodiments, the hybrid cleavable peptide sequence comprises RAKR (SEQ ID NO: 93) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 100-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RAKR (SEQ ID NO: 93) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 118-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RAKR (SEQ ID NO: 93) fused to a CHYSEL amino acid sequence of amino acid SEQ ID NO: 120, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the hybrid cleavable peptide is RAKRGSGA'TNFSLLKQAGDVEENPGP (SEQ ID NO: 123).

In some embodiments, the hybrid cleavable peptide sequence comprises RRRR (SEQ ID NO: 94) fused to a CHYSEL containing amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). In some embodiments, the hybrid cleavable peptide sequence comprises RRRR (SEQ ID NO: 94) fused to a CHYSEL amino acid sequence selected from the

group consisting of SEQ ID NOS: 100-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RRRR (SEQ ID NO: 93) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 118-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RRRR (SEQ ID NO: 94) fused to a CHYSEL amino acid sequence of amino acid SEQ ID NO: 120, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the hybrid cleavable peptide is RRRRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 124).

In some embodiments, the hybrid cleavable peptide sequence comprises RKRR (SEQ ID NO: 95) fused to a CHYSEL containing amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). In some embodiments, the hybrid cleavable peptide sequence comprises RKRR (SEQ ID NO: 95) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 100-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RKRR (SEQ ID NO: 95) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 118-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RKRR (SEQ ID NO: 95) fused to a CHYSEL amino acid sequence of amino acid SEQ ID NO: 120, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the hybrid cleavable peptide is RKRRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 125).

In some embodiments, the hybrid cleavable peptide sequence comprises RRKR (SEQ ID NO: 96) fused to a CHYSEL containing amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98) (Table 6). In some embodiments, the hybrid cleavable peptide sequence comprises RRKR (SEQ ID NO: 96) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 100-123, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RRKR (SEQ ID NO: 96) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 118-122, or peptide having an amino acid

sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RRKR (SEQ ID NO: 96) fused to a CHYSEL amino acid sequence of amino acid SEQ ID NO: 120, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the hybrid cleavable peptide is RRKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 126).

In some embodiments, the hybrid cleavable peptide sequence comprises RKKR (SEQ ID NO: 97) fused to a CHYSEL containing amino acid sequence D(V/I)EXNPGP, where X = any amino acid (SEQ ID NO: 98). In some embodiments, the hybrid cleavable peptide sequence comprises RKKR (SEQ ID NO: 97) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 100-123, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RKKR (SEQ ID NO: 97) fused to a CHYSEL amino acid sequence selected from the group consisting of SEQ ID NOS: 118-122, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the hybrid cleavable peptide sequence comprises RKKR (SEQ ID NO: 97) fused to a CHYSEL amino acid sequence of amino acid SEQ ID NO: 120, or peptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In particular embodiments, the hybrid cleavable peptide is RKKRGSGATNFSLLKQAGDVEENPGP (SEQ ID NO: 127).

Table 6 – Hybrid Cleavable Peptide Sequences

SEQ ID NO:	Peptide Sequence
123	RAKRGSGATNFSLLKQAGDVEENPGP
124	RRRRGSGATNFSLLKQAGDVEENPGP
125	RKRRGSGATNFSLLKQAGDVEENPGP
126	RRKRGSGATNFSLLKQAGDVEENPGP
127	RKKRGSGATNFSLLKQAGDVEENPGP

Regulatory Sequences

As provided herein, the immune checkpoint inhibitor peptides are expressed from a nucleic acid sequence inserted into a suitable location within the MVA genomic sequence. For the expression of the nucleic acid insert within the rMVA genomic backbone, it is necessary for

regulatory sequences such as promoters, which are required for the transcription of the polycistronic nucleic acid encoding the polyprotein, to be located in the 5' region of the nucleic acid insert adjacent to the transcription start site in order to initiate transcription. Wherein the nucleic acid insert is a polycistronic nucleic acid encoding multiple proteins/peptides as a single polyprotein, one or more promoters can be located 5' to the transcriptional start site of the ORF encoding the N-terminus most polypeptide of the polyprotein.

Because MVA is a cytoplasmic virus, suitable promoters, in some embodiments, include those derived from naturally occurring poxviral promoters. Poxviral genes, promoters, and transcription factors are divided into early, intermediate, and late classes, depending on their expression timing during poxvirus infections (see, e.g., Assarsson et al., Kinetic analysis of a complete poxvirus transcriptome reveals an immediate-early class of genes. PNAS 2008;105(6):2140–2145; Yang Z et al., Genome-wide analysis of the 5' and 3' ends of vaccinia virus early mRNAs delineates regulatory sequences of annotated and anomalous transcripts. J Virol. 2011;85(12):5897–5909). MVA replication in most mammalian cells (non-permissive cells) ceases during the assembly of progeny virions after all stages of expression occur. This supports the utility of all promoter classes, including late promoters, for controlling transgene expression (Sancho et al., The block in assembly of modified vaccinia virus Ankara in HeLa cells reveals new insights into vaccinia virus morphogenesis. J Virol. 2002;76(16):8318–8334; Geiben-Lynn et al., Kinetics of recombinant adenovirus type 5, vaccinia virus, modified vaccinia ankara virus, and DNA antigen expression in vivo and the induction of memory T-lymphocyte responses. Clin Vaccine Immunol. 2008;15(4):691–696). Some poxviral promoters have both early and late elements, allowing their open-reading frames (ORFs) or recombinant antigens to be expressed early in the virus infection and late after the viral genome replication, respectively (Broyles SS, Vaccinia virus transcription. J Gen Virol. 2003;84(Pt 9):2293–2303). Poxviral promoters can be utilized cross-strain (see Prideaux et al., Comparative analysis of vaccinia virus promoter activity in fowlpox and vaccinia virus recombinants. Virus Res. 1990;16(1):43–57; Tripathy et al., Regulation of foreign gene in fowlpox virus by a vaccinia virus promoter. Avian Dis. 1990;34(1):218–220).

Such MVA promoter sequences are known to those skilled in the art, and include for example the p11 promoter, which drives expression of the 11k protein encoded by the F17R ORF

(Wittek et al., Mapping of a gene coding for a major late structural polypeptide on the vaccinia virus genome. *J Virol.* 1984;49(2):371–378); the p7.5 promoter (Cochran et al., In vitro mutagenesis of the promoter region for a vaccinia virus gene: evidence for tandem early and late regulatory signals. *J Virol.* 1985;54(1):30–37); the p11L promoter (Schmitt et al., Sequence and transcriptional analysis of the vaccinia virus HindIII I fragment. *J Virol.* 1988;62(6):1889–1897); the pTK promoter (Weir and Moss, Determination of the promoter region of an early vaccinia virus gene encoding thymidine kinase. *Virology.* 1987;158(1):206–210); the pF7L promoter (Coupar et al., Effect of in vitro mutations in a vaccinia virus early promoter region monitored by herpes simplex virus thymidine kinase expression in recombinant vaccinia virus. *J Gen Virol.* 1987;68(Pt 9):2299–2309); the pH5 promoter (Perkus et al., Cloning and expression of foreign genes in vaccinia virus, using a host range selection system. *J Virol.* 1989;63(9):3829–3836); the short synthetic promoter pSyn (Chakrabarti et al., Compact, synthetic, vaccinia virus early/late promoter for protein expression. *Biotechniques.* 1997;23(6):1094–1097; Hammond et al., A synthetic vaccinia virus promoter with enhanced early and late activity. *J Virol Methods.* 1997;66(1):135–1380); the pmH5 promoter (Wyatt et al., Development of a replication-deficient recombinant vaccinia virus vaccine effective against parainfluenza virus 3 infection in an animal model. *Vaccine.* 1996;14(15):1451–1458); the pHyb promoter (Sancho et al., The block in assembly of modified vaccinia virus Ankara in HeLa cells reveals new insights into vaccinia virus morphogenesis. *J Virol.* 2002;76(16):8318–8334); the LEO promoter (Wyatt et al., Correlation of immunogenicities and in vitro expression levels of recombinant modified vaccinia virus Ankara HIV vaccines. *Vaccine.* 2008;26(4):486–493); the pB8 promoter (Orubu et al., Expression and cellular immunogenicity of a transgenic antigen driven by endogenous poxviral early promoters at their authentic loci in MVA. *PLoS One.* 2012;7(6):e40167); the pF11 promoter (Orubu et al., Expression and cellular immunogenicity of a transgenic antigen driven by endogenous poxviral early promoters at their authentic loci in MVA. *PLoS One.* 2012;7(6):e40167). In some embodiments, the promoter is selected from one or more of pmH5, p11, pSyn, pHyb, or a combination thereof.

In some embodiments, the promoter is the pH5 promoter
 AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAGCGAGA
 AATAATCATAA (SEQ ID NO: 128), or a nucleic acid sequence at least 85%, 90%, 95%, 97%,

or 99% identical thereto. In some embodiments, the promoter is the pH5 promoter AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAGCGAGA AATAATCATAAATT (SEQ ID NO: 129), or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

5 In some embodiments, the promoter is the modified pH5 promoter (pmH5) AAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAGCGAGA AATAATCATAA (SEQ ID NO: 130), or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the promoter is the modified pH5 promoter (pmH5) AAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTAAATTGAAAG
10 CGAGAAATAATCATAAATA (SEQ ID NO: 131), or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the promoter is the modified pH5 promoter (pmH5) AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAAATA (SEQ ID NO: 132), or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

15 Additional vaccinia virus promoters that may be particularly suitable as promoters in the present invention include those derived from natural promoter sequences, for example, as provided in Table 7 below, or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto, wherein the nomenclature for the gene locus is based on the ORF nomenclatures originally used for the WR and Copenhagen strains of vaccinia virus. In some embodiments, the promoter
20 is selected from one or more of SEQ ID. No. 133-308, or a combination thereof, or a nucleic acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

Table 7 - Additional Vaccinia Virus Promoters

SEQ ID. No.	Gene Locus	Promoter Sequence
128	pH5 promoter	AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAA
129	pH5 promoter	AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAAATT
130	modified pH5 promoter (pmH5)	AAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAA
131	modified pH5 promoter (pmH5)	AAAAAATTGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAAATA

132	modified pH5 promoter (pmH5)	AAAAAATGAAAATAAATACAAAGGTTCTTGAGGGTTGTGTTA AATTGAAAGCGAGAAATAATCATAAATA
133	C23L	AAAGTAGAAAATATA
134	Pseudogene	TATCCGGAGACGTCA
135	C11R	ATTACTGAATTAATA
136	C10L	GCAACGTAAAAACACA
137	no ortholog	AAAAAATAAAAAAAAA
138	no ortholog	AGTAAAAGAAAAAGAA
139	no ortholog	AAAATTGATAAATAA
140	no ortholog	AAATTAGACATTTGA
141	C9L	ATAACTGAAATGAAA
142	C7L	AAAGATGAAAAAGTA
143	C6L	ATTAATGAAATAATA
144	C5L	AAAAATGAAAATGGA
145	C4L	AAAACATAAAAAATTA
146	N2L	ATAACATAAAAAATAA
147	M2L	AAGATAGATTCCTA
148	K1L	AAAAATGAAAAAATA
149	K3L	GAAAAAGAAATTCCT
150	K5L	AATGGTGAAAAAATG
151	K6L	AAAACATAAAAAATAA
152	K7R	ATAATTGTAAAAACA
153	F7L	ATAATTGAAAATGGA
154	F8L	AAAAATTTAATTACA
155	F11L	AAAAGTGAAAAACAA
156	F12L	AAAAAAGAAAATAGA
157	F14L	GTAGAAGAAAATAAT
158	F15L	AAAAATGAAACGTAA
159	F16L	AAAAAACAAAATGAA
160	E1L	GAGACAGTAGTTTTA
161	E3L	AAAAATGATAAAATA
162	E4L	AATAATGAAAAAATA
163	E5R	ACAAAAGTGAATATA
164	E9L	TTAAATGAAAATATA
165	O1L	AATAATGAAAAAACA

166	I3L	TAAAGTGAAAATATA
167	I4L	ATTAATGAAAAGTTA
168	G2R	ATAACAAAAATAAAA
169	G5R	AAAAATGATAAGATA
170	G5.5R	AAAACGTAAACACGA
171	L2R	AAAACGTAAAATATA
172	J2R	TAAAGTGAACAATAA
173	J6R	AAAAGGGAAATTTGA
174	H3L	AGAATTGAAAACGAA
175	H5R	AAAAATGAAAATAAA
176	D1R	GTAAATGAAAAAAAAA
177	D4R	GAAAATGAAAAGGTA
178	D7R	AAAACGTATGAAATA
179	D9R	AAAAATGAAATGATA
180	D12L	AATAATGAAAACAAA
181	A4L	AATTCTGAACTAGA
182	A5R	AAAATTGAATTGCGA
183	A8R	TAAAGTGAAAATCTA
184	A18R	GCAATAGAAAAGATG
185	A20R	AAGAATGAAATAACA
186	A23R	AAAAATGTAATAACG
187	A29L	AAAGTCGAAAAAGAA
188	A31R	AAAACATAAATATAA
189	A33R	AATATGGAAAATAA
190	A35R	AAAAATGAATTAATA
191	A37R	AAAATTGAAGTAATA
192	A40R	AATACTTAAAATGTA
193	A41L	AAAATATAAAATAAA
194	268	AAAAATGAACTCTTA
195	A44L	AAAATAGAATAAGTA
196	A46R	ATAAATGAAAAGATA
197	A47L	AAAACGTAAAATAAA
198	A48R	AAATTGTAAAAAATA
199	A50R	AAATATTAAAAAATA
200	A52R	GAAATAAAAAACATA

201	A55R	AAAAATAAAAAATATA
202	A56R	AATTTTGTAAAAATA
203		ATTACATATTATATA
204	B1R	AAAACCTTAAAAATTTA
205	B2R	ATAAAAATTAAAAAA
206	B5R	ATATCTAAAAATCTT
207	B6R	AAAAATAATGACCAA
208	B8R	ATTATTCAAAATATG
209	B11R	GAAAATGAAAATATA
210	B12R	AAAACATAAAAAACA
211	B13R	AAGATTGAAATTATA
212	B17L	AAATATGTAAATATG
213	B19R	AAAACCTGATATTATA
214	Pseudogene	ATAAATGTAGACTCT
215	C12L	TAAACTGAAGTTTAA
216	K2L	ATTTTATACCGAACATAAAAAATAAGGTTAATTATTAATACCA TAAAATC
217	K4L	GGATTTTTAATAGAGTGAAGTGATATAGGATTATTCTTTTAAC AAATAAA
218	F13L	ATTCTAGAATCGTTGATAGAACAGGATGTATAAGTTTTATGT TAACTAA
219	E11L	TTTGTATCATTTGTCCATCAACGTCATTTCAATAATATTGGATG ATATAA
220	O2L	ACTAAAGAGTTAAATAAGTCGAGATAGTTTTATATCACTTAA TATATAA
221	O3L	GTGCCTAATATTACTATATCAAGTAATGCTGAATAAAAAATATT TATAAAT
222	I1L	TTCTACTACTATTGATATATTTGTATTTAAAAGTTGTTTGGTGA ACTTAA
223	I5L	ATACAACTAGGACTTTGTCCACATATTCTTTGATCTAATTTTTAG ATATAA
224	I6L	TGTGATATGTGATAAATTAACACAAAATTAAATAGAATAGTA AACGACG
225	G4L	CAGTGATTTATTTTCCAGCAGTAACGATTTTAAGTTTTTGATAC CCATAA
226	H1L	AATTACACGCGTTTACCGATAAAGTAGTTTTATCCATTTGTAC GTTATAA
227	H3L	AAAATATAACTCGTATTAAAGAGTTGTATATGATTAATTTCAA TAACTAA

228	D8L	AATTCCCATACTAAGAGCTATTTTTAAACAGTTATCATTTCATT TTTACT
229	D11L	TAAACTACTGCTGTGATTTTTAAAACATAGTTATTACTTATCAC TCATAA
230	D13L	GATATTTCTCTACGGAGTTTATTGTAAGCTTTTCCATTTTAA TAGAAA
231	A1L	AGGTTTTCTACTTGCTCATTAGAAGTATAAAAAAATAGTTCCG TAATTAA
232	A2L	AAAATGTTTTTATATAAAATATTGGACGACGAGATACGTAGAG TGTTAAC
233	A3L	AGATTGGATATTAAAAATCACGCTTTCGAGTAAAACTACGAAT ATAAATA
234	A6L	AACTCTGGAAGAGCACAAATAAATTAAACAACATAAATCTGTA AATAAATA
235	A12L	TATAATCTAGTTAAATCTTCTGTATAAATAAAAAATATTTTTAGC TTCTAA
236	A15L	CTATTTTATATCTATTTATTCGCGTCCTAAAATTAAACAAATG ATATAA
237	A16L	GATGTTGATATACCAACATTTAACAGTTTAAATACTGACGATT ATTAAGA
238	A19L	TTGCACGATCGTGTTATAGGGCATATTCTGACTTATTTTTTACT ACCTAA
239		AATTCGAAAGAAAAAGAATCACAGTCCTAAAAGCTGAACTTC GGAAATCT
240		ATCTAGAATATCAGATCTTGAAAGACAGTTGAACGACTGTAG ACGTAATA
241	A25L	TTATAATTACCCGATTGTAGTTAAGTTTTGAATAAAATTTTTTA TAATAA
242	A27L	TACCAAATATAAATAACGCAGAGTGTGAGTTTCTAAAATCTGT ACTTTAA
243	A30L	TCCATAAAAGACGAATAAGATACAAACACAAATGTTTATATA ATATTTAA
244	A30.5L	ATGTTTTTTCCAAAAACCTAAGTGTATTTAAAATAGATGCCAT GTAAAAA
245	A32L	TCCATATTTTGATTTATTATCAAATTAATTTAGTAACTGTAAAT ATAATT
246	A38L	CAAAATAGAATAAAATAAATAACAAAGGTATCATTTTAAATA AATAAAAA
247		GATATCCATGGTATAGACCAAACAATAACGATATATATCATAA ATAAATAA
248	E6R	TAATTATTAGAATAAGAGTGTAGTATCATAGATAACTCTCTTC TATAAAA

249	E7R	TATACATAGATATAATTATCACATATTAAAAATTCACACATTT TTGATAA
250	E8R	ACATAAAAACTCATTACATAGTTGATAAAAAAGCGGTAGGATA TAAATATT
251	I8R	TAGTTCTGGTATTTTACTAATTACTAAATCTGTATATCTTTCCA TTTATC
252	G8R	CGACGCTGTTCTGCAGCCATTTAACTTTAAATAATTTACAAAA ATTTAAA
253	L4R	TTTGTAACATCGGTACGGGTATTCATTTATCACAAAAAAAAC TCTCTAA
254	J1R	TAGTAAACCGATAGTGTATAAAGATTGTGCAAAGCTTTTGCGA TCAATAA
255	H7R	CTACGGATGGATGATATAGATCTTTACACAAATAATTACAAAA CCGATAA
256	D6R	ATCTCCGTAAATATATGCTCATATATTTATAGAAGATATCACA TATCTAA
257	D10R	GATAAATACGAATATCTGTCTTATATTTATAATATGCTAGTTA ATAGTAA
258	A22R	CAATATTGAAAATACTAATTGTTTAAATAACCCGAGTATTGAA ACTATAT
259	A34R	TATTTTTGTGTTAAAACAATGAACTAATATTTATTTTTGTACAT TAATAA
260		GATACGATACTATATGTATTCTTCGATAGTCCGCATTATGTAC CTATTCT
261	A42R	CAAGTTTATTCCAATAGATGTCTTATTA AAAACATATATAATA AATAACA
262	A43R	AACTGGTAATTAAAATAAAAAGTAATATTCATATGTAGTGTCA ATTTTAA
263	A53R	TTTTTGATGGTGGTTTAAACGTTTTAAAAAAGATTTTGTTATTG TAGTAT
264	B4R	TAACATTGTTAATTGAAAAGGGATAACATGTTACAGAATATAA ATTATAT
265	B9R	TGCATATTATACACTGGTTAACGCCCTTATAGGCTCTAACCAT TTTCAAG
266		TTGCAGTGTTCATCTCCCAACTGCAAGTGAAGGATTGATAACT GAAGGCA
267		CTCTTCTCCCTTTCCAGAAACAACTTTTTTTACCCACTATAA AATAAA
268	C13L	AATAGTATAAACTAAAAATTAAACAAATCGTTATTATAAGTAA TATCAAA
269	C19L	TTCTGTTTTTCTTTCACATCTTTAATTATGAAAAAGTAAATCAT TATGAG

270	C8L	CACTTACTAAATAGCCAAGGTGATTATTCGTATTTTTTTAAGG AGTAACC
271	C3L	TTTTATTATTTGTACGATGTCCAGGATAACATTTTACGGATAA ATAAAT
272	F9L	TAGTTTCTTGGA AAAAATTATTATGAGAGACATTTTCTCAGAC TGGATAA
273	F10L	TCTATCAAACCTGGACTTTCGTTTGTA AATTGGGGCTTTTTGTA CAATAA
274	I2L	ATGAATATGATGAAGATAGCGATAAAGAAAAGCCAATATTCA ATGTATAA
275	I7L	AACGCAGTTTGGA AAAAAGAAGATATCTGGTAAATTCTTTTCC ATGATAA
276	G1L	TACGATGATAACGACATACGAACATTACTTCCTATTTTACTCC TTAGTAA
277	G3L	ATCTTCTGTAAGTAGGAATTTGGACAAGTTGAACAAAATTAGA TCTCTAA
278	G7L	ATTTTTATACGGATGCTCATTTTAAATTTTGTA AATTATTTAA AGTTAA
279	L3L	ATGAGGTTTTCTAGCAGTAGACTCATTTAGAGAAGTTTTTTTG TGATAA
280	J5L	TTATTACA ACTATAAAAATAATAGTTATATTTACACTTTAAATT TTTATC
281	H4L	TAAAAAAATTATACATCATAAACCAATTTTCCTAGTTGTTTGTA ACTTTAA
282	D2L	CGTTATCGTCGTTATCTACTTTGGGATACTTATTATCCTTAACT ATAAAA
283	A2.5L	TATATTAGCGCTAGACATATTACAGAACTATTTTAGATTATGA TATTTAA
284	A7L	AAGACTTACATCATCGGTAGTAGATTTTCACTTACCCACGA TATAAAT
285	A9L	AAAATCTAAATATGACAGATGGTGACTCTGTCTCTTTTGATGA TGAATAA
286	A10L	ATCGTTTTGTATATCCGTCACTGGTACGGTCGTCATTTAATACT AAATAA
287	A13L	AAAAGATGATATATTGCATACTTGATCAATAGTGAAGTTATTG TCAATAA
288	A14L	GTTTATATTCCACTTTGTTTCATTCGGCGATTAAAAATTTTATT AGTTAA
289	A14.5L	ATTCGTATTATTTGAGCAAGAAAATATCCCACCACCTTTTCGT CTAGTAA
290	A17L	GGCATAAAGATTATACTCCATCTTTAATAGTGACATTTTTTAAT ATATAA

291	A21L	TGTACAGACTAAGTAATTCTTTTAAGTTAGTTAAATCAGCGCT AGAAGTC
292	A26L	ACTTAACTCTTTTGTTAATTAAAAGTATATTCAAAAAATGAGT TATATAA
293	A28L	CATTGTCTGATGCGTGTAaaaaaATTTTGTGAGCTTCTAATAG ATTATAA
294	F17R	TGTATGTAAAAATATAGTAGAATTTCAATTTGTTTTTTCTATG CTATAA
295	E10R	TAATGCACCGAACATCCATTTATAGAATTTAGAAATATATTTT CATTTAA
296	G6R	AGAACCTCAACGTAACCTAACAGTGCAACCTCTATTGGATATA AACTAAT
297	G9R	GATCAACATCTTTATGGCGTTTTTAGATTAATACTTTCAATGAG ATAAAT
298	L1R	TCAGTTTATTATCTCTCTTGGTAATATGGATACTAATTGTAGCT ATTTAA
299	L5R	AAAAGAATATTCCTCTAACAGATATTCCGACAAAGGATTGATT ACTATAA
300	H2R	GTAGTAGTAAGTATTTATACAACTTTTCTTATCCATTTATAAC GTACAA
301	H6R	AGGGAAAATCTAAAGTTGTTTCGTAAAAAAGTTAAACTTGTA AGAAGTAA
302	D3R	ATAAAATACTACTGTTGAGTAAATCAGTTATTTTTTTTATATCG ATATTG
303	A11L	TTGATCAAGAGTAACTATTGACTTAATAGGCATCATTATTTA GTATTAA
304	A39R	CCAATTTCCATCTAATATACTTTGTGCGATTATCTATAGTACAC GGAATA
305	A45R	CCATTGCTGCCACTCATAATATCAGACTACTTATTCTATTTTAC TAAATA
306	B7R	TTTGTATAAATAATTATTTCAATATACTAGTTAAAATTTTAAGA TTTTAA
307		TCCATCCACAGACGTTACCGAACCGATTAGTGATGTGACACCA TCGGTGG
308		ATACGAGGACGTGTATAGAGTAAGTAAAGAAAAAGAATGTGG AATTGCT

In addition, the nucleic acid sequence for insertion may further include suitable translation initiation sequences, such as for example, a Kozak consensus sequence (GCCACC/ATG).

In addition, the polycistronic nucleic acid sequence for insertion can include appropriate
5 stop codons, for example TAA, TAG, or TGA, or combinations or multiples thereof, at the 3' end

of the nucleic acid sequence following the last amino acid encoding sequence of the polypeptide. Furthermore, the nucleic acid sequence can include a vaccinia virus termination sequence 3' of the last stop codon of polyprotein. In addition, the nucleic acid sequence for insertion may further include restriction enzyme sites useful for generating shuttle vectors for ease of insertion of the immune checkpoint inhibitor encoding sequences.

Antigenic Targets

The provided rMVA viral constructs of the present invention can be used as an adjuvant for treating or preventing an infectious disease or cancer in a subject. In some embodiments, the rMVA viral construct is administered to a subject in need thereof, for example a human, in a prophylactic vaccination protocol to prevent an infectious disease, for example at a priming stage, a boosting stage, or both a priming stage and boosting stage. In an alternative embodiment, the rMVA viral construct is administered to a subject in need thereof, for example a human, in a treatment modality incorporating a vaccination protocol, for example, to treat a cancer. Accordingly, the rMVA viral construct can be administered in concert with one or more antigens intended to induce an immune response against an antigenic target in order to induce partial or complete immunization in a subject in need thereof.

Thus, the rMVA of the present invention can be administered with one or more antigens targeting an infectious disease or cancer. Examples of antigens and antigen delivery vehicles that the rMVA can be used with as an adjuvant include: an antigenic protein, polypeptide, or peptide, or fragment thereof; a nucleic acid, for example mRNA or DNA, encoding one or more antigens; a polysaccharide or a conjugate of a polysaccharide to a protein; glycolipids, for example gangliosides; a toxoid; a subunit (e.g., of a virus, bacterium, fungi, amoeba, parasite, etc.); a virus like particle; a live virus; a split virus; an attenuated virus; an inactivated virus; an enveloped virus; a viral vector expressing one or more antigens; a tumor associated antigen; or any combination thereof.

In particular aspects, the present invention provides a method of preventing or treating an infectious disease in a subject in need thereof, said method comprising administering an effective amount of the rMVA of the present invention in combination, alternation, or coordination with a prophylactically effective or therapeutically effective amount of one or more antigens, or antigen

expressing vectors, wherein the rMVA enhances immunity directed against the targeted infectious diseases.

In some embodiments, the targeted infection is a viral infection, including but not limited to: a double-stranded DNA virus, including but not limited to Adenoviruses, Herpesviruses, and Poxviruses; a single stranded DNA, including but not limited to Parvoviruses; a double stranded RNA virus, including but not limited to Reoviruses; a positive-single stranded RNA virus, including but not limited to Coronaviruses, Picornaviruses, and Togaviruses; a negative-single stranded RNA virus, including but not limited to Orthomyxoviruses, and Rhabdoviruses; a single-stranded RNA-Retrovirus, including but not limited to Retroviruses; or a double-stranded DNA-Retrovirus, including but not limited to Hepadnaviruses. In some embodiments, the targeted virus is adenovirus, avian influenza, coxsackievirus, cytomegalovirus, dengue fever virus, ebola virus, Epstein-Barr virus, equine encephalitis virus, flavivirus, hepadnavirus, hepatitis A virus, hepatitis B virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, herpes simplex virus, human immunodeficiency virus, human papillomavirus, influenza virus, Japanese encephalitis virus, JC virus, measles morbillivirus, marburg virus, Middle Eastern respiratory syndrome (MERS-CoV)-coronavirus, mumps rubulavirus, orthomyxovirus, papillomavirus, parainfluenza virus, parvovirus, picornavirus, poliovirus, pox virus, rabies virus, reovirus, respiratory syncytial virus, retrovirus, rhabdovirus, rhinovirus, Rift Valley fever virus, rotavirus, rubella virus, rubeola virus, severe acute respiratory syndrome-coronavirus 1 (SARS-CoV), severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), smallpox virus, togavirus, swine influenza virus, varicella-zoster virus, variola major, variola minor, and yellow fever virus. Examples of viruses that may be used as antigens also include measles virus, mumps virus (Mumps rubulavirus), Rubella virus, varicella zoster virus or a combination of all four or three thereof (e.g., measles, mumps, and rubella).

In some embodiments, the targeted infectious agent is a *Flaviviridae* virus, including infections with viruses of the genera *Flavivirus* and *Pestivirus*. *Flavivirus* infections include Dengue fever, Kyasanur Forest disease, Powassan disease, Wesselsbron disease, West Nile fever, yellow fever, Zika virus, Rio bravo, Rocio, Negishi, and the encephalitises including: California encephalitis, central European encephalitis, Ilheus virus, Murray Valley encephalitis, St. Louis encephalitis, Japanese B encephalitis, Louping ill, and Russian spring-rodents summer

encephalitis. *Pestivirus* infections include primarily livestock diseases, including swine fever in pigs, BVDV (bovine viral diarrhea virus) in cattle, or Border Disease virus infections.

In some embodiments, the targeted infectious agent is an Alphavirus virus, for example, Eastern equine encephalitis (EEE) virus, Venezuelan equine encephalitis (VEE) virus, Western equine encephalitis (WEE) virus, the Everglades virus, Chikungunya virus, Mayaro virus, Ockelbo virus, O'nyong-nyong virus, Ross River virus, Semliki Forest virus or Sindbis virus (SINV).

In some embodiments, the targeted infectious agent is the equine arteritis virus, bovine viral diarrhea virus (BVDV), hog cholera virus or border disease virus. The only member of the Rubivirus genus is the rubella virus.

In some embodiments, the targeted infectious agent a *Filoviridae* virus such as the Ebola virus and Marburg virus; a *Paramyxoviridae* virus such as Measles virus, Mumps virus, Nipah virus, Hendra virus, respiratory syncytial virus (RSV) and Newcastle disease virus (NDV); *Rhabdoviridae* virus such as Rabies virus; a *Nyamiviridae* virus such as Nyavirus, an *Arenaviridae* virus such as Lassa virus, a *Bunyaviridae* virus such as Hantavirus, Crimean-Congo hemorrhagic fever; or *Ophioviridae* and *Orthomyxoviridae* viruses such as influenza virus.

In one embodiment, an antigen is taken from one or more bacteria selected from *Borrelia* species, *Bacillus anthracis*, *Borrelia burgdorferi*, *Bordetella pertussis*, *Camphylobacter jejuni*, *Chlamydia* species, *Chlamydial psittaci*, *Chlamydial trachomatis*, *Clostridium* species, *Clostridium tetani*, *Clostridium botulinum*, *Clostridium perfringens*, *Corynebacterium diphtheriae*, *Coxiella* species, an *Enterococcus* species, *Ehrlichia* species, *Escherichia coli*, *Francisella tularensis*, *Haemophilus* species, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Lactobacillus* species, a *Legionella* species, *Legionella pneumophila*, *Leptospira interrogans*, *Listeria* species, *Listeria monocytogenes*, *Mycobacterium* species, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycoplasma* species, *Mycoplasma pneumoniae*, *Neisseria* species, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Pneumococcus* species, *Pseudomonas* species, *Pseudomonas aeruginosa*, *Salmonella* species, *Salmonella typhi*, *Salmonella enterica*, *Streptococcus* species, *Rickettsia* species, *Rickettsia rickettsii*, *Rickettsia typhi*, *Shigella* species, *Staphylococcus* species, *Staphylococcus aureus*, *Streptococcus* species, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Treponema* species, *Treponema*

pallidum, a *Vibrio* species, *Vibrio cholerae* and *Yersinia pestis*. Such bacteria may be a whole cell (e.g., live, attenuated or inactivated) or a polypeptide or polysaccharide of such a bacterium.

In some embodiments, the targeted infectious agent is a bacterium. The antigenic bacterial agent for targeting can be a polysaccharide-polypeptide antigen such as a pneumococcal (e.g., *S. pneumoniae*) polysaccharide (e.g., a cell capsule sugar)-protein (e.g., diphtheria protein) conjugate. In some embodiments, the conjugate comprises cell capture sugars of *S. pneumoniae* conjugated to a protein (e.g., diphtheria protein), e.g., wherein the cell capsule sugars are of seven serotypes of the bacteria *S. pneumoniae* (4, 6B, 9V, 14, 18C, 19F and 23F), conjugated with diphtheria proteins. In some embodiments, the conjugate comprises Pneumococcal polysaccharide serotype 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F conjugated to a protein such as protein D derived from non-typeable *Haemophilus influenza*, tetanus toxoid carrier protein and/or diphtheria toxoid carrier protein. In some embodiments, the conjugate comprises *Streptococcus pneumoniae* capsular polysaccharide conjugated to a diphtheria protein, e.g., *Streptococcus pneumoniae* type 1, 3, 4, 5, 6a, 6b, 7f, 9v, 14, 18c, 23f, 19a and 19f capsular polysaccharide conjugated to a protein such as diphtheria *crm197* protein. In some embodiments, one or more of the polysaccharide-protein conjugates comprising capsular polysaccharides from at least one of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6G, 6H, 7F, 7A, 7B, 7C, 8, 9A, 9L, 9N, 9V, 10F, 10A, 10B, 10C, 11F, 11A, 11B, 11C, 11D, 11E, 12F, 12A, 12B, 13, 14, 15F, 15A, 15B, 15C, 16F, 16A, 17F, 17A, 18F, 18A, 18B, 18C, 19F, 19A, 19B, 19C, 20A, 20B, 21, 22F, 22A, 23F, 23A, 23B, 24F, 24A, 24B, 25F, 25A, 27, 28F, 28A, 29, 31, 32F, 32A, 33F, 33A, 33B, 33C, 33D, 33E, 34, 35F, 35A, 35B, 35C, 36, 37, 38, 39, 40, 41F, 41A, 42, 43, 44, 45, 46, 47F, 47A, 48, CWPS1, CWPS2, CWPS3 of *Streptococcus pneumoniae* conjugated to one or more carrier proteins.

In some embodiments, the targeted infectious agent is a fungus, for example, but not limited to one or more fungus selected from an *Aspergillus* species, *Candida* species, *Candida albicans*, *Candida tropicalis*, *Cryptococcus* species, *Cryptococcus neoformans*, *Entamoeba histolytica*, *Histoplasma capsulatum*, *Leishmania* species, *Nocardia asteroides*, *Plasmodium falciparum*, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Toxoplasma* species, *Trypanosoma brucei*, *Schistosoma mansoni*, *Fusarium* species, and/or *Trichophyton* species. Such fungi may be a whole cell (e.g., live, attenuated or inactivated) or a polypeptide or polysaccharide of such a fungus.

In some embodiments, the targeted infectious agent is one or more parasites selected from Plasmodium species, Toxoplasma species, Entamoeba species, Babesia species, Trypanosoma species, Leshmania species, Pneumocystis species, Trichomonas species, Giardia species, and/or Schistosoma species. Such parasite antigens may be a whole cell (e.g., live, attenuated, or inactivated) or a polypeptide or polysaccharide of such a parasite.

In some embodiments, the antigenic agent is encoded by a nucleic acid. For example, in some embodiments, the antigenic agent is encoded by a nucleic acid is selected from DNA, RNA, mRNA, etc.

In some embodiments, the antigen is a toxoid. In some embodiments, the toxoid is diphtheria toxoid or tetanus toxoid or toxoids from C. Difficile.

In particular embodiments, the targeted antigen is derived from: the Ebola virus, for example, the envelope glycoprotein of Ebola virus Zaire strain (e.g., UniProtKB - P87671 (VGP_EBOEC)), the matrix protein VP40 of Ebola virus Zaire strain (e.g., UniProtKB - Q05128 (VP40_EBOZM)), or the matrix protein of Ebola virus Sudan strain (e.g., UniProtKB - Q7T9D9 (VGP_EBOSU)); the Lassa virus, for example, protein Z (e.g., UniProtKB - O73557 (Z_LASSJ)); the Zika virus, for example, non-structural protein 1 (NSP-1); the Marburg virus, for example, the Marburg virus glycoprotein (GenBank accession number AFV31202.1), the Marburg VP40 matrix protein (GenBank accession number JX458834); the *Plasmodium sp.* parasite, for example *Plasmodium falciparum*, for example, circumsporozoite protein (CSP), the Male gametocyte surface protein P230p (Pfs230 antigen), sporozoite micronemal protein essential for cell traversal (SPECT2), or GTP-binding protein, putative antigen (GenBank accession number PF3D7_1462300); the human immunodeficiency virus, for example an Env protein, for example gp41, gp120, gp160, a Gag protein, MA, CA, SP1, NC, SP2, P6, or a Pol protein RT, RNase H, IN, PR.

In an alternative embodiment, the rMVA viral construct is administered to a subject in need thereof, for example a human, in a treatment modality incorporating a vaccination protocol, for example, to treat a cancer. Accordingly, the rMVA viral construct can be administered in concert with one or more antigens intended to induce an immune response against an antigenic target in order to induce partial or complete immunization in a subject in need thereof.

Antigens used for cancer immunotherapy are generally intentionally selected based on either uniqueness to tumor cells, greater expression in tumor cells as compared to normal cells, or ability of normal cells with antigen expression to be adversely affected without significant compromise to normal cells or tissue. Tumor-associated antigens (TAA) can be loosely categorized as oncofetal (typically only expressed in fetal tissues and in cancerous somatic cells), oncoviral (encoded by tumorigenic transforming viruses), overexpressed/accumulated (expressed by both normal and neoplastic tissue, with the level of expression highly elevated in neoplasia), cancer-testis (expressed only by cancer cells and adult reproductive tissues such as testis and placenta), lineage-restricted (expressed largely by a single cancer histotype), mutated (only expressed by cancer as a result of genetic mutation or alteration in transcription), post-translationally altered (tumor-associated alterations in glycosylation, etc.), or idiotypic (highly polymorphic genes where a tumor cell expresses a specific “clonotype”, i.e., as in B cell, T cell lymphoma/leukemia resulting from clonal aberrancies). Although they are preferentially expressed by tumor cells, TAAs are oftentimes found in normal tissues. However, their expression differs from that of normal tissues by their degree of expression in the tumor, alterations in their protein structure in comparison with their normal counterparts or by their aberrant subcellular localization within malignant or tumor cells.

Examples of oncofetal tumor associated antigens include Carcinoembryonic antigen (CEA), immature laminin receptor, and tumor-associated glycoprotein (TAG) 72. Examples of overexpressed/accumulated include BING-4, calcium-activated chloride channel (CLCA) 2, Cyclin A₁, Cyclin B₁, 9D7, epithelial cell adhesion molecule (Ep-Cam), EphA3, Her2/neu, telomerase, mesothelin, orphan tyrosine kinase receptor (ROR1), stomach cancer-associated protein tyrosine phosphatase 1 (SAP-1), and survivin.

Examples of cancer-testis antigens include the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2. Examples of lineage restricted tumor antigens include melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen.

Examples of mutated tumor antigens include β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, and TGF- β RII. An example of a post-translationally altered tumor antigen is mucin (MUC) 1. Examples of idiotypic tumor antigens include immunoglobulin (Ig) and T cell receptor (TCR).

5 In some embodiments, the antigen associated with the disease or disorder is selected from the group consisting of CD19, CD20, CD22, hepatitis B surface antigen, anti-folate receptor, CD23, CD24, CD30, CD33, CD38, CD44, EGFR, EGP-2, EGP-4, 0EPHa2, ErbB2, 3, or 4, FBP, fetal acetylcholine receptor, HMW-MAA, IL-22R-alpha, IL-13R-alpha, kdr, kappa light chain, Lewis Y, MUC16 (CA-125), PSCA, NKG2D Ligands, oncofetal antigen, VEGF-R2, PSMA, 10 estrogen receptor, progesterone receptor, ephrinB2, CD123, CS-1, c-Met and/or biotinylated molecules, and/or molecules expressed by HIV, HCV, HBV or other pathogens.

Exemplary tumor antigens include at least the following: carcinoembryonic antigen (CEA) for bowel cancers; CA-125 for ovarian cancer; MUC1 or epithelial tumor antigen (ETA) or CA15-3 for breast cancer; tyrosinase or melanoma-associated antigen (MAGE) for malignant melanoma; 15 and abnormal products of ras, p53 for a variety of types of tumors; alphafetoprotein for hepatoma, ovarian, or testicular cancer; beta subunit of hCG for men with testicular cancer; prostate specific antigen for prostate cancer; beta 2 microglobulin for multiple myeloma and in some lymphomas; CA19-9 for colorectal, bile duct, and pancreatic cancer; chromogranin A for lung and prostate cancer; TA90 for melanoma, soft tissue sarcomas, and breast, colon, and lung cancer. Examples 20 of TAAs are known in the art, for example in N. Vigneron, "Human Tumor Antigens and Cancer Immunotherapy," BioMed Research International, vol. 2015, Article ID 948501, 17 pages, 2015. doi:10.1155/2015/948501; Ilyas et al., J Immunol. (2015) Dec 1; 195(11): 5117–5122; Coulie et al., Nature Reviews Cancer (2014) volume 14, pages 135–146; Cheever et al., Clin Cancer Res. (2009) Sep 1;15(17):5323-37, which are incorporated by reference herein in its entirety.

25 Examples of oncoviral TAAs include human papilloma virus (HPV) L1, E6 and E7, Epstein-Barr Virus (EBV) Epstein-Barr nuclear antigen (EBNA) 1 and 2, EBV viral capsid antigen (VCA) IgM or IgG, EBV early antigen (EA), latent membrane protein (LMP) 1 and 2, hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), hepatitis B core antigen (HBcAg), hepatitis B x antigen (HBxAg), hepatitis C core antigen (HCV core Ag), Human T- 30 Lymphotropic Virus Type 1 core antigen (HTLV-1 core antigen), HTLV-1 Tax antigen, HTLV-1

Group specific (Gag) antigens, HTLV-1 envelope (Env), HTLV-1 protease antigens (Pro), HTLV-1 Tof, HTLV-1 Rof, HTLV-1 polymerase (Pro) antigen, Human T-Lymphotropic Virus Type 2 core antigen (HTLV-2 core antigen), HTLV-2 Tax antigen, HTLV-2 Group specific (Gag) antigens, HTLV-2 envelope (Env), HTLV-2 protease antigens (Pro), HTLV-2 Tof, HTLV-2 Rof,
 5 HTLV-2 polymerase (Pro) antigen, latency-associated nuclear antigen (LANA), human herpesvirus-8 (HHV-8) K8.1, Merkel cell polyomavirus large T antigen (LTag), and Merkel cell polyomavirus small T antigen (sTAg).

Elevated expression of certain types of glycolipids, for example gangliosides, is associated with the promotion of tumor survival in certain types of cancers. Examples of gangliosides
 10 include, for example, GM1b, GD1c, GM3, GM2, GM1a, GD1a, GT1a, GD3, GD2, GD1b, GT1b, GQ1b, GT3, GT2, GT1c, GQ1c, and GP1c. Examples of ganglioside derivatives include, for example, 9-O-Ac-GD3, 9-O-Ac-GD2, 5-N-de-GM3, N-glycolyl GM3, NeuGcGM3, and fucosyl-GM1. Exemplary gangliosides that are often present in higher levels in tumors, for example melanoma, small-cell lung cancer, sarcoma, and neuroblastoma, include GD3, GM2, and GD2.

15 In addition to the TAAs described above, another class of TAAs is tumor-specific neoantigens, which arise via mutations that alter amino acid coding sequences (non-synonymous somatic mutations). Some of these mutated peptides can be expressed, processed and presented on the cell surface, and subsequently recognized by T cells. Because normal tissues do not possess these somatic mutations, neoantigen-specific T cells are not subject to central and peripheral
 20 tolerance, and also lack the ability to induce normal tissue destruction. See, e.g., Lu & Robins, Cancer Immunotherapy Targeting Neoantigens, Seminars in Immunology, Volume 28, Issue 1, February 2016, Pages 22-27, incorporated herein by reference.

In some embodiments, the TAA is specific to an oncofetal TAA selected from a group consisting of Carcinoembryonic antigen (CEA), immature laminin receptor, orphan tyrosine
 25 kinase receptor (ROR1), and tumor-associated glycoprotein (TAG) 72.

In some embodiments, a TAA is specific to an oncoviral TAA selected from a group consisting of human papilloma virus (HPV) E6 and E7, Epstein-Barr Virus (EBV) Epstein-Barr nuclear antigen (EBNA) 1 and 2, latent membrane protein (LMP) 1, and LMP2.

In some embodiments, the TAA is specific to an overexpressed/accumulated TAA selected
 30 from a group consisting of BING-4, calcium-activated chloride channel (CLCA) 2, CyclinA1,

Cyclin B1, 9D7, epithelial cell adhesion molecule (Ep-Cam), EphA3, Her2/neu, L1 cell adhesion molecule (L1-Cam), telomerase, mesothelin, stomach cancer-associated protein tyrosine phosphatase 1 (SAP-1), and survivin.

In some embodiments, the TAA is specific to a cancer-testis antigen selected from the group consisting of the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, cutaneous T cell lymphoma associated antigen family (cTAGE), Interleukin-13 receptor subunit alpha-1 (IL13RA), CT9, Putative tumor antigen NA88-A, leucine zipper protein 4 (LUZP4), NY-ESO-1, L antigen (LAGE) 1, helicase antigen (HAGE), lipase I (LIPI), Melanoma antigen preferentially expressed in tumors (PRAME), synovial sarcoma X (SSX) family, sperm protein associated with the nucleus on the chromosome X (SPANX) family, cancer/testis antigen 2 (CTAG2), calcium-binding tyrosine phosphorylation-regulated fibrous sheath protein (CABYR), acrosin binding protein (ACRBP), centrosomal protein 55 (CEP55) and Synaptonemal Complex Protein 1 (SYCP1).

In some embodiments, the TAA is specific to a lineage restricted tumor antigen selected from the group consisting of melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosinase, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen.

In some embodiments, the TAA is specific to a mutated TAA selected from a group consisting of β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, MART-2, p53, Ras, TGF- β RII, and truncated epithelial growth factor (tEGFR).

In some embodiments, the TAA is specific to the post-translationally altered TAA mucin (MUC) 1.

In some embodiments, the TAA is specific to an idiotypic TAA selected from a group consisting of immunoglobulin (Ig) and T cell receptor (TCR).

In some embodiments, the TAA is specific to BCMA. In some embodiments, at least one T-cell subpopulation is specific to BCMA.

In some embodiments, the TAA is specific to CS1.

In some embodiments, the TAA is specific to XBP-1

In some embodiments, the TAA is specific to CD138.

In some embodiments, the TAA is specific to WT1, PRAME, Survivin, NY-ESO-1, MAGE-A3, MAGE-A4, Pr3, Cyclin A1, SSX2, Neutrophil Elastase (NE), HPV E6, HPV E7, EBV LMP1, EBV LMP2, EBV EBNA1, or EBV EBNA2.

5 In addition to the TAAs described above, another class of TAAs is tumor-specific neoantigens, which arise via mutations that alter amino acid coding sequences (non-synonymous somatic mutations). Some of these mutated peptides can be expressed, processed and presented on the cell surface, and subsequently recognized by T cells. Because normal tissues do not possess these somatic mutations, neoantigen-specific T cells are not subject to central and peripheral
10 tolerance, and also lack the ability to induce normal tissue destruction. See, e.g., Lu & Robins, Cancer Immunotherapy Targeting Neoantigens, Seminars in Immunology, Volume 28, Issue 1, February 2016, Pages 22-27, incorporated herein by reference.

In specific embodiments, the TAA is derived from Mucin 1 (MUC1)(UniProtKB - P15941 (MUC1_HUMAN)). In some embodiments, the TAA is derived from Cyclin B1 (UniProtKB -
15 P14635 (CCNB1_HUMAN)).

rMVA Viral Vectors

As provided herein is an rMVA viral vector comprising a heterologous nucleic acid insert encoding an immune checkpoint inhibitor capable of being secreted from the cell.

20 In some embodiments, the rMVA viral vector comprises a heterologous nucleic acid insert encoding a polypeptide wherein the polypeptide comprises (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor)_x, wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein M = methionine.

In some embodiments, the rMVA viral vector comprises a heterologous polycistronic
25 nucleic acid insert encoding a polypeptide wherein the polypeptide comprises a tandem repeat sequence (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x, wherein $x = 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine (see, e.g., FIGs. 1A-1B).

In some embodiments, provided herein is an rMVA viral vector comprising a heterologous
30 polycistronic nucleic acid insert encoding one or more polypeptides in a tandem repeat sequence

and an additional polypeptide fused to the C-terminus of the last polypeptide in the tandem repeat sequence ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine (see, e.g., FIGs. 2A-2B). In particular embodiments, the encoded polypeptide comprises (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x, wherein $x = 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, or in an alternative embodiment ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein M = methionine, and wherein the Secretion Signal Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 57-90, the Immune Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 1-56, and the Cleavable Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 91-127. In some embodiments, the Secretion Signal Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 65 and 66, the Immune Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 1 and 5, and the Cleavable Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 93-97, 120, and 123-127.

In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1.

In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 2-10$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of

SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 4, 5, \text{ or } 6$.

In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5.

In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 2-10$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 4, 5, \text{ or } 6$.

In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid of Table 8 below, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NOS: 309-340 or SEQ ID NOS: 341-348, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 309, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 310, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 3110, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or

99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 312, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 313, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 314, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 315, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 316, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 317, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 318, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 319, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 320, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 321, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical

thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 322, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 323, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 324, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 325, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 326, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 327, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 328, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 329, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 330, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 331, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical

thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 332, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 333, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 334, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 335, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 336, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 337, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 338, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 339, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 340, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 341, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical

thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 342, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 343, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 344, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 345, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 346, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 347, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an amino acid selected from the amino acid sequences of SEQ ID NO: 348, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

Table 8 - rMVA Viral Vectors

SEQ ID NO:	Sequence Description	Encoded Polypeptide
309	(M)(tPA + LD01 + RAKR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
310	(M)(tPA + LD01 + RRRR cleavable	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSGATNFSLLKQAGDVEENPGP)x,

	sequence + 2A/2A-like cleavage sequence)x	wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
311	(M)(tPA + LD01 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRRTSTGQISTL RVNITAPLSQRKRKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
312	(M)(tPA + LD01 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRRTSTGQISTL RVNITAPLSQRKRKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
313	(M)(tPA + LD10 + RAKR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTL RVNIT APLSQRAKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
314	(M)(tPA + LD10 + RRRR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTL RVNIT APLSQRRRRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
315	(M)(tPA + LD10 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTL RVNIT APLSQRKRKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
316	(M)(tPA + LD10 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTL RVNIT APLSQRKRKRGSGATNFSLLKQAGDVEENPGP)x, wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
317	(M)(tPA + LD01 + RAKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD01)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRRTSTGQISTL RVNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGP)x(DAMKRGLCCV LLLCGAVFVSPSQEIHFRRGARCRRRTSTGQISTL RVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

318	(M)(tPA + LD01 + RRRR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD01)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQRRRRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
319	(M)(tPA + LD01 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD01)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQRKRKRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
320	(M)(tPA + LD01 + RRKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD01)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQRRKRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
321	(M)(tPA + LD10 + RAKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD10)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
322	(M)(tPA + LD10 + RRRR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD10)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQRRRRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
323	(M)(tPA + LD10 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD10)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQRKRKRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
324	(M)(tPA + LD10 + RRKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD10)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQRKRKRGSGATNFSLLKQAGDVEENPGP) _x (DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

	sequence)x(tPA + LD10)	
325	(M)(tPA + LD01 + RAKR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRAKRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR GARCRRSTSTGQISTLRVNITAPLSQRAKRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLR VNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC CGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRAKRGSG ATNFSLLKQAGDVEENPGP
326	(M)(tPA + LD01 + RRRR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR GARCRRSTSTGQISTLRVNITAPLSQRRRRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLR VNITAPLSQRRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC CGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSG ATNFSLLKQAGDVEENPGP
327	(M)(tPA + LD01 + RKRR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRKRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRKRRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR GARCRRSTSTGQISTLRVNITAPLSQRKRRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLR VNITAPLSQRKRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC CGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRKRRGSG ATNFSLLKQAGDVEENPGP
328	(M)(tPA + LD01 + RRKR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRRKRSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRRKRSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR GARCRRSTSTGQISTLRVNITAPLSQRRKRSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLR VNITAPLSQRRKRSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC CGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRRKRSG ATNFSLLKQAGDVEENPGP
329	(M)(tPA + LD01 + RAKR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD01)	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQRAKRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRR GARCRRSTSTGQISTLRVNITAPLSQRAKRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLR VNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC CGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRVNITAPLSQ
330	(M)(tPA + LD01 + RRRR cleavable	MDAMKRGLCCVLLLCGAVFVSPSQEIHARFRRGARCRRSTSTGQISTLRV NITAPLSQRRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC

	sequence + 2A/2A-like cleavage sequence)4(tPA + LD01)	GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRR GARCRRSTSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLR VNITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLL CGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQ
331	(M)(tPA + LD01 + RRKR cleavable sequence + 2A/2A-like cleavage sequence)4(tPA + LD01)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRV NITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRR GARCRRSTSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLR VNITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLL CGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQ
332	(M)(tPA + LD01 + RRKR cleavable sequence + 2A/2A-like cleavage sequence)4(tPA + LD01)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRV NITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRRRRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRR GARCRRSTSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLR VNITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLL CGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQ
333	(M)(tPA + LD10 + RAKR cleavable sequence + 2A/2A-like cleavage sequence)5	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRAKRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRAKRGSGATNFSLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRAKRGSGATNFSLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRAKRGSG ATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQRAKRGSGATNFSLKQAGDVEENPGP
334	(M)(tPA + LD10 + RRRR cleavable sequence + 2A/2A-like cleavage sequence)5	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRGSG ATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENPGP
335	(M)(tPA + LD10 + RKRR cleavable sequence + 2A/2A-like cleavage sequence)5	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRGSG ATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQRRRRGSGATNFSLKQAGDVEENPGP
336	(M)(tPA + LD10 + RRKR cleavable	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRRRRGSGATNFSLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF

	sequence + 2A/2A-like cleavage sequence) ₅	VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRKRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRRKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRKRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQRRKRGSGATNFSLLKQAGDVEENPGP
337	(M)(tPA + LD10 + RAKR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD10)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRAKRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRAKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRAKRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQ
338	(M)(tPA + LD10 + RRRR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD10)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRRRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRRRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRRRRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQ
339	(M)(tPA + LD10 + RKRR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD10)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRKRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKRRRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRKRRRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKRRRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQ
340	(M)(tPA + LD10 + RRKR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD10)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRRKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRKRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRRKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRRKRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQ
341	(M)(tPA + LD01 + RKKR cleavable sequence + 2A/2A-like cleavage sequence) _x	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRTSTGQISTL RVNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGP) _x , wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
342	(M)(tPA + LD10 + RKKR cleavable sequence + 2A/2A-like	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNIT APLSQRKKRGSGATNFSLLKQAGDVEENPGP) _x , wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.

	cleavage sequence)x	
343	(M)(tPA + LD01 + RKKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD01)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTL RVNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGP)x(DAMKRGLCCV LLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
344	(M)(tPA + LD10 + RKKR cleavable sequence + 2A/2A-like cleavage sequence)x(tPA + LD10)	(M)(DAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNIT APLSQRKKRGSGATNFSLLKQAGDVEENPGP)x(DAMKRGLCCVLLLCG AVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQ), wherein x=2, 3, 4, 5, 6, 7, 8, 9, 10, or more.
345	(M)(tPA + LD01 + RKKR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRV NITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRKKRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRR GARCRRSTSTGQISTLRVNITAPLSQRKKRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLR VNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRKKRGSG ATNFSLLKQAGDVEENPGP
346	(M)(tPA + LD10 + RKKR cleavable sequence + 2A/2A-like cleavage sequence) ₅	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKKRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKKRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGP
347	(M)(tPA + LD01 + RKKR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD01)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLRV NITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQRKKRGSGA TNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRR GARCRRSTSTGQISTLRVNITAPLSQRKKRGSGATNFSLLKQAGDVEENP GPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARCRRSTSTGQISTLR VNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLC GAVFVSPSQEIHFRRGARCRRSTSTGQISTLRVNITAPLSQ
348	(M)(tPA + LD10 + RKKR cleavable sequence + 2A/2A-like cleavage sequence) ₄ (tPA + LD10)	MDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITA PLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVF VSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKKRGSGATNFSLLKQ AGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHFRRGARSTGQI STLRVNITAPLSQRKKRGSGATNFSLLKQAGDVEENPGPDAMKRGLCC VLLLCGAVFVSPSQEIHFRRGARSTGQISTLRVNITAPLSQRKKRGSG ATNFSLLKQAGDVEENPGPDAMKRGLCCVLLLCGAVFVSPSQEIHF RRGARSTGQISTLRVNITAPLSQ

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As provided herein, the polycistronic nucleic acid insert encoding the immune checkpoint inhibitor polypeptide as described herein can be inserted into the MVA genome at any suitable location, for example, a natural deletion site, a modified natural deletion site, in a non-essential MVA gene, for example the MVA thymidine kinase locus, or in an intergenic region between essential or non-essential MVA genes. Suitable insertion sites have been described, for example, in U.S. Pat. No. 6,998,252, U.S. Pat. No. 9,133,478, Ober et al., Immunogenicity and safety of defective vaccinia virus lister: comparison with modified vaccinia virus Ankara. J. Virol., Aug. 2002 (pg. 7713-7723), U.S. Pat. No. 9,133,480, U.S. Pat. No. 8,288,125, each of which is incorporated herein by reference.

In some embodiments, the polycistronic nucleic acid insert encoding the immune checkpoint inhibitor polypeptide as described herein is inserted into a natural deletion site, for example a deletion site selected from the natural deletion sites I, II, III, IV, V or VI, a modified natural deletion site, for example the restructured and modified deletion III site between the MVA genes A50R and B1R (see, e.g., U.S. 9,133,480), between non-essential MVA genes, between essential MVA genes, for example I8R and G1L or A5R and A6L or other suitable insertion site, in a non-essential locus, for example in the MVA TK locus, or a combination thereof.

In alternative embodiments, the rMVA viral vectors of the present invention, in addition to the ability to express multiple immune checkpoint inhibitor peptides, may further be constructed to encode and express one or more antigen peptides. The one or more antigenic peptides can be encoded on one or more separate nucleic acid inserts, or in an alternative embodiment, the one or more antigenic peptides are encoded on the same polycistronic nucleic acid insert as the multiple immune checkpoint inhibitor peptides.

In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Antigenic Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine. In some embodiments, the antigenic peptide is contained in a chimeric polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable

Peptide)_x(Secretion Signal Peptide-Antigenic Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine (see, e.g., FIGs. 4A-4B). In some embodiments, the antigenic peptide is also provided so that 2 or more antigenic peptides are encoded in the polycistronic nucleic acid insert, with each chimeric polypeptide separated by a cleavable peptide described herein. In some embodiments, the antigenic peptide is contained in a chimeric polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the antigenic peptide, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_y), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine. In some embodiments, the antigen containing chimeric polypeptide fused to the C-terminus of the last antigen containing chimeric polypeptide does not include a cleavable sequence, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine. In some embodiments, the antigenic peptide contained in the chimeric polypeptide comprising a secretion signal peptide fused to the N-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the antigenic peptide can be oriented in the polycistronic nucleic acid insert so that the antigen containing chimeric polypeptide encoding nucleic acid is located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example ((M)(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x) or, alternatively ((M)(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, and wherein M = methionine.

In some embodiments, the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated

antigen, or an antigen derived from an agent described in the section titled Antigenic Targets above, which is expressly incorporatd into this section.

In some embodiments, the polycistronic nucleic acid insert encodes a polypeptide comprising an antigenic amino acid of Table 9 below, or polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto. In some embodiments, the polycistronic nucleic acid insert encodes a antigen comprising an amino acid derived from an amino acid sequence selected from SEQ ID NOS: 349-396, 398, 400, 402, or 405, or a fragment thereof, or a polypeptide having an amino acid sequence at least 85%, 90%, 95%, 97%, or 99% identical thereto.

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Table 9 - Antigenic Peptides

SEQ ID NO:	Antigen	Amino Acid Sequence
349	Human Mucin 1	TPGTQSPFFLLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEK NAVSMTSSVLSSHSPGSGSSTTQGQDVTLPATEPASGSAATWGQDVT SVPVTRPALGSTTPPAHDVTSAPDNKPAPGSTAPPAHGVTSAPDTRPAP GSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGV VTSAPDTRPAPGSTAPPAHGVTSAPDNRPALGSTAPPVHNVTASGSAS GSASTLVHNGTSARATTTASKSTPFSIPSHSDTPTTLASHSTKTDASS THHSTVPPLTSSNHSTSPQLSTGVSFLLSFHISNLQFNSSLEDPSTDYYQ ELQRDISEMFLQIYKQGGFLGLSNIKFRPGSVVVQLTLAFREGTINVHD VETQFNQYKTEAASRYNLTISDVSVDVPFPFSAQSGAGV
350	Cyclin B1	LPGMALRVTRNSKINAENKAKINMAGAKRVPTAPAATSKPGLRPRTA LGDIGNKVSEQLQAKMPMKKEAKPSATGKVIDKKLPKPLEKVPMLVP VPVSEPVPPEPEPEPEPEPVKEEKLSEPEILVDTASPSMETSGCAPAEEDL CQAFSDVILAVNDVDAEDGADPNLCSEYVKDIYAYLRQLEEEQAVRP KYLLGREVTGNMRAILIDWL VQVQMKFRLLQETMYMTVSIIDRFMQN NCVPPKKMLQLVGV TAMFIASKYEEMYPFEIGDAFVTDNTYTKHQIRQ MEMKILRALNFG LGRPLPLHFLRRASKIGEVDVEQHTLAKYLMELTML DYDMVHFPPSQIAAGAFCLALKILDNGEWTPTLQHYLSYTEESLLPVM QHLAKNVVMVNQGLTKHMTVKNKYATSKHAKISTLPQLNSALVQDL AKAVAKV
351	HBV PresS2	QWNSTTFHQTLQDPRVRGLYFPAGGSSSGAVNPVPTTASPLSSIFSRI DPALNMENITSGFLGPLLVLQAGFLLTRIL TIPQSLDSWWTSNLFLGG TTVCLGQNSQSPTSNSPTS CPPTCPGYRWMCLRRFIIFLLCLIFLL VLLDYQGMLPVCPLIPGSSTTSTGPCRTCMTTAQGTSMYPSCCCTKPS DGNCTCIPIPSSWAFGKFLWEWASARFSWLSLLVPFVQWFVGLSPTVW LSVIWMMWYWGPSLYSILSPFLPLPIFFCLWVYI
352	HBV PreCore/Core	HLFHLCLIISSCPTVQASKLCLGWLWGMDIDPYKEFGATVELLSFLPS DFFPSVRDLLDTASALYREALESPEHCSPHHTALRQAILCWGELMTLA TWVGVNLEDPASRDLVVS YVNTNMGLKFRQLLWFHISCLTFGRETVIE YLVSGVWIRTPPAYRPPNAPILSTLPETT VVRRRGRSPRRRTPSPRRR SOSPRRRRSOSREPOC

353	HBV Truncated X Gene Product	AARLCCQLDPARDVLCRLPVGAESCGRPFSGSLETLSPPSPSAVPTDHG AHLRLRGLPAMSTTDLEAYFKDCLFKDWEELGEEIRLKVFVLGGCRHK LVCAPAPCTFFTSA
354	HBV PreS ₁ HA (chimeric fusion including the signal peptide of influenza HA, preS ₁ and the transmembrane/cytoplasmic domains of influenza HA)	EAKLFLVLCFAFTALKAMGTNLSVPNPLGFFPDHQLDPAFGANSNNPD WDFNPIKDHWPAAANQVGVGAFGPGLTPPHGGILGWSPQAQGILTTVST IPPPASTNRQSGRQPTPISPLRDSHPQAMQWNSTAFHQALQDPRVRGL YLPAGGSSSGTVNPAPNIAISHISSISARTGDPVTNKLESVGVHQILAIYS TVASSLVLLVSLGAISFWMCNGLSLQCRICI
355	Plasmodium sp. CSP	ARPGMMRKLAILSVSSFLFVEALFQEYQCYGSSSNTRVLNELNYDNAG TNLYNELEMNYYGKQENWYSLKKNSRSLGENDDGNNEDNEKLKPKK HKLLKQPADGNPDPPGGSNKNNQGNQGHNMPNDPNRNVNENANA NSAVKNNNNNEEPSDKHIKEYLNKIQNSLSTEWSPCSVTGNGIQVRIKP GSANKPKDEL DYANDIEKKICKMEKCSSVFNVVNS
356	Plasmodium sp. CSP CSP21R (21 Repeats)	PIPGMMRKLAILSVSSFLFVEALFQEYQCYGSSSNTRVLNELNYDNAGT NLNELEMNYYGKQENWYSLKKNSRSLGENDDGNNEDNEKLKPKKH KKLLKQPADGNPDPPNANPNVDPNANPNVDPNANPNVDPNANPNANPN ANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPNANPN PNANPNANPNANPNANPNVDPNANPNKNNQGNQGHNMPNDPNRNVNEN ANANSAVKNNNNNEEPSDKHIKEYLNKIQNSLSTEWSPCSVTGNGIQV RIKPGSANKPKDEL DYANDIEKKICKMEKCSSVFNVVNS
357	Plasmodium sp. Pf5230	PGATMSVLQSGALPSVGVDELDKIDLSYETTESGDTAVSEDSYDKYAS NNTNKEYVCDFTDQLKPTESGPKVKKCEVKVNEPLIKVKIICPLKGSVE KLYDNIEYVPKKSPPYVVLTKETTKLKEKLLSKLIYGLLISPTVNEKENN FKEGVIEFTLPPVVHKATVFYFICDNSKTEDDNKKGNRGIVEVYVEPY GNKING
358	Human Mucin-I extracellular domain fragment	AHGVTSAPDTRPAPGSTAPP
359	Human Mucin-I extracellular domain fragment	AHGVTSAPDNRPALGSTAPP
360	Human Mucin-I extracellular domain fragment	AHGVTSAPDTRPAPGSTAPPAHGVTSAPDNRPALGSTAPP
361	Human Mucin-I extracellular domain fragment	AHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAP DTRPAPGSTAPPAHGVTSAPDTRPAPGSTAPPAHGVTSAPDNRPALGST APP
362	Human Mucin-I intracellular domain fragment	RRKNYGQLDIFPARDTYHPMSEYPTYHTHGRYVPPSSSTRSPYEKVSA GNGGSSLSYTNPAVAATSANL
363	Human Mucin-I 1 tandem repeat	TPGTQSPFFLLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEK NAVSMSTSSVLSSHSPGSGSSTTQGQDVT LAPATEPASGSAATWGQDVT SVPVTRPALGSTTPPAHDVTSAPDNKPAPGSTAPPIAHGVTSAPDTRPA

		PGSTAPAAHGVT SAPDNRPALGSTAPPVHNVTASGSASGSASTLVHN GTSARATTTTPASKSTPFSSIPSHHSDTPTTLASHSTKTDASSTHHSTVPPLT SSNHSTSPQLSTGVSSFFLSFHISNLQFNSSLEDPSDYYQELQRDISEMF LQIYKQGGFLGLSNIKFRPGSVVVQLTLAFREGTINVHDVETQFNQYKT EAASRYNLTISDVSVDVPPFSAQSGAGVPGWGIALLVLCVLVALAI VYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEPTYHTHGRYVPPSS TDRSPYEKVSAGNGGSSLS YTNPAVAATSANL
364	Human Mucin-I 4 tandem repeat	TPGTQSPFFLLLLLTVLTVVTGSGHASSTPGGEKETSATQRSSVPSSTEK NAVSMSTSSVLSSHSPSGSGSSTTQGQDVT LAPATEPASGSAATWGQDVT SVPVTRPALGSTTPPAHDVTSAPDNKPAPGSTAPPAHGVT SAPDTRPAP GSTPPAHGVT SAPDTRPAPGSTAPPAHGVT SAPDTRPAPGSTAPPAHGVT TSAPDTRPAPGSTAPPHGVT SAPDNRPALGSTAPPVHNVTASGSASGS ASTLVHNGTSARATTTTPASKSTPFSSIPSHHSDTPTTLASHSTKTDASSTH HSTVPPLTSSNHSTSPQLSTGVSSFFLSFHISNLQFNSSLEDPSDYYQEL QRDISEMF LQIYKQGGFLGLSNIKFRPGSVVVQLTLAFREGTINVHDVE TQFNQYKTEAASRYNLTISDVSVDVPPFSAQSGAGVPGWGIALLVLCVL VALAIVYLIALAVCQCRRKNYGQLDIFPARDTYHPMSEPTYHT HGRYVPPSS TDRSPYEKVSAGNGGSSLS YTNPAVAATSANL
365	Lassa virus Glycoprotein	GQIVTFFQE VPHVIEEVMNIVLIALSVLAVLKGLYNFATCGLVGLVTFL LLCGRSCTTSLYKGVYELQTELELNMETLNMTPLSCTKNNSHHYIMV GNETGLELTLTNTSIINHFKCNLSDAHKKNLVDHALMSIISTFHLSIPNF NQYEAMSCDFNGGKISVQYNLSHSYAGDAANHC GTVANGVLQTFMR MAWGGSYIALDSGRGNWDCIMTSYQYLIHQNTT WEDHCQFSRPSPIGY LGLLSQRTRDIYISRRL LGTFTWTLSDSEGKDPGGYCLTRWMLIEAEL KCFGNTAVAKCNEKHDEEFC DMLRLDFDNKQAIQRLKAE AQMSIQLIN KAVNALINDQLIMKNHLRDIMGIPYCNYSKYWYLNHTTTGRTSLPKC WLVSNGSYLNETHFSDDIEQQADNMITEMLQKEYMERQGKTPLGLVD LFVFSTSFYLISIFLHLVKIPTHRHI V GKSCP KPHRLNHMGICSCGLYKQP GVPVKWKR
366	Lassa virus Z protein	GNKQAKAPESKDS PRASLIPDATHLGPQFCKSCWFENKGLVECNHHL CLNCLTLLLSVSNRC PICKMPLPTKL RPSAAPTAPPTGAADSIRPPPYSP
367	Ebola virus Glycoprotein	GVTGILQLPRDRFKRTSFFLWVILFQRTFSIPLGVIHNSTLQVSDVDKL VCRDKLSSTNQLR SVGLNLEGNGVATDVPSVTKRWGFRSGVPPKVVN YEAGEWAENCYNLEIKKPDGSECLPAAPDGIRGFPCRYVHKVSGTGP CAGDFAFHKEGAFFLYDRLASTVIYRGTTFAEGVVAFLILPQAKKDDFFS SHPLREPVNATEDPSSGYSTTIRYQATGFGTNETEYLF EVDNLTYVQL ESRFTPOFLLQLNETIYASGKRSNTTGKLIWKVNPEIDTTIGEWAFWET KKNLTRKIRSEELSFTAVSNGPKNISGQSPARTSSDPETNTNEDHKIM ASENSSAMVQVHSQGRKAAVSHLTTLATISTSPQPPTTKTGPDNSTHN TPVYKLDISEATQVGQHHRRADNDSTASDTPPATTAAGPLKAENTNTS KSADSLDLATTTSPQNYSETAGNNNTHHQDTGEESASSGKLGLITNTIA GVAGLITGGRRTRE VIVNAQPKCNP NLHYWTTQDEGAAIGLAWIPYF GPAAEGIYTEGLMHNQDGLICGLRQLANETTQALQLFLRATTELRTFSI LNRKAIDFLLQRWGGTCHILGPDCCIEPHDWTKNITDKIDQIIHDFVDK TLPDQGDNDNWWTGWRQWIPAGIGVTGVIIAVALFCICKFVF
368	Ebola Virus VP40 protein	RRVILPTAPPEYMEAIYPARSNSTIARGNSNTGFLTPEVNGDTPSNPL RPIADDTIDHASHTPGSVSSAFIEAMVNVISGPKVLMKQIPWLPLGVA DQKTYSFDSSTTAAIMLASYTITHFGKATNPLVRVNR L GP GIPDHPLRL RIGNQAFLQEFVLPVQLPQYFTFDLTALKLITQPLPAATWTDDTPTGS NGALRPGISFHPKL RPILLPNKSGKKGNSADLTSPEKIQAIMTSLQDFKI

		VPIDPTKNIMGIEVPETLVHKLTKKKVTSKNGQPIIPVLLPKYIGLDPVA PGDLTMVITQDCDCHSPASLPAVVEK
369	Zika virus - native polyprotein sequence for Zika, from GenBank (ALX35659)	KNPKKSSGGFRIVNMLKRGVARVSPFGGLKRLPAGLLLGHGPIRMVL AILAFLRFTAIPSLGLINRWGSGVKKKEAMEIKKFKKDLAAMLRIINAR KEKKRRGADTSVGIVGLLLTTAMAAEVTRRGSAYYMYLDRNDAGEAI SFPTTLGMNKCYYQIMDLGHTCDATMSYECPLMDEGEVPPDDVDCWCN TTSTWVVYGTCHHHKGEARRSRRAVTLPSHSTRKLQTRSQTWLESRE YTKHLIRVENWIFRNPGFALAAAIAWLLGSSTSQKVIYLVILLIAPA YSIRCIGVSNRDFVEGMSGGTWVDVLEHGGCVTVMAQDKPTVDIEL VTTTVSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVC KRTLVDRGWGNCGFLFGKGSLVTCAKFACSKKMTGKSIQENLEYRI MLSVHGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLG LDCEPRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGT PHWNNKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMD GAKGRLSSGHLKCRCLKMDKLRLKGVSYSLCTAFTFTKIPAEITLHGTV TVEVQYAGTDGPKCVPAQMAVDMQTLTPVGRILTANPVITESTENSK MMLELDPFPGDSYIVIGVGEKKITHHWHRSSTIGKAFEAIVRGAKRM AVLGDTAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIG TLLMWLGLNKNKSGISLMCLALGGVLIFLSTAVSADVGCSDVFSKKT RCGTGVFVYNDVEAWRDYKYHPDSPRRLAAAVKQAWEDGICGISS VSRMENIMWRSVEGELNAILEENGVLTVVVGSVKNPMWRGPORLP VPVNELPHGWKAWGKSYFVRAAKTNSFVVDGDTLKECPLKHHAW NSFLVEDHGFVFHTSVWLKVREDYSLECDPAVIGTAVKGKEAVHSD LGYWIESEKNDTWRLKRAHLIEMKTCEWPKSHTLWTDGIEESDLIPKS LAGPLSHHNTREGYRTQMKGPWHSEELIRFECEPGTKVHVEETCGTR GPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGCWYGMEIRPRKEPE SNLVRSMVTAGSTDHMDHFSGLVLLVMVQEGLLKRMTHKIIISTSM AVLVAMILGGFSMSDLAKLAILMGATFAEMNTGGDVAHLALIAAFKV RPALLVSFIFRANWTPRESMLLALASCLLQTAISALEGDLMLVINGFAL AWLAIRAMVVPRTDNITLAILAALTPLARGTLLVAWRAGLATCGGFM LLSLKKGKGSVKKNLPFVMAALGLTAVRLVDPINVVGLLLTRSGKRSWP PSEVLTAVGLICALAGGFAKADIEMAGPMAAVGLLIVSYVVEGKSDV MYIERAGDITWEKDAEVTGNSPRLDVALDESGDFSLVEDDGPPMREIL KVVLMTICGMNPIAIPFAAGAWYVYVKTGKRSGALWDVPAPKEVKK GETTDGVYRVMTRRLLGSTQVGVGMQEGVFHTMWHVTKGSALRS GEGRLDPYWGDVKQDLVSYCGPWKLDAAWDGHSEVQLLAVPPGER ARNIQTLPGIFKTKDGDIGAVALDYPAGTSGSPILDKCGRVIGLYGNGV VIKNGSYVSAITQGRREEETPVECFEPSMLKKKQLTVLDLHPGACKTR RVLPEIVREAIKTRLRTVILAPTRVVAEMEEALRGLPVRYMTTAVNV THSGTEIVDLMCHATFTSRLLQPIRVPNYNLYIMDEAHFTDPSSIAARG YISTRVEMGEAAAFMTATPPGTRDAFPDSNSPIMDTEVEVPERAWSSG FDWVTDHSGKTVWFVPSVRNGNEIAACLTKAGKRVQLSRKTFETEFQ KTKHQEWDFVVTDDISEMGANFKADRVIDSRRLKPVILDGERVILAG PMPVTHASAAQRRGRIGRNPKNKPGDEYLYGGGCAETDEDHAHWLEA RMLLDNIYLQDGLIASLYRPEADKVAIEGEFKLRTEQRKTFVELMKR GDLPVWLAYQVASAGITYTDRRWCFDGTNTTIMEDSVPAEYWTRH GEKRVLKPRWMDARVCSHAALKSFKEFAAGKRGAAFGVMEALGTL PGHMTERFQEAIDNLAVLMRAETGSRPYKAAAQLPETLETIMLLGLL GTVSLGIFVLMRNKGIGKMGFGMVTLGASAWLMWLSEIEPARIACV LIVVFLLLVVLPEPEKQRSPQDNQMAIIIMVAVGLLGLITANELGWLER TKSDLSHLMGRREEGATIGFSMDIDLRPASAWAIYAALTTFITPAVQHA VTTSYNNYSLMAMATQAGVLFGMGKGMFPFYAWDFGVPLLMIGCYSQ LTPLTLIVAIILLVAHYMYLIPGLQAAAARAAQKRRTAAGIMKNPVVDGI

		VVTDIDTMTIDPQVEKKMGQVLLIAVAVSSAILSRTAWGWGEAGALIT AATSTLWEGSPNKYWNSSSTATSLCNIFRGSYLAGASLIYTVTRNAGLV KRRGGGTGETLGEKWKARLNQMSALEFYSYKKS GITEVCREEARRAL KDG VATGGH AVSRGSAKLRLWVERGYLQPYGKVIDLGCGRGGWSYY AATIRK VQEVKGYTKGGPGHEEPVLVQSYGWNIVRLKSGVDVFHMA AEPD TLLCDIGESSSSPEVEEARTLRVLSMVGDWLEKRPGAFCIKVLC PYTSTM METLERLQRRYGGGLVRVPLSRNSTHEMYWVSGAKSNTIKS VSTTSQ LLLGRMDGPRRPVKY EEDVNLGSGTRAVVSCAEAPNMKIIGN RIERIRSEHAETWFFDENHPYRTWAYHGSYEAPTQGSASSLINGVVRL SKPWDVVTGVTGIAMTDTTPYGOQRVFKEKVDTRVPDPQEGTRQVM SMVSSWLWKELGKHKRPRVCTKEEFINKVRSNAALGAIFEEKEWKT AVEAVNDPRFWALVDKEREHHLRGECQSCVYNMMGKREKKQGEFG KAKGSR AIWYMWLGARFLEFEALGFLNEDHWMGRENSGGGVEGLGL QRLGYVLEEMSRIPGGRMYADDTAGWDTRISRFDLENEALITNQMEK GHRALALAIKITYQNKVVKVLRPAEKGKTVMDIISRQDQRGSGQVV TYALNTFTNLVVQLIRNMEAEEVLEMQDLWLLRSEKVTNWLQSNQ WDR LKRM AVSGDDCVVKPIDDRFAHALRFLNDMGMKVRKDTQEWKPS TGWDNWEEVPFCSHHFNKLHLKDGRSIVVPCRHQDELIGRARVSPGA GWSIRETACLAKSYAQMWQLLYFHRRDLRLMANAICSSVPVDWVPT GRTTWSIHGKGEWMTTEDMLVWVNRVWIEENDHMEDKTPVTKWTD IPYLGKREDLWCGSLIGHRPRTTWAENIKNTVNMVRRIGDEEKYMDY LSTQVRYLGEEGSTPGVL
370	Zika virus - PrM + E	TRRGSAYYMYLDRNDAGEAISFPTTLGMNKC YIQIMDLGHTCDATMS YEC PMLDEGVEPDDVDCWCNTTSTWVVYGTCHHKKGEARRSRAV LPSHSTRKLQTRSQTWLESREYTKHLIRVENWIFRNPGFALAAAAIAW LLGSSTSQKV IYLMILLIAPAYSIR CIGVSNRDFVEGMSGGTWVDVVL EHGGCVTVMAQDKPTVDIELVTTTVSNMAEVRSYCYEASISDMASDS RCPTQGEAYLDKQSDTQYVCKRTLVDRGWGNCGCLFGKGSLVTCAK FACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHETDENRAK VEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYYLT MNKNHVLVH KEWFHDIPLPWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGS QEGAVHTALAGALEAEMDGAKGRLSSGHLKCR LKMDKLRLKGVSYS LCTAAFTFTKIPAE TLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLT PVGRLITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHR SGSTIGKA FEATVRGAKRMAVLGDTAWDFGSGVGGALNSLGKGIHQIF GA AFKSLFGGMSWFSQILIGTLLMWLGLNAKNGSISLMCLALGGVLIF LSTAVSA
371	Zika virus - JEV signal	GKRSAGSIMWLASLAVVIACAGA
372	Zika virus - JEV signal + PrM + E	GKRSAGSIMWLASLAVVIACAGATRRGSAYMYLDRNDAGEAISFPT TLGMNKC YIQIMDLGHTCDATMSYEC PMLDEGVEPDDVDCWCNTTS TWVVYGTCHHKKGEARRSRAVTLPSHSTRKLQTRSQTWLESREYTK HLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKV IYLMILLIAPAYSIR CIGVSNRDFVEGMSGGTWVDVVL EHGGCVTVMAQDKPTVDIELVTTT VSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTL VDRGWGNCGCLFGKGSLVTCAKFACSKKMTGKSIQPENLEYRIMLSV HGSQHSGMIVNDTGHETDENRAKVEITPNSPRAEATLGGFGSLGLDCE PRTGLDFSDLYYLT MNKNHVLVHKEWFHDIPLPWHAGADTGTPHWN NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGR LSSGHLKCR LKMDKLRLKGVSYS LCTAAFTFTKIPAE TLHGTVTVEVQ YAGTDGPCKVPAQMAVDMQTLTPVGRLITANPVITESTENSKMMLEL DPPFGDSYIVIGVGEKKITHHWHRS GSTIGKA FEATVRGAKRMAVLGD

		TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMW LGLNAKNGSISLMCLALGGVLIFLSTAVSA
373	Zika virus - full-length Zika virus NS1 protein sequence	VGCSVDFSKKETRCGTGVFVYNDVEAWRDYKYHPDSPRRLAAAVK QAWEDGICGISSVSRMENIMWRSVEGELNAILEENGVLQTVVVGSVK NPMWRGPQRLPVPVNLPHGWKAWGKSYFVRAAKTNSNFVVDGDTL KECPLKHRAWNSFLVEDHGFVFHTSVWLKVREDYSLECDPAVIGTA VKGKEAVHSDLGWIESEKNDTWRLKRAHLIEMKTCEWPKSHTLWT DGIEESDLIIPKSLAGPLSHHNTREGYRTQMKGPDWHSEELIRFEECPGT KVHVEETCGTRGPSLRSTTASGRVIEEWCCRECTMPPLSFRAKDGWCWY GMEIRPRKEPESNLVRSMVTAG
374	Zika virus - Zika virus polyprotein JEV signal + prM + E + K643-S644	GKRSAGSIMWLASLAVVIACAGATRRGSAYMYLDRNDAGEAISFPT TLGMNKCYYIQIMDLGHTCDATMSYECPLMDEGVPEDDVDCWCNTTS TWVVYGTCHHKKGAEARRSRAVTLPSHSTRKLQTRSQTWLESREYTK HLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMLLIAPAYSIR CIGVSNRDFVEGMSGGTWVDVVI.EHGGCVTVMAQDKPTVDIEL.VTTT VSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTL VDRGWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSV HGSQHSGMIVNDTGHEHNDENRAKVEITPNSPRAEATLGGFGSLGLDCE PRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGR LSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAEHLHGTVTVEVQ YAGTDGPCKVPAQMAVDMQTLTPVGRITANPVITESTENSKMMLEL DPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFAEATVRGAKRMAVLGD TAWDFGSGVGGALNSLGKGIHQIFGAAFKSLFGGMSWFSQILIGTLLMW LGLNAKNGSISLMCLALGGVLIFLSTAVSA
375	Zika virus - gene product	GKRSAGSIMWLASLAVVIACAGATRRGSAYMYLDRNDAGEAISFPT TLGMNKCYYIQIMDLGHTCDATMSYECPLMDEGVPEDDVDCWCNTTS TWVVYGTCHHKKGAEARRSRAVTLPSHSTRKLQTRSQTWLESREYTK HLIRVENWIFRNPGFALAAAAIAWLLGSSTSQKVIYLVMLLIAPAYSIR CIGVSNRDFVEGMSGGTWVDVVI.EHGGCVTVMAQDKPTVDIEL.VTTT VSNMAEVRSYCYEASISDMASDSRCPTQGEAYLDKQSDTQYVCKRTL VDRGWGNGCGLFGKGS�VTCAKFACSKKMTGKSIQPENLEYRIMLSV HGSQHSGMIVNDTGHEHNDENRAKVEITPNSPRAEATLGGFGSLGLDCE PRTGLDFSDLYLTMNNKHWLVHKEWFHDIPLPWHAGADTGTPHWN NKEALVEFKDAHAKRQTVVVLGSQEGAVHTALAGALEAEMDGAKGR LSSGHLKCRKMDKLRKGVSYSLCTAAFTFTKIPAEHLHGTVTVEVQ YAGTDGPCKVPAQMAVDMQTLTPVGRITANPVITESTENSKMMLEL DPPFGDSYIVIGVGEKKITHHWHRSSTIGKAFAEATVRGAKRMAVLGD TAWDFGSGVGGALNSLGKGIHQIFGAAFK
376	Zika virus - prME	TRRGSAAYMYLDRNDAGEAISFPTTLGMNKCYYIQIMDLGHTCDATMS YECPLMDEGVPEDDVDCWCNTTSTWVVYGTCHHKKGAEARRSRAV LPSHSTRKLQTRSQTWLESREYTKHLIRVENWIFRNPGFALAAAAIAW LLGSSTSQKVIYLVMLLIAPAYSIRCIGVSNRDFVEGMSGGTWVDVVL EHGGCVTVMAQDKPTVDIEL.VTTTVSNMAEVRSYCYEASISDMASDS RCPTQGEAYLDKQSDTQYVCKRTL.VDRGWGNGCGLFGKGS�VTCAK FACSKKMTGKSIQPENLEYRIMLSVHGSQHSGMIVNDTGHEHNDENRAK VEITPNSPRAEATLGGFGSLGLDCEPRTGLDFSDLYLTMNNKHWLVH KEWFHDIPLPWHAGADTGTPHWNKEALVEFKDAHAKRQTVVVLGS QEGAVHTALAGALEAEMDGAKGRLSSGHLKCRKMDKLRKGVSYSL CTAAFTFTKIPAEHLHGTVTVEVQYAGTDGPCKVPAQMAVDMQTLT PVGRITANPVITESTENSKMMLELDPPFGDSYIVIGVGEKKITHHWHR SGSTIGKAFAEATVRGAKRMAVLGD.TAWDFGSGVGGALNSLGKGIHQIF GAAFK

377	SARS-CoV2 full-length S protein – Wuhan Strain	FVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASTEKS NIIRGWIFGTTLDSKTQSL LIVNNA TNVVIKVCE FQFCNDPFLGVYYHK NNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVF KNIDGYFKIYSKHTPINLVRDL PQGFSALEPLVDLPIGINITRFQTLLALH RSYLT PGDSSSGWTAGAAAYYVGYLQPR TFL LKYNENG TITDAV DCA LDPLSETKCTLSFTVEKG IYQTSNFRVQPTESIVRFPNITNLCPFGEVFN ATRFASVYAWNRRKRISNCVADYSVL YNSASFSTFKCYGVSP TKLNDLC FTNVYADSFVIRGDEV RQIAPGQTGKIADYNYKLPDDFTGCVIAWNSN NLDSKVGGNYNYLYRLFRKSNLKPFERDISTE IYQAGSTPCNGVEGFN CYFPLQSYGFQPTNGVGYQPYRVV VLSFELLHAPATVCGPKKSTNLVK NKC VNFNFENGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTG SNVFQTRAGCLIGA EHVNN SYECDIPIGAGICASYQTQTNSP RRARSVASQSIIAYTMSLGAENS VAYSNN SIAIPTNFTISVTTEILPVSM T KTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLAD AGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMI AQYTSALLA GTITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF NSAIGKIQDSL SSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV LNDIL SRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLA ATKMSECVLGQSKRVDFCGKGYHLSFQPSAPHGVVFLHVTYVPAQE KNFTTAPAICH DGKAHFPRG VFVSNGTHW FVTQRNFYEPQIITDNTF VSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDIS GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWL GFIAGLIAIVMVTIMLCCMTSCCSCLKGCCSCGSCCKFDEDDSEPVLKG VKLHYT
378	SARS-CoV2 full-length S protein - K417T, E484K, and N501Y	FVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVS GTNGTKRFDNPVLPFNDGVYFASTEKS NIIRGWIFGTTLDSKTQSL LIVNNA TNVVIKVCE FQFCNDPFLGVYYHK NNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVF KNIDGYFKIYSKHTPINLVRDL PQGFSALEPLVDLPIGINITRFQTLLALH RSYLT PGDSSSGWTAGAAAYYVGYLQPR TFL LKYNENG TITDAV DCA LDPLSETKCTLSFTVEKG IYQTSNFRVQPTESIVRFPNITNLCPFGEVFN ATRFASVYAWNRRKRISNCVADYSVL YNSASFSTFKCYGVSP TKLNDLC FTNVYADSFVIRGDEV RQIAPGQTG TIADYNYKLPDDFTGCVIAWNSN NLDSKVGGNYNYLYRLFRKSNLKPFERDISTE IYQAGSTPCNGVKGFN CYFPLQSYGFQPTYGVGYQPYRVV VLSFELLHAPATVCGPKKSTNLVK NKC VNFNFENGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTG SNVFQTRAGCLIGA EHVNN SYECDIPIGAGICASYQTQTNSP RRARSVASQSIIAYTMSLGAENS VAYSNN SIAIPTNFTISVTTEILPVSM T KTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLAD AGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMI AQYTSALLA GTITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF NSAIGKIQDSL SSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV LNDIL SRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLA ATKMSECVLGQSKRVDFCGKGYHLSFQPSAPHGVVFLHVTYVPAQE KNFTTAPAICH DGKAHFPRG VFVSNGTHW FVTQRNFYEPQIITDNTF VSGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDIS GINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWL

		GFIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKG VKLHYT
379	SARS-CoV2 full-length S protein - delta variant	FVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASIEKSN IIRGWIFGTTLDSTQSLIVNNATNVVIK VCEFQFCNDPFLDVYYHKN NKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNI DGYFKIYSKHTPINLVRDL PQGFSALEPLVDLPIGINITRFQTLLALHRSY LTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGITITDAVDCALDP LSETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNAT RFASVYAWNKRKISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFT NVYADSFVIRGDEV RQIAPGQTGKIADYNYKL PDDFTGCVIAWNSNNL DSKVGGNYNRYRLFRKSNLKPFERDISTEYQAGSKPCNGVEGFNCY FPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNK CVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILD ITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWR VYSTGSNVFQTRAGCLIGAEHVNNSECDIPAGICASYQTQTNRRRR ARSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKT SVDCTMYICGDSTEC SNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVF AQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAG FIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGT ITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNS AIGKIQDSLSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISSVLN DILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAAT KMSECVLGQSKRVDFCGKGYHLMSPQSAHPGVFLHVTVVPAQEK NFTTAPAICHGDKAHFPREGV FVSNGTHWFVTQRNFYEPQIITDNTFV SGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISG INASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLG FIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGV KLHYT
380	SARS-CoV2 full-length S protein - delta variant plus	FVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHFSGTNGTKRFDNPVLPFNDGVYFASIEKSN IRGWIFGTTLDSTQSLIVNNATNVVIK VCEFQFCNDPFLDVYYHKN KSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNID GYFKIYSKHTPINLVRDL PQGFSVLEPLVDLPIGINITRFQTLLALHRSY LTPGDSSSGLTAGAAAYYVGYLQPRTFLLKYNENGITITDAVDCALDPLS ETKCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRF ASVYAWNKRKISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNV YADSFVIRGDEV RQIAPGQTGNIADYNYKL PDDFTGCVIAWNSNNLDS KVGGNYNRYRLFRKSNLKPFERDISTEYQAGSKPCNGVEGFNCYFP LQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNK VNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDI TPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRV YSTGSNVFQTRAGCLIGAEHVNNSECDIPAGICASYQTQTNRRRA RSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMTKTS VDC'TMYICGDSTEC SNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFA QVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFI KQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTIT SGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFN AIGKIQDSLSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISSVLND ILSRDLKVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATK MSECVLGQSKRVDFCGKGYHLMSPQSAHPGVFLHVTVVPAQEKNF TTAPAICHGDKAHFPREGV FVSNGTHWFVTQRNFYEPQIITDNTFVSG

		NCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGIN ASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGF AGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVKGVK LHYT
381	SARS-CoV2 full-length protein – stabilized with 2 proline substitutions	FVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKS NIIRGWIFGTTLDSTQSLIVNNAATNVVIKVCEQFCNDPFLGVYYHK NNKSWMESEFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVF KNIDGYFKIYSKHTPINLVRDLPOGFSALEPLVDLPIGINITRFQTLLALH RSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCAL LDPLSETKCTLSFTVEKGITYQTSNFRVQPTESIVRFPNITNLCPFGEVFN ATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLC FTNVYADSFVIRGDEVQRAPGQTGKIADYNYKLPDDFTGCVIAWNSN NLDSKVGGNYNLYLRLFRKSNLKPFERDISTEYQAGSTPCNGVEGFN CYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVK NKCYNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTN RRARSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSM KTSVDCTMYICGDSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSPKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMAQYTSALLA GTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF NSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV LNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAA TKMSECVLGQSKRVDFCGKGYHLMSPQSAPHGVVFLHVTVYVPAQEK NFTTAPAICHGDKAHFPREGVVFVSNNGTHWFTVQRNFYEPQIITDNTFV SGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGIN ASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGF AGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVKGVK LHYT
382	SARS-CoV2 full-length protein - K417T, E484K, and N501Y	FVFLVLLPLVSSQCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKS NIIRGWIFGTTLDSTQSLIVNNAATNVVIKVCEQFCNDPFLGVYYHK NNKSWMESEFRVYSSANNCTFEYVSQPFMDLEGKQGNFKNLREFVF KNIDGYFKIYSKHTPINLVRDLPOGFSALEPLVDLPIGINITRFQTLLALH RSYLTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGTITDAVDCAL LDPLSETKCTLSFTVEKGITYQTSNFRVQPTESIVRFPNITNLCPFGEVFN ATRFASVYAWNRKRISNCVADYSVLYNSASFSTFKCYGVSPTKLNDLC FTNVYADSFVIRGDEVQRAPGQTGKIADYNYKLPDDFTGCVIAWNSN NLDSKVGGNYNLYLRLFRKSNLKPFERDISTEYQAGSTPCNGVKGFN CYFPLQSYGFQPTYGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVK NKCYNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLE ILDITPCSFGGVSVITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPT WRVYSTGSNVFQTRAGCLIGAEHVNNSYECDIPIGAGICASYQTQTN RRARSVASQSIIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSM KTSVDCTMYICGDSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQE VFAQVKQIYKTPPIKDFGGFNFSQILPDPSPKPSKRSFIEDLLFNKVT LADAGFIKQYGDCLGDIAARDLCAQKFNGLTVLPPLLTDEMAQYTSALLA GTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQF NSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNFGAISSV LNDILSRLDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAA

		TKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEK NFTTAPAICHGDKAHFPREGVVFVSNNGTHWFWTQRNFYEPQIITDNTFV SGNCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISG INASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWL FIAGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGV KLHYT
383	SARS-CoV2 full-length stabilized S protein Delta variant	FVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVSNGTNGTKRFDNPVLPFNDGVYFASIEKSN IIRGWIFGTTLDSTQSLNATNVVIKVFCEQFCNDPFLDVYYHKN NKSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNI DGYFKIYSKHTPINLVRDLPGQFSALEPLVDLPIGINITRFQTLLALHRSY LTPGDSSSGWTAGAAAYYVGYLQPRTFLLKYNENGITITDAVDCALDP LSEKCTLKSTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNAT RFASVYAWNKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFT NVYADSFVIRGDEVRIAPGQTGKIADYNYKLPPDFTGCVIAWNSNNL DSKVGGNYNRYRLFRKSNLKPFERDISTEYQAGSKPCNGVEGFNCY FPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNK CVNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILD ITPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWR VYSTGSNVFQTRAGCLIGAHEVNNSYECDIPIGAGICASYQTQTNRRR ARSVASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMKT SVDCTMYICGDSSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVF AQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTADAG FIKQYGDCLGDIAARDLCAQKFNGLTVLPLLTDEMIAQYTSALLAGT ITSGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNS AIGKIQDSLSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAIVSVLN DILSRDPPEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATK MSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNF TTAPAICHGDKAHFPREGVVFVSNNGTHWFWTQRNFYEPQIITDNTFVSG NCDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPDVLGDISGIN ASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWPWYIWLGI AGLIAIVMVTIMLCCMTSCCCLKGCCSCGSCCKFDEDDSEPVLKGVK LHYT
384	SARS-CoV2 full-length stabilized S protein Delta variant, plus	FVFLVLLPLVSSQCVNLRTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHS TQDLFLPFFSNVTWFHAIHVSNGTNGTKRFDNPVLPFNDGVYFASIEKSN IRGWIFGTTLDSTQSLNATNVVIKVFCEQFCNDPFLDVYYHKN KSWMESGVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLREFVFKNID GYFKIYSKHTPINLVRDLPGQFVLEPLVDLPIGINITRFQTLLALHRSY LTPGDSSSGLTAGAAAYYVGYLQPRTFLLKYNENGITITDAVDCALDPLS ETKCTLKSTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRF ASVYAWNKRISNCVADYSVLVNSASFSTFKCYGVSPTKLNDLCFTNV YADSFVIRGDEVRIAPGQTGNIADYNYKLPPDFTGCVIAWNSNNLDS KVGGNYNRYRLFRKSNLKPFERDISTEYQAGSKPCNGVEGFNCYFP LQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKNKC VNFNFNGLTGTGVLTESNKKFLPFQFGRDIADTTDAVRDPQTLEILDI TPCSFGGVSVITPGTNTSNQVAVLYQGVNCTEVPVAIHADQLTPTWRV YSTGSNVFQTRAGCLIGAHEVNNSYECDIPIGAGICASYQTQTNRRR RSVASQSIAYTMSLGAENSVAYSNNIAIPTNFTISVTTEILPVSMKT VDCTMYICGDSSTECNLLLQYGSFCTQLNRALTGIAVEQDKNTQEVFA QVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTADAGFI KQYGDCLGDIAARDLCAQKFNGLTVLPLLTDEMIAQYTSALLAGTIT SGWTFGAGAAALQIPFAMQMAYRFNGIGVTQNVLYENQKLIANQFNSA

		IGKIQDSLSTASALGKLQNVVNQNAQALNTLVKQLSSNFGAISSVLND ILSRLDPPAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKM SECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEKNT TAPAICHGDKAHFPREGVVFVSNNGTHWFVTQRNFYEPQIITDNTFVSGN CDVVIGIVNNTVYDPLQPELDSFKEELDKYFKNHTSPD VDLGDISGINA SVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEYQYIKWPWYIWLGFIA GLIAIVMVTIMLCCMTSCCSCCLKGCCSCGSCCKFDEDDSEPVLKGVKL HYT
385	SARS-CoV2 E protein amino acid sequence	YSFVSEETGTLIVNSVLLFLAFVVFLVTLAILTALRLCAYCCNIVNVSL VKPSFYVYSRVKNLNSSRPDLLV
386	SARS-CoV2 M protein amino acid sequence	ADSNGTITVEELKKLLEQWNLVIGFLFTWICLLQFAYANRNRFLYIHK LIFLWLLWPVTLACFVLAAYRINWITGGIAIAMACL VGLMWLSYFIA SFRI.FARTRSMWSFNPETNII.I.NVPI.HGTIILTRPI.I.SFI.VIGAVII.RGH LRIAGHHLGRCDIKDLPEKITVATSRTLSYYKL GASQRVAGDSGFAAY SRYRIGNYKLN TDHSSSDNIALLVQ
387	SARS-CoV2 PP1ab polyprotein amino acid sequence	ESLVPGFNEKTHVQSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKDG TCGLVEVEKGVLPQLEQPYVFIKRS DARTAPHGHVMVELVAELEGIQY GRSGETLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFD LGDELGTDPYEDFQENWNTKHSSGV TRELMRELNGGAYTRYVDNNF CGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTKRGVYCCREHEHEIA WYTERSEKSYELQTPFEIKLAKKFDTFNGECPNFVPLNSIIKTIQPRVE KKKLDGFMGRIRSVPV ASPNECNQMCLSTLMKCDHCGETSWQTGDF VKATCEFCGTENLTKEGATTCGYLPQNAVVKIYCPACHNSEVGPHESL AEYHNESGLKTILRKGGRTIAFGGCVFSYVGCHNKCAYWVPRASANIG CNHTGVVGESEGLNDNLLLEILQKEKVNINIVGDFKLNEEIAIILASFSA STSAFVETVKGLDYKAFKQIVESCGNFKVTKGKAKKGAWNIGEOKSIL SPLYAFASEAARVVRISIFSR TLETAQNSVRVLQKAAITILDGISQYSRLI DAMMFTSDLATNNLVVMAYITGGVVQLTSQWL TNIFGTVYEKLPVL DWLEEFKEGVEFLRDGWEIVKFISTCACEIVGGQIVTCAKEIKESVQT FFKL VNKFLALCADSIIIGGAKLKALNLGETFVTHSKGLYRKCVKSREE TGLLMPLKAPKEIIFLEGETLPTEVLTEEVLTKTGDLPLEQTPSEAVEA PLVGTTPVCINGLMLLEIKDTEKYCALAPNMMVTNNTFTLKGGAPTKV TFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEVNEFA CVVADAVIKTLQP VSELLTPLGIDLDEWSMATYYLFDESGEFKLASHM YCSFYPPDEDEEEGDCEEEFEPTQY EYGTEDDYQGKPLEFGATSAAL QPEEEQEEDWLDDDSQQT VGGQDGS EDNQT TTIQTIVEVQPQLEMELT PVVQTIEVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYL KHGGGVAGALNKATNNAMQVESDDYIATNGPLKVGGSCVLSGHNLA KHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPI HSLRVCVDTVRTNVYLA VFDKNLYDKLVSSFLEMKSEKQVEQKIAEIP KEEVKPFITESKPSVEQRKQDDKKIKACVEEVTTTLEETKFLTENLLLVI DINGNLHPDSATLVSDIDITFLKKDAPYIVGDVVQEGVLTAVVIPTKKA GGTTEMLAKALRKVPTDNYITTPGQGLNGYTVEEAKTVLKKCKSAF YILPSIISNEKQEILGT VSWNLREMLAHAEETRKLMPVCVETKAIVSTIQ RKYKGIKIQEGVVDYGARFYFYTSKTTVASLINTLNDLNETLVTMPLG YVTHGLNLEEAARYMRS LKVPATVSVSPDAVTA YNGYLTSSSKTPEE HFIIETISLAGSYKDSYSGQSTQLGIEFLKRGDKSVYYTSNPTTFHLDG EVITFDNLKTLLSLREVRTIKVFTTVDNINLHTQVVDM SMTY GQQFGP TYLDGADVTKIKPHNSHEGKTFYVLPNDDTLRVEAFEYHYHTTDP SFLG RYMSALNHTKKWKYPQV NGLTSIKWADNNCYLATALLTLOQIELKFN PPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRETMSYLFQH

		ANLDSCKRVLNVVCKTCGQQQTTLKGVEAVMYMGTLSEYQFKKGVO IPCTCGKQATKYL VQQESPVMMSAPPAQYELKHGHTFCASEYTGNY QCGHYKHITSKETLYCIDGALLTKSSEYKGPITDV FYKENSYTTTIKPV YKLDGVVCTEIDPKLDNYYKKDNSYFTEQPIDLVNPQYPNASFDNFK FVCDNIKFADDLNQLTGYKKPASRELKVTFPPDLNGDVVAIDYKHYTP SFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVETSNS FDVLKSEDAQGMNDLACEDLKPVSEEVENPTIQKD VLECNVKTTEV VGDIILKPANNSLKITEEVGHTDLM AAYVDNSSLTIKKPNELSRVLGLK TLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRCLNRVCTNY MPYFFTL LLQLCTFTRSTNSRIKASMPPTIAKNTVKS VGKFCLEASFNY LKSPNFSKLINIIWFLLLSVCLGSLIYSTAALGVLM SNLGMPSYCTGYR EGYLNSTNVTIATYCTGSIPCSVCLSGLDSDLTYP SLETIQITISSFKWDL TAFGLVAEWFLAYILFTRFFYVLGLAAIMQLFFSYFAVHFISNSWLMW LIINLVQMAPISAMVRMYIFFASFYYVWKS YVHVVDGCNSSTCMMC KRN RATRVECTTIVNGVRRSFYVYANGGKGFC LHNWNCVNCDTFC AGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVT VKNGSIHLFYDKAG QKTYERHSLSHFVNLDNLRANNTKGSLPINVIVFDGKSKCESSAKSAS VYYSQ LMCQPILLLDQALVSDVGDSA EVAVKMFDAYVNTFSSTFNP MEKLKTLVATAEAE LAKNVSLDNVLS TFISAARQGFVDS DVETKDVV ECLKLSHQSDIEVTGDS CNNYMLTYNKVENMTPRDLGACIDCSARHIN AQVAKSHNIALIWNVKDFMSLSEQLRKQIRSA AKKNNLPFKLTCATTR QVVNVVT KIALKGGKIVNNWLKQLIKVTLVFLVAAIFYLITPVHVM SKHTDFSSEIIGYKAIDGGVTRDIASDTCFANKHADFDTWFSQRGGSY TNDKACPLIAAVITREVG FVVPGLPGTILRTTNGDFLHFLPRVFSAVGNI CYTPSKLIEYTD FATSA CVLAAECTIFK DASGKPVPCYD TNVLEGSVA YESLRPDTRYVLMDGSIIQFPNTYLEGSVRVVTTFDSEYCRHGTCERSE AGVCVSTSGRWVLNNDY YRSLPGVFCGVD AVNLLTNMFTPLIQPIGAL DISASIVAGGIVAIVVTCLAYYFMRFRRAFGEYSHVVA FN TLLFLMSFT VLCLTPVYSFLPGVYSVIYLYLTFYLTNDVSFLAHIQWMVMFTPLVPF WITAIYIICISTKHFYWFBSNYLKRRVVFNGVSFSTFEEAALCTFLLNKE MYLKLRS DVLPLTQYNRYLALYNKYKYFSGAMDTTSYREAA CCHL AKALNDFSNSGSDVLYQPPQTSITSAVLQSGFRKMAFP SGKVEGCMVQ VTCGTTTLNGLWLD DVVYCPRHVICTSEDMLNP NYEDLLIRKSNHNFL VQAGNVQLRVIGH SMQNCVLKLKVD TANPKTPKYKFVRIQPGQTF SV LACYNGSPSGVYQCAMRPNFTIKGSFLNGSCGSGVFNIDYDCVSFCYM HHMELPTGVHAGTDLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWL YAAVINGDRWFLNRFTTTLNDFNLVAMKYN YEPLTQDHVDILGPLSA QTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPF DVVRQCSGV TFQSAVKRTIKGTHHWLLL TILTSLLVLVQSTQWSLFFFLYENAFLPFA MGIIAMSAFAMMFVKHKAFLCLFLLPSLATVAYFNMVYMPASWVM RIMTWLDMVDTSLSGFKLKDCVMYASAVLLILMTARTVYDDGARR VWTLMNVLTLVYKVYYGNALDQAISMWALIISVTSNYSGVVTTVMFL ARGIVFMCVEYCPIFFITGNTLQCIMLVYCF LGYFCTCYGFLFCLLNRY FRLTLGVYDYL VSTQEFYRMNSQGLLPPKNSIDAFKLN IKLLGVGGKP CIKVATVQSKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCQVLHNDI LLAKDTTEAFEK MVSLLSVLLSMQGA VDINKLCEEMLDNRATLQAIAS EFSSLPSYAAFATAQEAYEQAVANGDSEVVLK LKLSLVAKSEFDR DAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTM LFTML RKLDNDALNNIINNARDGCVPLNIPLTTAAKL MVVPDYN TYKNTCD GTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALRA NSAVKLQNNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGR FVLALLSDLQDLKWARFPKSDGTGTIYTELEPPCRFVTDTPKGPVKY LYFIKGLNNLNRGMVLGSLAATVRLQAGNATEVPANSTVLSFCFAFV DAAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTPEANMDQESF GGASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPVGFTLKNTV
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		CTVCGMWKGYGCSCDQLREPMQLQSADAQSFLNRVCGVSAARLTPCG TGTSTDVVYRAFDIYNNDKVAGFAKFLKTNCCRFQEKDEDDNLDISYFV VKRHTFSNYQHEETIYNLLKDCPAVAKHDFKFRIDGDMVPHISRQRL TKYTMADLVYALRHFDEGNCDTLKEILVTYNCCDDDYFNKKDWYDF VENPDILRVYANLGERVRQALLKTVQFCDAMRNAGIVGVLTLDNQDL NGNWDYDFGDFIQTTTPGSGVPVVDSSYSLMPILTLTRALTAESHVDTD LTKPYIKWDLKDYDFTEERLKLFDRYFKYWDQTYHPNCVNCDDRCI LHCANFNVLFSSTVFPTSFGLVRKIFVDGVPFVSTGYHFRELGVVHN QDVNLHSSRLSFKELLVYAADPAMHAASGNLLDKRTTCFSVAALTN NVAFQTVKPGNFNKFDFYDFAVSKGFFKEGSSVELKHFFFAQDGNAAIS DYDYRYRNLPTMCDIRQLLFVVEVVDKYFDCYDGGCINANQVIVNNL DKSAGFPFNKWKARLYYDSMSYEDQDALFAYTKRNVIPITITQMNK YASAKNRARTVAGVSICSTMTNRQFHQKLLKSIAATRGTATVIGTSKF YGGWHNMLKTVYSDVENPHLMGWDYPKCDRAMPNMLRIMASLVLA RKHTTCCSLSHRFYRLANECQVLSMVVMCGGSLYVKPGGTSSGDAT TAYANSVFNICQAVTANVNALLSTDGNKIADKYVRNLQHRLYECLYR NRDVDTDVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIK NFKSVLYYQNNVFMSEAKCWTETDLTKGPHEFCSQHTMLVKQGDDY VYLPYDPDSRILGAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTKHPNQ EYADVFLYLQYIRKLHDELTHGMLDMYSVMLTNDNTSRWYEFY EAMYPHTVLQAVGACVLCNSQTSRLRCGACIRRPFLCCKCCYDHVIST SHKLVLVSNPYVCNAPGCDVTDVTQLYLGGMSYCYCKSHKPPISFPLCA NGQVFLGYKNTCVGSDNVTDENAIATCDWTNAGDYILANTCTERLKL FAAETLKATEETFKLSYGIATVREVLSDRELHLSWEVGKPRPPLNRNY VFTGYRVTKNSKVQIGEYTFEKGDYGDVAVYRGTTTYKLVNGDYFVL TSHTVMPLSAPTLVPQEHYVRITGLYPTLNISDEFSSNVANYQKVGMO KYSTLQGPPTGKSHFAIGLALYPSARIVYTACSHAAVDALCEKALK YLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCVNALPETTADIVVF DEISMATNYDLSVVNARLRAKHYYIGDPAQLPAPRTLLTKGTLEPEY FNSVCRLMKITGPDMLGTCCRCPAEIVDVSALVYDNKLKAHKDKS AQCCKMFYKGVITHDVSSAINRPQIGVVREFLTRNPAWRKAVFISPYNS QNAVASKILGLPTQTVDSQSEYDYVIFTQTTETAHSCNVNRFNVAIT RAKVGILCIMSDDRLYDKLQFTSLEIPRRNVATLCAENVGTGLFKDCSK VITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPKDMTYRRLISMGMFK MNYQVNGYPNMFITREEAIRHVRAWIGFDVEGCHATREAVGTNLPLQ LGFSTGVNLVAVPTGYVDTNNTDFSRVSAKPPPGDQFKHLIPLMYKG LPWNVVRIKIVQMLSDTLKNLSDRVVFLWAHGFELTSMKYFVKIGPE RTCCLCDRRATCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTG NLQSNHDLYCQVHGNHVASCDAIMTRCLAVHECFVKRVDWTIEYPII GDELKINAACRKVQHMVVKAAALLADKFPVLHDIGNPKAIKCVPPQADV EWKFYDAQPCSDKAYKIEELFYSYATHSDKFTDGVCLFWNCNVDRYP ANSIVCRFDTRVLSNLPNPGCDGGSLYVNKHAFHTPAFDKSAFVNLKQ LPFFYYSDSPCESHGKQVVSIDYVPLKSATCITRCNLGGAVCRHHAN EYRLYLDAYNMMISAGFSLWVYKQFDTYNLWNTFTRLQSLNVAFN VVNKGHFDGQGEVPSIINNTVYTKVDGVDVELFENKTTLPVNVAF ELWAKRNIKPVEVKILNNLGVDIAANTVIWDYKRDAPAHISTIGVCS MTDIAKKPTETICAPLTVFFDGRVDGQVDLFRNARNGVLITEGSVKGL QPSVGPKQASLNGVTLIGEA VKTQFNYYKKVDGVVQQLPETYFTQSR NLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDVSHSQLGG LHLIIGLAKRFKESPFLEDFIPMDSTVKNYFITDAQTGSSKCVCSVIDL LLDDFVEIISQDLSVVSXVVKVTIDYTEISFMLWCKDGHVETFPYKLG SSQAWQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKGIMMNV KYTQLCQYLNLTALVPYNMRVIHFGAGSDKGVAPGTAVLRQWLPTG TLLVDSLDNDFVSDADSTLIGDCATVHTANKWDLIISDMYDPKTKNVT KENDSKEGFFTYICGFIQQLALGGSVAIKITEHSWNADLYKLMGHFA
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		WWTAFVTNVNASSSEAFILGNCNYLGKPREQIDGYVMHANYIFWRNTN PIQLSSYSLFDMSKFPLKLRGTAVMSLKEGQINDMILSLLSKGRLIIREN NRVVISDVL VNN
388	SARS-CoV2 PP1a polyprotein amino acid sequence. (Wuhan-Hu-1)	ESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLLKDG TCGLVEVEKGVLPQLEQPYVFIKRSDARTAPHGHVMVELVAELEGIQY GRSGETLGVLVPHVGEIPVAYRKVLLRKNNGNKGAGGHSYGADLKSFD LGDELGTDPYEDFQENWNTKHSSGVTRELMRELNGGAYTRYVDNMF CGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTKRGVYCCREHEHEIA WYTERSEKSYELQTPFEIKLAKKFDTFNGECPNFVPLNSIIKTIQPRVE KKKLDGFMGRIRSVYPVASPNECNQMCLSTLMKCDHCGETSWQTGDF VKATCEFCGTENLTKEGATTCGYLPQNAVVKIYCPACHNSEVGPEHSL AEYHNESGLKTILRKGGRTIAFGGCVFSYVGCHNKCAYWVPRASANIG CNHTGVVGESEGLNDNLLLEILQKEKVNINIVGDFKLNELIAILASFSA STSAFVETVKGLDYKAFKQIVESCNGFVKTKGKAKKGAWNIGEQQSIL SPLYAFASEAARVVRISFRTLETAQNSVRVLQKAAITILDGISQYSLRLI DAMMFTSDLATNNLVVMAYITGGVVQLTSQWLTNIFGTVEYKLVKPV DWLEEKFKEGVEFLRDGWEIVKFISTCACEIVGGQIVTCAKEIKSVQT FFKL VNKFLALCADSIIGGAKLKALNLGETFVTHSKGLYRKCVKSREE TGLLMPLKAPKEIIFLEGETLPTEVLTEEVLKTDGLQPLEQPTSEAVEA PLVGTPVCINGLMLLEIKDTEKYCALAPNMMVTNNFTLKGGAFTKV TFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEVNEFA CVVADAVIKTLQPVSELLTPLGIDLDEWSMATYYLFDSEGEFKLASHM YCSFYPPDEDEEEGDCEEEFEFSTQYEYGTEDDYQGKPLEFGATSAL QPEEEQEEDWLDDDSQQTVGQDQSEDNQTTTIQTIVEVQPQLEMELT PVVQTIENVNSFSGYLKLTNDNVYIKNADIVEEAKKVKPTVVVNAANVYL KHGGGVAGALNKATNNAMQVESDDYIATNGPLKVGGSCLVSGHNLA KHCLHVVGPNVKNKGEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPI HSLRVCVDTVRTNVYLA VFDKNLYDKLVSSFLEMKSEKQVEQKIAEIP KEEVKPFITESKPSVEQRKQDDKKIKACVVEVTTTLEETKFLTENLLL YI DINGNLHPDSATLVSDIDITFLKKDAPYIVGDVVQEGVLTA VVIPTKKA GGTTEMLAKALRKVPTDNYITTPGQGLNGYTVEEAKTVLKKCKSAF YILPSISNEKQEILGTVSWNLREMLAHAEETRKLMPVCVETKAIIVTIQ RKYKGIKIQEGVVDYGARFYFYTSKTTVASLINTLNDLNETLVTMPLG YVTHGLNLEEAARYMRSLKVPATVSVSSPDAVTAYNGYLTSSSKTPEE HFIETISLAGSYKDWYSYSGQSTQLGIEFLKRGDKSVYYTSNPTTFHLDG EVITFDNLKTLLSLREVRTIKVFTTVDNINLHTQVVDMSMTYGGQFGP TYLDGADVTKIKPHNSHEGKTFYVLPNDDTLRVEAFEYYHTTDP SFLG RYMSALNHTKKWKYPQVNGLT SIKWADNNCYLATALTLQQLKFN PPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRETMSYLFQH ANLDSCKRVLNVVCKTCGQQQTTLKGVEAVMYMGTLSEYQFKKG VQ IPCTCGKQATKYL VQQESPVMMSAPPAQYELKHGFTFCASEYTGNY QCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSYTTTIKPV YKLDGVVCTEIDPKLDNYYKKDNSYFTEQPIDLPVNPYPNASFDNFK FVCDNIKFADDLNLQTLGYKKPASRELKVTFPDLNGDVVAIDYKHYP SFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVETSNS FDVLKSEDAQGMNDLACEDLKPVSEEVVENPTIQKDVLNCKVTTEV VGDILKPANNSLKITEEVGHTDLMAAYVDNSSLTIKKPNELSRVLGLK TLATHGLAAVNSVPWDTIANYAKPFLNKVSTTTNIVTRCLNRVCTNY MPYFFTL LLQLCTFTRSTNSRIKASMPPTIAKNTVKS VGKFCLEASFNY LKSPNFSKLINIIWFLLLSVCLGSLIYSTAALGVLMNSLGMPSYCTGYR EGYLNSTNVTIATYCTGSIPCSVCLSGLDSDLTYPSELETIQITISSFKWDL TAFGLVAEWFLAYILFTRFFYVLGLAAIMQLFFSYFAVHFISNSWLMW LIINLVQMAPISAMVRMYIFFASFYYVWKS YVHVVDGCNSSSTCMICY

		KRNRATRVECTTIVNGVRRSFYVYANGGKGFCCLHNWNCVNCDTFC AGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVTVKNGSIHL YFDKAG QKTYERHSLSHFVNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSAS VYYSQLMCQPILLLDQALVSDVGDSA EVAVKMFDAYVNTFSSTFNVP MEKLKTLVATAEAELAKNVSLDNVLS TFISAARQGFVDSVETKDVV ECLKLSHQSDIEVTGDSNNYMLTYNKVENMTPRDLGACIDCSARHIN AQVAKSHNIALIWNV KDFMSLSEQLRKQIRSAKKNL PFKLTCATTR QVVNVVTTKIALKGGKIVNNWLKQLIKVTLVFLVAAIFYLITPVHVM SKHTDFSSEIIIGYKAIDGGVTRDIASDTCFANKHADFDTWFSQRGGSY TNDKACPLIAAVITREVGFVVPGLPGTILRTTNGDFLHFLPRVFSAVGNI CYTPSKLIEYTD FATSA CVLAAECTIFK DASGKPVPCYDTNVLEGSVA YESLRPDTRYVLMDGSIIQFPNTYLEGSVRVVTTFDSEYCRHGT CERSE AGVCVSTSGRWVLNNDYYRSLPGVFCGVD AVNLLTNMFTPLIQPIGAL DISASIVAGGIVAIVVTCLAYYFMRFRRAFGEYSHVVAFNLLFLMSFT VLCLTPVYSFLPGVYSVIYLYLTFYL TNDVSFLAHIQWMVMFTPLVPF WITIA YIICISTKH FYWFFSNYLKRRVVFN GVSFSTFEEAALCTFLLNKE MYLKLRS DVLPLTQYNRYLALYNKYKYFSGAMDTTSYREAAACCHL AKALNDFSNSGSDVLYQPPQTSITS AVLQSGFRKMAFPSGKVEGCMVQ VTCGTTTLNGLWLDDV VYCPRHVICTSEDMLNPNYEDLLIRKSNHNFL VQAGNVQLRVIGHSMQNCVLKLKVD TANPKTPKYKFVRIQPGQTFSV LACYNGSPSGVYQCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYM HHMELPTGVHAGTDLEGNFYGPFVDRQTAQAAGTDTTITVNVLAWL YAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILGPLSA QTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDDVVRQCSGV TFQSAVKRTIKGTHHWLLL TILTSLLVLSQTSQWSLFFFLYENAFLPFA MGIIAMSAFAMMFVKHKHAFLCLFLLPSLATVAYFNMVMYPASWVM RIMTWLDMVDTSLSGFKLKDCVMYASAVVLLILMTARTVYDDGARR VWTLMNVLTLVYKVYYGNALDQAISMWALISVTSNYSGVVTTVMFL ARGIVFMCVEYCPIFFITGNTLQCIMLVYCFLGYFCTCYFGLFCLLNRY FRLTLGVYDYL VSTQEFYMN SQGLLPPKNSIDAFKLNKLLGVGGKP CIKVATVQSKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDI LLAKDTEAFEKMSVLLSVLLSMQGA VDINKLCEEMLDNRATLQAIAS EFSSLPSYA AFATAQEAYEQAVANGDSEVVLLKLLKSLNVAKSEFDR DAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTM LFTML RKLDNDALNNIINNARDGCVPLNIPLTTAAKLMVIPDYNTYKNTCD GTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALRA NSAVKLQNNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGR FVLALLSDLQDLWARFPKSDGTGTIYTELEPPCRFVTDTPKGPKVKYL YFIKGLNNLNRMVGLGSLAATVRLQAGNATEVPANSTVLSFCFAVD AAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTPEANMDQESFG GASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPVGFTLKNTVC TVCGMWKGYGCSCDQLREPM LQSADAQSFLNGFAV
389	SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1)	ESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQH LKDG TCGLVEVEKGVLPQLEQPYVFIKRS DARTAPHGHVMVELVAELEGIQY GRSGETLGVLVPHVGEIPVAYRKVLLRKNGNKGAGGHSYGADLKSFD LGDELGTDPYEDFQENWNTKHSSGV TRELMRELNGGAYTRYVDNNF CGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTKRGVYCCREHEHEIA WYTERSEKSYELQTPFEIKLAKKFDTFNGECPNFVFLNLSIIKTIQPRVE KKKLDGFMGRIRSVYPV ASPNECNQMCLSTLMKCDHCGETSWQTGDF VKATCEFCGTENLTKEGATTCGYLPQNAVVKIYCPACHNSEVGPEHSL AEYHNESGLKTILRKGGRTIAFGGCVFSYVGCHNK CAYWVPRASANIG CNHTGVVGESEGLNDNLLEILQKEKVNINIVGDFKLNEEIAIILASFSA STSAFVETVKGLDYKAFKQIVESC GNFKVTKGKAKKGAWNIGE QKSIL

		SPLYAFASEAARVVRISFRTLETAQNSVRVLQKAAITILDGISQYSRLRI DAMMFTSDLATNNLVVMAYITGGVVQLTSQWL TNIFGTVEKLPVL DWLEEFKEGVEFLRDGWEIVKFISTCACEIVGGQIVTCAKEIKESVQT FFKL VNKFLALCADSIIGGAKLKALNLGETFVTHSKGLYRKCVKSREE TGLLMPLKAPKEIIFLEGETLPTEVL TEEVVLKTGDLQPLEQPTSEAVEA PLVGTTPVCINGLMLLEIKDTEKY CALAPNMMVTNNFTLKGGAPTKV TFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEVNEFA CVVADAVIKTLQPVSELL TPLGIDLDEWSMATYYLFDESGEFKLASHM YCSFYPPDEDEEEEGDCEEEEFEPTQYIEYGTEDDYQGKPLEFGATSAAL QPEEEQEEDWLDDDSQQTVGQDGSSEDNQTTTIQTIVEVQPQLEMELT PVVQTIENVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYL KHGGGVAGALNKATNNAMQVESDDYIATNGPLKVGGS CVLSGHNLA KHCHLVVGPVNVKGEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPI HSLRVCVDTVRTNVYLA VFDKNLYDKLVSSFLEMKSEKQVEQKIAEIP KEEVKPFITESKPSVEQRKQDDKKIKACVEEVTTTLEETKFLTENLLLYI DINGNLHPDSATLVSDIDITFLKKDAPYIVGDVVQEGVLTAVVIPTKKA GGTTEMLAKALRKVPTDNYITTYPGQGLNGYTVVEAKTVLKKCKSAF YILPSIISNEKQEILGTVSWNLREMLAHAEETRKLMPVCVETKAIVSTIQ RKYKGIKIQEGVVDYGARFYFYTSKTTVASLINTLNDLNETLVTMPLG YVTHGLNLEEAARYMRS LKVPATVSVSPDAVTAYNGYLTSSSKTPEE HFIIETISLAGSYKDWYSYSGQSTQLGIEFLKRGDKSVYYTSNPTTFHLDG EVITFDNLKTLLSLREVRTIKVFTTVDNINLHTQVVDMSMTYGOQFGP TYLDGADVTKIKPHNSHEGKTFYVLPNDDTLRVEAFEYYHTTDP SFLG RYMSALNHTKKWKYPQVNGLTSIKWADNNCYLATALLTLQQIELKFN PPALQDAYYRARAGEAANFCALILAYCNKT VGE LGDVRETMSYLFQH ANLDSCKRVLNVVCKTCGQQQTTLKGVEAVMYMGTLSEYQFKKG VQ IPCTCGKQATKYL VQQESPVMMSAPPAQYELKHGTFTCASEYTGNY QCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSYTTTIK PVT YKLDGVVCTEIDPKLDNYYKKDNSYFTEQPIDLVPNPYPNASFDNFK FVCDNIKFADDLNQLTG YKKPASRELKVTFPDLNGDVVAIDYKHYTP SFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVETSNS FDVLKSEDAQGMNDLACEDLKPVSEEVVENPTIQKD VLECNVKTTEV VGDIILKPANNLSLKITEEVGHTDLMAAYVDNSSLTIKKPNELSRVLGLK TLATHGLAAVNSVPWDTIANYAKPFLNKVVSTTTNIVTRCLNRVCTNY MPYFFTLLLQLCTFTRSTNSRIKASMPPTIAKNTVKS VGKFCLEASFNY LKSPNFSKLINIIWFLLLSVCLGSLIYSTAALGV LMSNLGMPSYCTGYR EGYLNSTNVTIATYCTGSIPCSVCLSGLDSDLTYP SLETIQITISSFKWDL TAFGLVAEWFLAYILFTRFFYVLGLAAIMQLFFSYFAVHFISNSWLMW LIINLVQMAPISAMVRMYIFFASFYYVWKS YVHVVDGCNSSTCMMC Y KRNRA TRVECTIVNGVRRSFYVYANGGKGFC LHNWNCVNCDTFC AGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVT VKNGSIHL YFDKAG QKTYERHSLSHFVNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSAS VYYSQLMCQPILLLDQALVSDVGDSA EVAVKMFDA YVNTFSSTFNVP MEKLKTLVATAEAE LAKNVSLDNVLSTFISAARQGFVDS DVETKD VV ECLKLSHQSDIEVTGDSCNNYMLTYNKVENMTPRDLGACIDCSARHIN AQVAKSHNIALIWNVKDFMSLSEQLRKQIRSA AKKNNLPFKLTCATTR QVVNVVTTKIALKGG
390	SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1)	KIVNNWLKQLIKVTLVFLFVA AIFYLITPVH VMSKHTDFSSEIIGYKAID GGVTRDIASTDTCFANKHADFDTWFSORGGSYTNDKACPLIAAVITRE VGFVVPGLPGTILRTTNGDFLHFLPRVFS AVGNICYTPSKLIEYTD FATS ACVLAAECTIFK DASGKVPYCYDTNVLEGS VAYESLRPDTRY VLMD GSIIQFPNTYLEGSVRVVTTFDSEYCRHGT CERSEAGVCVSTSGRWVLN NDYYRSLPGVFCGVDAVNLLTNMFTPLIQIPIGALDISASIVAGGIVAIVV

		TCLAYYYFMRFRRAFGGEYSHVVAFNLTLLFLMSFTVLCLTPVYSFLPGVY SVIYLYLTFYLTNDVSFLAHIQWMVMFTPLVPFWITIAIICISTKHFYW FFSNYLKRRVVFNGVSFSTFEEAALCTFLLNKEMYKLRSVDLLPLTQ YNRYLALYNKYKYFSGAMDTTSYREAACCHLAKALNDFSNSGSDVL YQPPQTSITSAVLQSGFRKMAFSPSGKVEGCMVQVTCGTTTLNGLWLD DVVYCPRHVICTSEDMLNPNYEDLLIRKSNHNFLVQAGNVQLRVIGHS MQNCVLKLVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVYQC AMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHMELPTGVHAGT DLEGNFYGPVDRQTAQAAGDTTITVNVLAWLAAVINGDRWFLNR FTTTLNDFNLVAMKYNIEPLTQDHVDILGPLSAQTGIAVLDMCASLKE LLQNGMNGRTILGSALLEDEFTPFDDVVRQCSGVTFQSAVKRTIKGTHH WLLL TILTSLLVLVQSTQWSLFFFLYENAFLPFAMGIIAMSAFAMMFV KHKHAFLCLFLLPSLATVAYFNMVYMPASWVMRIMTWLDMVDTSL GFKLKDCVMYASAVVLLILMTARTVYDDGARRVWTLNMNVLTLYK VYYGNALDQAISMWALIISVTSNYSGVVTTVMFLARGIVFMCVEYCPI FFITGNTLQCIMLVYCFGLGYFCTCYFGLFCLLNRYFRLTLGVYDYLST QEFRYMNSQGLLPKNSIDAFKLNKLLGVGGKPCIKVATVQSKMSDV KCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEAFEKMY SLLSVLLSMQGAVDINKLCEEMLDNRATLQAIASEFSSLSYAAAFATAQ EAYEQAVANGDSEVVLKLLKSLNVAKSEFDRDAAMQRKLEKMA QAMTQMYKQARSEDKRAKVTSAMQTMFLTMLRKLDNDALNNIINNA RDGCVPLNIPLTTAAKLMVVIPDYNTYKNTCDGTFTFYASALWEIQ VVDADSKIVQLSEISMDNSPNLAWPLIVTALRANSVAVKLQNNELSPVA LRQMSCAAGTTQTACTDDNALAYYNTTKGGRFVYALLSDLQDLKWA RFPKSDGTGTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNNLNRGMVL GSLAATVRLQAGNATEVPANSTVLSFCAFAVDAKAYKDYLASGGQP ITNCVKMLCTHTGTGQAITVTPEANMDQESFGGASCCLYCRCHIDHPN PKGFCDLKGKYVQIPTTCANDPVGFTLKNVCTVCGMWKGYGCSCD QLREPMLQSADAQSFLNGFAV
391	SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1)	SADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAK FLKTNCCRFQEKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCPA VAKHDFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCDTL KEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKT VQFCDAMRNAGIVGVLTLDNQDLNGNWYDFGDFIQTTPGSGVPVVD YYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLKYDFTEERLKLFD YFKYWDQTYHPNCVNCLDDRCILHCANFNVLSTVFPPTSFGPLVRKIF VDGVPFVYSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAADPAMH AASGNLLLDKRTTCFSVAALTNNVAFQTVKPGNFNKDFYDFAVSKGF FKEGSSVELKHFFFAQDGNAAISDYDYRYNLPTMCDIRQLLFVVEV DKYFDCYDGGCINANQVIVNNLDKSAGFPFNKWKARLYYDSMSYE DQDALFAYTKRNVIPITITQMNLYAISAKNRARTVAGVSICSTMTNRQ FHQKLLKSIAATRGATTVVIGTSKFYGGWHNMLKTVYSDVENPHLMG WDYPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANCAQV LSEMVMCGGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVNALLS TDGNKIADKYVRNLQHRLYECLYRNRDVTDFVNEFYAYLRKHFSM MILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKCWE TDLTKGPHEFCSQHTMLVKQGGDYVYLPYPDPSTRILGAGCFVDDIVKT DGTLMIERFVSLAIDAYPLTKHPNQEYADVHLYLQYIRKLHDELTHG MLDMYSVMLTNDNTSRWEPFYEAMYTPHTVLAQVAGACVLCNSQT SLRCGACIRRPFLCCKCCYDHVISTSHKLVLVSNPYVCNAPGCDVTDV TQLYLGGMSYYCKSHKPPISFPLCANGQVFGLYKNTCVGSDNVTD FN AIATCDWTNAGDYILANTCTERLKLFAAETLKATEETFKLSYGIATVRE VLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQIGEYTFEKGD

		YGDVAVYRGTTTYKLVNVDYFVLTSHVTMPLSAPTLVPQEHYVRITG LYPTLNISSDEFSSNVANYQKVGMQKYSTLQGGPGTGKSHFAIGLALYY PSARIVYTACSHAAYDALCEKALKYLPIDKCSRIIPARARVECFDKFKV NSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNARLRKHY VYIGDPAQLPAPRTLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCP AEIVDTVSALVYDNKLKAHKDKSAQCCKMFYKGVITHDVSSAINRPQI GVVREFLTRNPAPWRKAVFISPYNSQNAVASKILGLPTQTVDSQSQSEY DYVIFTQTTETAHSCNVNRFNVAITRAKVGILCIMSDDRDLYDKLQFTSL EIPRRNVATLQAENVGTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGL CVDIPGIPKDMTYRRLISMGMGFKMNYQVNGYPNMFITREEAIRHVRA WIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDTPNNTD FSRVSAKPPPGDQFKHLIPLMYKGLPWNVVRKIVQMLSDTLKNLSR VVFVLWAHGFELTSMKYFVKIGPERTCCLCDRRATCFSTASDTYACW HHSIGFDYVYNPFMIDVQQWGFTGNLQSNHDLVYQVHGNHVASCD AIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAAACRKVQHMVKAAL LADKFPVLHDIGNPKAIKCVQADVEWKFYDAQPCSDKAYKIEELFYS YATHSDKFTDGVCLFWNCNVDRYPANSIVCRFDTRVLSNLSLPGCDG GSLYVNKHAFHTPAFDKSAFVNLKQLPFFYYSDSPCESHGKQVVSIDID YVPLKSATCITRCNLGGAVCRHHANEYRLYLDAYNMMISAGFSLWVY KQFDTYNLWNTFTRLQSLNVAFNVNKGHFDGQQGEVPVSIINNTV YTKVDGVDVELFENKTTLPVNVAFELWAKRNIKPVPEVKILNNLGVDI AANTVIWDYKRDAHAHISTIGVCSMTDIKKPTETICAPLTVFFDGRVD GQVDLFRNARNGVLITEGSVKGLQPSVGPKQASLNGVTLIGEAVKTQF NYYKKVDGVVQQLPETYFTQSRNLQEFKPRSQMEIDFLELAMDEFIER YKLEGYAFEHIVYGDFSHSQLGGLHLLIGLAKRFKESPFLEDFIPMDS TVKNYFITDAQTGSSKCVCSVIDLLLDDFVEIHKSQLSVSVKVVKVTID YTEISFMLWCKDGHVETFPKQLQSSQAWQPGVAMPNLYKMORMLLE KCDLQNYGDSATLPKGIMMNVAKYTQLCQYLNLTAVPYNMRVIHF GAGSDKGVAPGTAVLRQWLPTGTLLVSDSLNDFVSDADSTLIGDCAT VHTANKWDLISDMYDPKTKNVTKEKDSKEGFFTYICGFIQKQLALGG SVAIKITEHSWNADLYKLMGHFAWWTAFVTNVNASSSEAFILGCNYL GKPREQIDGYVMHANYIFWRNTNPIQLSSYSLFDMSKFPKLKRGTAVM SLKEGQINDMILSLLSKGRLIHRENNRVVISSDVLVNN
392	SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1)	SADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAK FLKTNCCRFQEKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCPA VAKHDFFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFDEGNCDTL KEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKT VQFCDAMRNAGIVGVLTLDNQDLNGNWDYDFGDFIQTTTPGSGVPVDS YYSLLMPILTLTRALTAESHVDTDLTKPYIKWDLKYDFTEERLKLFDL YFKYWDQTYHPNCVNCLDDRCILHCANFNVLSTVFPTSFGLVRKIF VDGVPFVYSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAADPAMH AASGNLLLDKRTTCFSAALTNVAFQTVKPGNFNKDFYDFAVSKGF FKEGSSVELKHFFFAQDGNAAISDYDYRYNLPTMCDIRQLLFVVEVV DKYFDCYDGGCINANQVIVNNLDKSAGFPFNKWGKARLYYDSMSYE DQDALFAYTKRNVIPITITQMNLYAISAKNRARTVAGVSICTMTNRQ FHQKLLKSIAATRGA TVVIGTSKIFYGGWHNMLKTVYSDVENPHLMG WDYPKCDRAMPNMLRIMASLVLARKHTTCCSLSHRFYRLANECQV LSEMVMCGSLYVKPGGTSSGDAATTAYANSVFNICQAVTANVALLS TDGNKIADKYVRNLQHRLEYECLYRNRDVTDFVNEFYAYLRKHFSM MILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKCWTE TDLTKGPHEFCSQHTMLVKQGDDYVYLPYPDPSRILGAGCFVDDIVKT DGTLMIERFVSLAIDAYPLTKHPNQEYADVFLHYLQYIRKLHDELTHG MLDMYSVMLTNDNTSRYWEPEFYFAMYPHTVLQ

393	SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1)	AVGACVLCNSQTSRLRCGACIRRPFLCCKCCYDHVISTSHKLVL SVN PY VCNAPGCDVTDVTQLYLGGMSYYCKSHKPPISFPLCANGQVFGLYKN TCVGSNDVTDFAIATCDWTNAGDYILANTCTERLKLFAAETLKATEE TFKLSYGIATVREVLSRELHLSWEVGKPRPLNRNYVFTGYRVTKNS KVQIGEYTFEKGDYGDVAVYRGTTTYKLVNGDYFVLTSHVTMPLSAP TLVPQEHYVRITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQGGPGT GKSHFAIGLALYYPSARIVYTACSHAAVDALCEKALKYLPIDKCSRIP ARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNYD LSVVNARLRKHYVYIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKT IGPDMFLGTCRRCPAEIVDVSALVYDNKLLKAHKDKSAQCCKMFYKG VITHDVSSAINRPQIGVVREFLTRNPAWRKAVFISPYNSQNAVASKILG LPTQTVDSQGEYDYVIFTQTTETAHSCNVNRFNVAITRAKVGILCIM SDRDLYDKLQFTSLEIPRRNVATLQAENVGTGLFKDCSKVITGLHPTQAP THLSVDTKFKTEGLCVDIPGPKDMTYRRLISMMGFKMNYQVNGYPN MFITREEAIRHVRAWIGFDVEGCHATREAVGTNPLQLGFSTGVNLVA VPTGYVDTNNTDFSRVSAKPPPGDQFKHLIPLMYKGLPWNVVRKIV QMLSDTLKNLSRVVFLWAHGFELTSMKYFVKIGPERTCCLCDRRA TCFSTASDTYACWHHSIGFDYVYNPFMIDVQQWGFTGNLQSNHDLYC QVHGNAHVASCDAIMTRCLAVHECFVKRVDWTIEYPIIGDELKINAAC RKVQHMVVKAAALLADKFPVLHDIGNPKAIKCVPAQADVEWKFYDAQP CSDKAYKIEELFYSYATHSDKFTDGVCLFWNCNVDRYPANSIVCRFDT RVLSNLNLPGLDGGSLYVNKHAFHTPAFDKSAFVNLKQLPFFYYS DSP CESHGKQVVSIDIDYVPLKSATCITRCNLGGAVCRHHANEYRLYLDAY NMMISAGFSLWVYKQFDTYNLWNTFTRLQ
394	SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1)	SLENVAFNVVNKGHFDGQQGEVPVSIHNTVYTKVDGVDVELFENKT TLPVNVAFELWAKRNIKPVPEVKILNNLGV DIAANTVIWDYKRDAPAH ISTIGVCSMTDIAKKPTETICAPLTVFFDGRVDGQVDLFRNARNGVLITE GSVKGLQPSVGPQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPET YFTQSRNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGD FS HSQLGGLHLLIGLAKRFKESPFELDFIPMDSTVKNYFITDAQTGSSKC VCSVIDLLDDFVEIISQDLSVSVKVKVTIDYTEISFMLWCKDGHVE TFYPKLQSSQAWQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKG IMMNVAKYTQLCQYLNLT LAVPYNMRVIHFGAGSDKGVA PGTA VL RQWLPTGTLLVDSLNDFVSDADSTLIGDCATVHTANKWDLIISDMY DPKTKNVTKENDSKEGFFTYICGFIQQLALGGSVAIKITEHSWNADLY KLMGHFAWWTAFVTNVNASSSEAF LIGCN YLGKPREQIDGYVMHAN YIFWRNTNPIQLSSYSLFDMSKFPLKLRGTAVMSLKEGQINDMILSLLS KGRLLIRENNRVVISSDVLVNN

In some embodiments, any of the above SEQ ID NOS:349-395 or 401, further includes the amino acid residue methionine (M) as the first amino acid residue.

In some embodiments, the antigenic insert is derived from a tumor associated antigen. In some embodiments, the antigenic insert is derived from human mucin-1, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NO: 349, 358-364, or 403, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the antigenic insert is derived from a human cyclin B1 protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NO: 350, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

5 In some embodiments, the antigenic insert is derived from a hepatitis B virus protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NOS: 351-354, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

10 In some embodiments, the antigenic insert is derived from a Plasmodium sp. protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NOS: 355-357, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

15 In some embodiments, the antigenic insert is derived from a Lassa virus protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NOS: 365-366, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

20 In some embodiments, the antigenic insert is derived from a ebola virus protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NOS: 367-368, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the antigenic insert is derived from a Zika virus protein, or a fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid sequence selected from SEQ ID NOS: 369-376, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

25 In some embodiments, the antigenic insert is derived from one or more SARS-CoV-2 proteins or polypeptides, for example, a protein or peptide derived from one or more of the spike (S) (NCBI Reference Sequence YP_009724390), membrane (M) (NCBI Reference Sequence YP_009724393), envelope (E) (NCBI Reference Sequence YP_009724392), nucleoside (N) (NCBI Reference Sequence YP_009724397), ORF1AB (NCBI Reference Sequence YP_009724389), ORF3a (NCBI Reference Sequence YP_009724391), ORF6 (NCBI Reference
30 YP_009724389), ORF3a (NCBI Reference Sequence YP_009724391), ORF6 (NCBI Reference

Sequence YP_009724394), ORF7a (NCBI Reference Sequence YP_009724395), ORF7b (NCBI Reference Sequence YP_009725318), ORF8 (NCBI Reference Sequence YP_009724396), or ORF10 (NCBI Reference Sequence YP_009725255). In certain embodiments, the antigenic insert is derived from SARS-CoV2 S protein, or a variant thereof. In some embodiments, the S protein is expressed as a full-length protein and contains one or more amino acid substitutions compared to NCBI Reference Sequence YP_009724390. In some embodiments, the S protein is derived from the amino acid sequence of SEQ ID NO:377, or fragment thereof, or amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the S protein is expressed as a full-length protein and contains one or more substitutions selected from K417T, E484K or N501Y of SEQ ID NO:377. In some embodiments, the S protein is expressed as a full-length protein and contains the following substitutions: K417T, E484K, and N501Y of SEQ ID NO:377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising substitutions at L452R, T478K, or P681R, or a combination thereof of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising substitutions at L452R, T478K, and P681R of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at N440K, S443A, G476S, E484R, and/or G502P, or combinations thereof of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at one or more of T19R, G142D, R158G, K417N, L452R, T478K, E484Q, D614G, P681R, D950N, E156del, F157del, N501Y, spike deletion 69-70del, spike deletion 144del, A570D, T716I, S982A, D1118H, P681H, L18F, D80A, D215G, 242-244del, R246I, K471N, E484K, A701V, N440K, S443A, G476S, E484R, and G502P, or any combinations thereof of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at T19R, T95I, G142D, E156del, F157del, R158G, L452R, T478K, D614G, P681R, and D950N of SEQ ID NO: 377. In some embodiments, the substitution is K417N. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at T19R, V70F, T95I, G142D, E156del, F157del, R158G, A222V, W258L, K417N, L452R, T478K, D614G, P681R, and D950N of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein

further comprising a substitution at N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at K417N, E484K, N501Y, D614G, and A701V of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at K417T, E484K, N501Y, D614G, and H655Y of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, T478K, D614G, and P681R of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, D614G, and Q677H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, E484Q, D614G, and P681R of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at S477N, E484K, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at R346K, E484K, N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452Q, F490S, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, E484Q, D614G, and P681R of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at Q414K, N450K, ins214TDR, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at V367F, E484K, and Q613H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, N501Y, A653V, and H655Y of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further

comprising a substitution at E484K, N501T, and H655Y of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at P384L, K417N, E484K, N501Y, D614G, and A701V of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at K417N, E484K, N501Y, E516Q, D614G, and A701V of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at S494P, N501Y, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, D614G, and Q677H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, D614G, N679K, and ins679GIAL of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, D614G, and A701V of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at S477N, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at E484K, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at T478K, and D614G of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at N439K, E484K, D614G, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at D614G, E484K, H655Y, K417T,

N501Y, and P681H of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at L452R, T478K, D614G, P681R, and K417N of SEQ ID NO: 377. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the S protein further comprising a substitution at D614G,
 5 E484K, H655Y, N501Y, N679K, and Y449H of SEQ ID NO: 377.

In some embodiments, the S protein is expressed as a full-length protein and has a deletion of one or more spike protein amino acids H69, V70, or Y144, or combinations thereof, of SEQ ID NO: 377. In some embodiments, the S protein is expressed as a full-length protein and contains one or more substitutions selected from D614G, A570D, P681H, T716I, S982A, D1118H, K417N
 10 or K417T, D215G, A701V, L18F, R246I, Y453F, I692V, M1229I, N439K, A222V, S477N, or A376T, or combinations thereof, of SEQ ID NO: 377. In some embodiments, the variant strain is a SARS-CoV2 virus which has a spike protein deletion at amino acids 242-244 of SEQ ID NO: 377. In some embodiments, the S protein is expressed as a full-length protein and contains the following deletions and substitutions: deletion of amino acids 69-70, deletion of amino acid Y144,
 15 amino acid substitution N501Y, amino acid substitution A570D, amino acid substitution D614G, amino acid substitution P681H, amino acid substitution T716I, amino acid substitution S982A, and amino acid substitution D1118H, or SEQ ID NO: 377. In some embodiments, the S protein is expressed as a full-length protein and contains the following deletions and substitutions: N501Y, K417N or K417T, E484K, D80A, A701V, L18F, and amino acid deletion at amino acids 242-244,
 20 of SEQ ID NO: 377. In some embodiments, the S protein is expressed as a full-length protein and contains one or more of the following substitutions: D614G; D936Y; P1263L; L5F; N439K; R21I; D839Y; L54F; A879S; L18F; F1121L; R847K; L452R; T478I; A829T; Q675H; S477N; H49Y; T29I; G769V; G1124V; V1176F; K1073N; P479S; S1252P; Y145 deletion; E583D; R214L; A1020V; Q1208H; D215G; H146Y; S98F; T95I; G1219C; A846V; I197V; R102I; V367F; T572I;
 25 A1078S; A831V; P1162L; T73I; A845S; G1219V; H245Y; L8V; Q675R; S254F; V483A; Q677H; D138H; D80Y; M1237I; D1146H; E654D; H655Y; S50L; S939F; S943P; G485R; Q613H; T76I; V341I; M153I; S221L; T859I; W258L; L242F; P681L; V289I; A520S; V1104L; V1228L; L176F; M1237I; T307I; T716I; L141; M1229I; A1087S; P26S; P330S; P384L; R765L; S940F; T323I; V826L; E1202Q; L1203F; L611F; V615I; A262S; A522V; A688V; A706V;
 30 A892S; E554D; Q836H; T1027I; T22I; A222V; A27S; A626V; C1247F; K1191N; M731I; P26L;

S1147L; S1252F; S255F; V1264L; V308L; D80A; I670L; P251L; P631S; *1274Q; A344S; A771S; A879T; D1084Y; D253G; H1101Y; L1200F; Q14H; Q239K; A623V; D215Y; E1150D; G476S; K77M; M177I; P812S; S704L; T51I; T547I; T791I; V1122L; Y145H; D574Y; G142D; G181V; I834T; N370S; P812L; S12F; T791P; V90F; W152L; A292S; A570V; A647S; A845V; D1163Y; G181R; L84I; L938F; P1143L; P809S; R78M; T1160I; V1133F; V213L; V615F; A831V; D839Y; D839N; D839E; S943P; P1263L; S13I; or V622F; and combinations thereof, of SEQ ID NO: 377.

In some embodiments, the S protein is selected from SEQ ID NOS: 377-384, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains one or more substitutions selected from K417T, E484K or N501Y of SEQ ID NO: 381. In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains the following substitutions: K417T, E484K, and N501Y of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising substitutions at L452R, T478K, or P681R, or a combination thereof of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising substitutions at L452R, T478K, and P681R of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at N440K, S443A, G476S, E484R, and/or G502P, or combinations thereof of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at one or more of T19R, G142D, R158G, K417N, L452R, T478K, E484Q, D614G, P681R, D950N, E156del, F157del, N501Y, spike deletion 69-70del, spike deletion 144del, A570D, T716I, S982A, D1118H, P681H, L18F, D80A, D215G, 242-244del, R246I, K471N, E484K, A701V, N440K, S443A, G476S, E484R, and G502P, or any combinations thereof of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at T19R, T95I, G142D, E156del, F157del, R158G, L452R, T478K, D614G, P681R, and D950N of SEQ ID NO: 381. In some embodiments, the substitution is K417N. In some embodiments, the rMVA contains a nucleic acid sequence which

encodes the Stabilized S protein further comprising a substitution at T19R, V70F, T95I, G142D, E156del, F157del, R158G, A222V, W258L, K417N, L452R, T478K, D614G, P681R, and D950N of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at K417N, E484K, N501Y, D614G, and A701V of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at K417T, E484K, N501Y, D614G, and H655Y of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, T478K, D614G, and P681R of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, D614G, and Q677H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, E484Q, D614G, and P681R of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at S477N, E484K, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at R346K, E484K, N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452Q, F490S, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, E484Q, D614G, and P681R of SEQ ID NO: 8. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at Q414K, N450K, ins214TDR, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA

contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at V367F, E484K, and Q613H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, N501Y, A653V, and H655Y of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, N501T, and H655Y of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at P384L, K417N, E484K, N501Y, D614G, and A701V of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at K417N, E484K, N501Y, E516Q, D614G, and A701V of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at S494P, N501Y, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, D614G, and Q677H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, D614G, N679K, and ins679GIAL of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, D614G, and A701V of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, and D614G of SEQ ID NO: 8. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at S477N, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at E484K, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising

a substitution at E484K, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at T478K, and D614G of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at N439K, E484K, D614G, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at D614G, E484K, H655Y, K417T, N501Y, and P681H of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at L452R, T478K, D614G, P681R, and K417N of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the Stabilized S protein further comprising a substitution at D614G, E484K, H655Y, N501Y, N679K, and Y449H of SEQ ID NO: 381.

In some embodiments, the Stabilized S protein is expressed as a full-length protein and has a deletion of one or more spike protein amino acids H69, V70, or Y144, or combinations thereof, of SEQ ID NO: 381. In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains one or more substitutions selected from D614G, A570D, P681H, T716I, S982A, D1118H, K417N or K417T, D215G, A701V, L18F, R246I, Y453F, I692V, M1229I, N439K, A222V, S477N, or A376T, or combinations thereof, of SEQ ID NO: 381. In some embodiments, the variant strain is a SARS-CoV2 virus which has a spike protein deletion at amino acids 242-244 of SEQ ID NO: 381. In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains the following deletions and substitutions: deletion of amino acids 69-70, deletion of amino acid Y144, amino acid substitution N501Y, amino acid substitution A570D, amino acid substitution D614G, amino acid substitution P681H, amino acid substitution T716I, amino acid substitution S982A, and amino acid substitution D1118H, or SEQ ID NO: 381. In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains the following deletions and substitutions: N501Y, K417N or K417I, E484K, D80A, A701V, L18F, and amino acid deletion at amino acids 242-244, of SEQ ID NO: 381. In some embodiments, the S protein is expressed as a full-length protein and has a deletion of one or more spike protein amino acids H69, V70, or Y144, or combinations thereof, of SEQ ID NO: 381. In some embodiments, the S protein is expressed as a full-length protein and contains one or more

substitutions selected from D614G, A570D, P681H, T716I, S982A, D1118H, K417N, K417T, D215G, A701V, L18F, R246I, Y453F, I692V, M1229I, N439K, A222V, S477N, or A376T, or combinations thereof, of SEQ ID NO: 381. In some embodiments, the spike protein includes a deletion at amino acids 242-244 of SEQ ID NO: 381. In some embodiments, the S protein is expressed as a full-length protein and contains the following deletions and substitutions: deletion of amino acids 69-70, deletion of amino acid Y144, amino acid substitution N501Y, amino acid substitution A570D, amino acid substitution D614G, amino acid substitution P681H, amino acid substitution T716I, amino acid substitution S982A, and amino acid substitution D1118H, of SEQ ID NO: 381. In some embodiments, the S protein is expressed as a full-length protein and contains the following deletions and substitutions: N501Y, K417N or K417T, E484K, D80A, A701V, L18F, and amino acid deletion at amino acids 242-244, of SEQ ID NO: 381. encodes the stabilized S protein further comprising substitutions at L452R, T478K, and P681R of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the stabilized S protein further comprising a substitution at N440K, S443A, G476S, E484R, and/or G502P, or combinations thereof of SEQ ID NO: 381. In some embodiments, the rMVA contains a nucleic acid sequence which encodes the stabilized S protein further comprising a substitution at one or more of T19R, G142D, R158G, K417N, L452R, T478K, E484Q, D614G, P681R, D950N, E156del, F157del, N501Y, spike deletion 69-70del, spike deletion 144del, A570D, T716I, S982A, D1118H, P681H, L18F, D80A, D215G, 242-244del, R246I, K471N, E484K, A701V, N440K, S443A, G476S, E484R, and G502P, or any combinations thereof of SEQ ID NO: 381.

In some embodiments, the Stabilized S protein is expressed as a full-length protein and contains one or more of the following substitutions: D614G; D936Y; P1263L; L5F; N439K; R21I; D839Y; L54F; A879S; L18F; F1121L; R847K; L452R; T478I; A829T; Q675H; S477N; H49Y; T29I; G769V; G1124V; V1176F; K1073N; P479S; S1252P; Y145 deletion; E583D; R214L; A1020V; Q1208H; D215G; H146Y; S98F; T95I; G1219C; A846V; I197V; R102I; V367F; T572I; A1078S; A831V; P1162L; T73I; A845S; G1219V; H245Y; L8V; Q675R; S254F; V483A; Q677H; D138H; D80Y; M1237T; D1146H; E654D; H655Y; S50L; S939F; S943P; G485R; Q613H; T76I; V341I; M153I; S221L; T859I; W258L; L242F; P681L; V289I; A520S; V1104L; V1228L; L176F; M1237I; T307I; T716I; L141; M1229I; A1087S; P26S; P330S; P384L; R765L; S940F; T323I; V826L; E1202Q; L1203F; L611F; V615I; A262S; A522V; A688V; A706V;

A892S; E554D; Q836H; T1027I; T22I; A222V; A27S; A626V; C1247F; K1191N; M731I; P26L; S1147L; S1252F; S255F; V1264L; V308L; D80A; I670L; P251L; P631S; *1274Q; A344S; A771S; A879T; D1084Y; D253G; H1101Y; L1200F; Q14H; Q239K; A623V; D215Y; E1150D; G476S; K77M; M177I; P812S; S704L; T51I; T547I; T791I; V1122L; Y145H; D574Y; G142D; G181V; I834T; N370S; P812L; S12F; T791P; V90F; W152L; A292S; A570V; A647S; A845V; D1163Y; G181R; L84I; L938F; P1143L; P809S; R78M; T1160I; V1133F; V213L; V615F; A831V; D839Y; D839N; D839E; S943P; P1263L; S13I; or V622F; and combinations thereof, of SEQ ID NO: 381.

In some embodiments, the stabilized S protein is expressed as a full-length protein of SEQ ID NO: 378, 379, 380, 381, 382, 383, or 384, or an amino acid sequence 80%, 85%, 90%, 95%, 98%, or 99% homologous thereto.

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus that causes coronavirus disease 2019 (COVID-19). Virus particles include the RNA genetic material and structural proteins needed for invasion of host cells. Once inside the cell the infecting RNA is used to encode structural proteins that make up virus particles, nonstructural proteins that direct virus assembly, transcription, replication and host control and accessory proteins whose function has not been determined. ORF1ab, the largest gene, contains overlapping open reading frames that encode polyproteins PP1ab and PP1a. The polyproteins are cleaved to yield 16 nonstructural proteins, NSP1-16. Production of the longer (PP1ab) or shorter protein (PP1a) depends on a -1 ribosomal frameshifting event. The proteins, based on similarity to other coronaviruses, include the papain-like proteinase protein (NSP3), 3C-like proteinase (NSP5), RNA-dependent RNA polymerase (NSP12, RdRp), helicase (NSP13, HEL), endoRNase (NSP15), 2'-O-Ribose-Methyltransferase (NSP16) and other nonstructural proteins. A description of the various NSPs encoded by ORF1ab can be found, for example, in Arya et al., Structural insights into SARS-CoV-2 proteins. J Mol Biol. 2021 Jan 22; 433(2): 166725, incorporated herein by reference. In some embodiments provided herein, the rMVA antigenic insert is derived from one or more SARS-CoV-2 proteins or polypeptides selected from SEQ ID NOS:377-394.

In some embodiments, the antigenic insert is derived from a Marburg virus protein, or fragment thereof. In some embodiments, the antigenic insert is derived from an amino acid

sequence selected from SEQ ID NO: 395-396, 398, or 400, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In particular embodiments, the encoded polypeptide comprises, in various alternative embodiments, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Antigenic Peptide)), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide)), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_y), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide-Cleavable Peptide)_y), wherein $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, wherein M = methionine, and wherein the Secretion Signal Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 57-90, the Immune Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 1-56, the Cleavable Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 91-127, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen. In some embodiments, the Secretion Signal Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 65 and 66, the Immune Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 1 and 5, and the Cleavable Peptide is selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 93, 120, and 123. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 2-10$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having

an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 4, 5, \text{ or } 6$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 2-10$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, wherein $x = 4, 5, \text{ or } 6$. In some embodiments, the antigenic peptide is selected from SEQ ID NOS: 349-394.

In some embodiments, the antigenic peptide encoded by the polycistronic nucleic acid insert in the rMVA is contained in a chimeric polypeptide that includes a viral glycoprotein signal sequence fused to the N-terminus of the antigenic peptide, and a viral glycoprotein transmembrane domain fused to the C-terminus of the antigenic peptide, and the rMVA is further constructed to encode a viral matrix protein, wherein upon translational cleavage of the antigenic containing chimeric peptide, the viral matrix protein and antigen-viral glycoprotein chimeric polypeptide are capable of forming a non-infectious virus-like particle (VLP). In some embodiments, provided herein is an rMVA viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide) $_x$ (Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, \text{ or more than } 10$, and wherein M = methionine (see, e.g., Fig. 5A & 5B). In some embodiments, the antigenic peptide is contained in a chimeric polypeptide comprising a viral glycoprotein signal sequence fused to the N-terminus of the antigenic peptide, and a viral glycoprotein transmembrane domain fused to the C-terminus of the antigenic peptide, and a cleavable peptide fused to the C-terminus of the viral glycoprotein transmembrane domain, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide) $_x$ (Glycoprotein Signal Peptide-

Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine. In some embodiments, the antigen containing chimeric polypeptide fused to the C-terminus of the last antigen containing chimeric polypeptide does not include a cleavable sequence, for example ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, wherein y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and wherein M = methionine. In some embodiments, the (Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y, wherein y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, can be oriented in the polycistronic nucleic acid insert so that the antigen containing chimeric polypeptide encoding nucleic acid is located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x) or, alternatively ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine. In yet a further embodiment, the polycistronic nucleic acid insert of the rMVA further encodes the viral matrix protein, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)(Viral Matrix Protein)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine (see, e.g., Fig. 6A & 6B). In alternative embodiments, the coding sequences for both the antigen containing chimeric polypeptide and the viral matrix protein are contained in the polycistronic nucleic acid in one or more copies, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y=1, 2, 3, 4,

5, 6, 7, 8, 9, 10, or more than 10, and M = methionine. In some embodiments, the most C-terminus viral matrix protein lacks a cleavable peptide, for example, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x(Viral Matrix Protein-Cleavable Peptide)_y(Viral Matrix Protein)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y=1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine. In some embodiments, the ((Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y), wherein y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine, can be oriented in the polycistronic nucleic acid insert so that the sequences are located 5' of the immune checkpoint inhibitor peptide containing chimeric polypeptides, for example ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x) or, alternatively ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more than 10, and M = methionine.

In particular embodiments, the glycoprotein and matrix proteins are derived from Marburg virus (MARV). In particular embodiments, the glycoprotein is derived from the MARV GP protein (Genbank accession number AFV31202.1). The amino acid sequence of the MARV GP protein is provided as SEQ ID. No. 395 in Table 10 below. In particular embodiments, the MARV GPS domain comprises amino acids 2 to 19 of the glycoprotein (WTTCFFISLILIQGIKTL) (SEQ ID. No. 396, which can be encoded by, for example the MVA optimized nucleic acid sequence of SEQ ID. No. 397), the GPTM domain comprises amino acid sequences 644-673 of the glycoprotein (WWTSDWGVLTNLGILLLLLSIAVLIALSCICRIFTKYIG) (SEQ ID. No. 398, which can be encoded by, for example the MVA optimized nucleic acid sequence of SEQ ID. No. 399), or a nucleic acid sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some embodiments, the MARV GPS signal further comprises a methionine as the first amino acid.

The MARV VP40 amino acid sequence is available at GenBank accession number JX458834, and provided below in Table 10 as SEQ ID. No. 400, which can be encoded by, for example, the MVA optimized nucleic acid sequence of SEQ ID. No. 401, or a nucleic acid sequence 70%, 75%, 80%, 85%, 90%, 95% or more identical thereto. In some embodiments, the MARV VP40 amino acid sequence further comprises a methionine as the first amino acid.

Table 10 - MARV Glycoprotein Domains and VP40 Protein

SEQ ID NO:	Sequence
395 – GP MARV amino acid sequence	WTTCFFISLILIQGIKTLPILEIASNDQPQNVDSVCSGTLQKTEDVHLMGFTLSGQKV ADSPLEASKRWAFRTGVPPKNVEYTEGEEAKTCYNISVTDPSGKSLLLDPPTNVDRD YPKCKTIHHIQGQNPHAQGIALHLWGAFFLYDRIASSTMYRGKVFTEGNIAAMIVN KTVHKMIFSRQGGQYRHMNLTSTNKYWTSSNGTQTNDTGCFGLQEYNSTKNQT CAPSKTPPPPTAHPEIKPTSTPTD ATRLNTNPNSSDDEDLTTS GSGSGEQEPYTTSD AVTKQGLSSTMPPTLSPQPGTPQGGNNTNHSQDAATELDNTNTTAQPPMPSHNT TTISTNNTSKHNLSTLSEPPQNTTNPNTQSMATENEKTSAPPKTTLPPTESPTTEKST NNTKSPTTMEPNTTNGHFTSPSSTPNSTTQHLYFRRKRSILWREGDMFPFLDGLIN APIDFDPVPNTKTIFDESSSSGASAEEDQHASSNISLTL SYLPHTSENTAYSGENEND CDAELRIWSVQEDDLAAGLSWIPFFGPGIEGLYTAGLIKNQNNLVCRLRRLANQTA KSLELLLRVTTEERTFSLINRHAIDFLLTRWGGTCKVLGPDCIGIEDLSRNISEQID QIKKDEQKEGTGWGLGGKWWTSDWGVLTNLGILLLLSIAVLIALSCICRIFTKYIG
396 – Signal peptide amino acid sequence of GP MARV	WTTCFFISLILIQGIKTL
397 – Signal peptide nucleic acid sequence of GP MARV - optimized	TGGACGACCTGCTTCTTCATCTCCCTAATCCTAATCCAGGGAATCAAGACCCTA
398 – Transmembrane domain amino acid sequence of GP MARV	WWTSDWGVLTNLGILLLLSIAVLIALSCICRIFTKYIG
399 – Transmembrane domain nucleic acid sequence of GP MARV – optimized	TGGTGGACATCTGACTGGGGAGTCCTAACGAACCTAGGAATCCTACTACTATT GTCGATCGCGGTCTAATCGCGCTATCCTGTATCTGTAGAATCTTCACCAAGTA CATCGGA
400 – MARVVP 40 amino acid sequence	ASSSNYNTYMQYLNPPPYADHGANQLIPADQLSNQHGITPNYVGDNLDDQFKGN VCHAFTLEAIIDISAYNERTVKGVPAWLPLGIMSNFEYPLAHTVAALLTGSYTITQF THNGQKFVRVNRLGTGIPAHPLRMLREGNQAFIQNMVIPRNFSTNQFTYNLTNLVL SVQKLPPDDAWRPSKDKLIGNTMHPAISHPNLPPIVLPVKKQAYRQHKNPNNGPL LAISGILHQLRVEKVPEKTSLFRISLPADMFSVKEGMMKKRGESSPVVYFQAPENFP LNGFNRRQVVLAYANPTLSAI
401 – MARVVP 40 nucleic acid	GCGTCTAGTTCTAATTATAACTTATATGCAATATCTAAATCCACCACCATAT GCGGATCATGGTGCTAATCAACTAATTCAGCGGATCAACTATCTAATCAACA TGGAATTACACCAAATTATGTTGGAGATCTAAATCTAGATGATCAGTTTAAAG

sequence optimized	-	GAAATGTTTGTTCATGCGTTTACACTAGAAGCGATTATTGATATTTCTGCGTATA ATGAAAGAACAGTAAAGGTGTACCAGCTTGGCTACCACTAGGAATTATGTCT AATTTTGAATATCCACTAGCGCATACAGTAGCGGCGCTATTGACAGGATCTTAT ACAATTACACAGTTTACACATAATGGACAAAAGTTTGTTAGAGTAAATAGACT AGGAACTGGAATACCAGCGCATCCACTAAGAATGCTAAGAGAAGGAAATCAA GCTTTTATTCAAAATATGGTTATTCCAAGAAATTTCTCTACAAATCAGTTTACTT ATAATCTAACTAATCTAGTACTATCTGTACAAAAGCTACCAGATGATGCTTGGG GACCATCTAAAGATAAACTAATTGGAAATACAATGCATCCAGCGATTCTATT CATCCAAATCTACCACCAATAGTACTACCAACTGTAAAGAAACAAGCGTATAG ACAACATAAGAAATCCAATAATGGACCACTATTGGCGATTCTGGAATTCTACA TCAACTAAGAGTAGAAAAGTACCAGAAAAGACATCTTTGTTTAGAATTTCTCT ACCAGCGGATATGTTTCTGTAAAAGAAGGAATGATGAAGAAAAGAGGAGAAT CTTCTCCAGTAGTATATTTTCAAGCGCCAGAAAATTTCCATTGAATGGTTTAA ATAATAGACAAGTAGTACTAGCGTATGCGAATCCAACACTATCTGCGATATAA TAA
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In some embodiments, any of the above SEQ ID NOS:395-396 and 400, further includes the amino acid residue methionine (M) as the first amino acid residue. In some embodiments, any of the above SEQ ID NOS:397 ad 401, further includes the nucleic acid codon ATG as the first codon of the coding sequence. In particular embodiments, the encoded polypeptide comprises, in various alternative embodiments, ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain)), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain)), ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x), ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)(Viral Matrix Protein)), ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-

Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y), ((M)(Secretion Signal Peptide-
 Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-
 Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_x(Viral Matrix
 Protein-Cleavable Peptide)_y(Viral Matrix Protein)), ((M)(Glycoprotein Signal Peptide-Antigenic
 5 Peptide-Glycoprotein Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-
 Cleavable Peptide)_y), ((M)(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein
 Transmembrane Domain-Cleavable Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion
 Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x), or ((M)(Glycoprotein
 Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Domain-Cleavable
 10 Peptide)_y(Viral Matrix Protein-Cleavable Peptide)_y(Secretion Signal Peptide-Immune Checkpoint
 Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide- Immune Checkpoint Inhibitor
 Peptide)), wherein $x = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$, or more than 10, $y = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10$,
 or more than 10, M = methionine, and wherein the Secretion Signal Peptide is selected from a
 peptide having an amino acid sequence selected from SEQ ID NOS: 57-90, the Immune
 15 Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid sequence selected
 from SEQ ID NOS: 1-56, the Cleavable Peptide is selected from a peptide having an amino acid
 sequence selected from SEQ ID NOS: 91-127, the Glycoprotein Signal Peptide is a peptide having
 the amino acid sequence of SEQ ID NO. 396, the Glycoprotein Transmembrane Domain is a
 peptide having the amino acid sequence of SEQ ID NO. 398, the Viral Matrix Protein, when
 20 present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide
 is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or
 toxoid, or alternatively, a tumor associated antigen. In some embodiments, the antigenic peptide
 is selected from SEQ ID NOS: 349-394. In some embodiments, the Secretion Signal Peptide is
 selected from a peptide having an amino acid sequence selected from SEQ ID NOS: 65 and 66,
 25 the Immune Checkpoint Inhibitor Peptide is selected from a peptide having an amino acid
 sequence selected from SEQ ID NOS: 1 and 5, the Cleavable Peptide is selected from a peptide
 having an amino acid sequence selected from SEQ ID NOS: 93, 120, and 123, the Glycoprotein
 Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO. 396, the Glycoprotein
 Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO. 398, and the
 30 Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO:

400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394, and wherein $x = 1-10$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, and the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 1, the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394, and wherein $x = 4, 5$, or

6. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394, wherein $x =$ 1-10. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, the antigenic peptide is selected from SEQ ID NOS: 349-394, wherein $x \geq 4$. In some embodiments, the Secretion Signal Peptide is a peptide having an amino acid sequence of SEQ ID NO: 66, the Immune Checkpoint Inhibitor Peptide is a peptide having an amino acid sequence of SEQ ID NO: 5, the Cleavable Peptide is a peptide having an amino acid sequence of SEQ ID NO: 123, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO: 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394, wherein $x = 4, 5, \text{ or } 6$. In some embodiments, the encoded polypeptide comprises SEQ ID NOS: 325 or 333, the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO: 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid

sequence of SEQ ID NO. 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO: 400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus, bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394. In some
5 embodiments, the encoded polypeptide comprises SEQ ID NO. 329 or 337, , the Glycoprotein Signal Peptide is a peptide having the amino acid sequence of SEQ ID NO. 396, the Glycoprotein Transmembrane Domain is a peptide having the amino acid sequence of SEQ ID NO. 398, and the Viral Matrix Protein, when present, is a peptide having the amino acid sequence of SEQ ID NO:
400, and the antigenic peptide is a peptide derived from an infectious agent, for example a virus,
10 bacteria, parasite, fungus, or toxoid, or alternatively, a tumor associated antigen, or the antigenic peptide is selected from SEQ ID NOS: 349-394.

In alternative embodiments, the rMVA viral vectors of the present invention, in addition to the ability to express multiple immune checkpoint inhibitor peptides, may further be constructed to encode and express one or more antigen peptides encoded on one or more separate nucleic acid
15 inserts. In some embodiments, the nucleic acid sequence encoding multiple immune checkpoint inhibitor peptides as described herein is inserted into one gene locus of the rMVA, and one or more heterologous nucleic acid sequences encoding an antigenic peptide is inserted into a separate gene locus of the rMVA. The one or more antigen peptides can be derived from any of the targets described in the section Antigenic Targets, incorporated into this section in its entirety for all
20 purposes. In some embodiments, the antigen peptides are derived from any of the amino acid sequences selected from SEQ ID NOS: 349-396, 398, or 400, or a fragment derived therefrom, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. If inserted as a separate nucleic acid insert, a start codon encoding the amino acid residue methionine (M) can be included as the first residue of the antigen peptides are derived from any of the amino
25 acid sequences selected from SEQ ID NOS: 349-396, 398, or 400, or a fragment derived therefrom, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

In certain embodiments, the rMVA, in addition to the polycistronic nucleic acid encoding the immune checkpoint inhibitor polypeptides described herein, further encodes an antigenic peptide comprising a chimeric peptide comprising an extracellular domain of an antigen and a
30 transmembrane domain of a viral glycoprotein, and further encodes a viral matrix protein, wherein

the chimeric peptide and viral matrix protein, when expressed, are capable of forming a virus-like particle (VLP) *in vivo*. In some embodiments, the transmembrane domain of the viral glycoprotein is derived from the amino acid of SEQ ID NO: 398, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some

5 embodiments, the viral matrix protein is derived from Marburg virus VP40 protein, for example, as provided in SEQ ID NO: 404, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto. In some embodiments, the rMVA encodes for the amino acid sequence of SEQ ID NO:329, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, the amino acid sequence of

10 SEQ ID NO: 402, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto, and the amino acid sequence of SEQ ID NO:404, or a fragment thereof, or an amino acid sequence at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical thereto.

15 **Table 11 –MUC-Insert Sequences**

SEQ ID NO: 402 - MUC-1– MARV GPTM- optimized nucleic acid sequence	ATGACACCTGGAACACAATCTCCATTCTTCCTACTACTACTATTGACAGTACTAACA GTAGTAACAGGATCTGGACATGCGTCTAGTACACCAGGTGGAGAGAAGGAAACAT CTGCGACTCAAAGATCTTCTGTACCATCTTCTACAGAGAAGAATGCGGTATCTATG ACATCTAGTGTACTATCTTCTCATTCTCCTGGATCTGGATCTTCTACTACACAAGGA CAAGATGTAACTAGCGCCAGCTACAGAACAGCTTCTGGATCTGCTGCTACTTG GGGTCAAGATGTTACTTCTGTTCCAGTAACAAGACCAGCGCTAGGATCTACAACAC CACCAGCGCATGATGTAACAAGTGCGCCAGATAATAAGCCAGCGCCTGGTTCTACT GCTCCACCAGCTCATGGTGTACTTTCAGCGCCTGATACAAGACCCGACCCGGATC TACCGCTCCGCCTGCACACGGCGTCACATCTGCTCCCGACACTCGTCCAGCTCCTGG TAGCACAGCACCTCCAGCGCATGGAGTAACCAGTGCACCAGATACCCGACCTGCGC CGGGCAGTACTGCCCCACCGGCCCACGGGGTGACGAGCGCCCCGGACACGCGCCC AGCTCCAGGGTCAACGGCGCCCCCTGCTCATGGTGTACAAAGTGCACCTGATAATA GACCTGCGTTGGGATCTACTGCGCCTCCAGTTCATAATGTAACATCAGCGTCTGGA AGTGCGTCTGGTTCTGCGTCTACATTGGTTCATAATGGTACATCTGCGAGAGCGAC AACAACTCCAGCGTCTAAGTCTACACCATCTCTATTCCATCTCATCATTCTGATAC ACCAACAACATTGGCGAGTCATTCTACAAAGACAGATGCGAGTTCTACACATCATT CTACTGTACCACCACTAACATCTTCTAATCATAGTACATCTCCACAACCTATCTACTG GTGTATCTTTCTTCTTCCTATCCTTTTCATATTCTAATCTACAGTTCAATTCTAGTTT GGAAGATCCATCTACAGATTATTATCAAGAACTACAAAGAGATATTTCTGAAATGT TTCTACAAATATATAAACAAGGAGGATTTCTAGGACTATCTAATATTAAGTTTAGA CCAGGATCTGTAGTAGTTCAACTAACTCTAGCGTTTAGAGAAGGTACTATTAATGT ACATGATGTTGAAACACAGTTTAATCAATATAAGACAGAAGCGGCGTCTAGATATA ATCTAACAATTTCTGATGTATCTGTATCTGATGTTCCATTTCATTCTCTGCGCAATC TGGTGTGTTGTATGGTGGACATCTGATTGGGGAGTACTAACTAATCTAGGAATTC TACTATTGCTATCTATTGCGGTACTAATTGCGCTATCTTGATATGTAGAAGAAAGA ATTATGGACAACCTAGATATTTTCCCAGCGAGAGATACTTATCATCCAATGTCTGAA TATCCAACATATCATACACATGGAAGATATGTACCACCTTCTTCAACAGATAGATC
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[illegible]

Sequence Optimization

One or more nucleic acid sequences comprising the polycistronic nucleic acid insert of the rMVA provided herein may be optimized for use in an MVA vector. Optimization includes codon optimization, which employs silent mutations to change selected codons from the native sequences into synonymous codons that are optimally expressed by the host-vector system. Other types of optimization include the use of silent mutations to interrupt homopolymer stretches or transcription terminator motifs. Each of these optimization strategies can improve the stability of the gene, improve the stability of the transcript, or improve the level of protein expression from the sequence. In exemplary embodiments, the number of homopolymer stretches in the heterologous

DNA insert sequence will be reduced to stabilize the construct. A silent mutation may be provided for anything similar to a vaccinia termination signal.

In exemplary embodiments, the sequences are codon optimized for expression in MVA; sequences with runs of > 5 deoxyguanosines, > 5 deoxycytidines, > 5 deoxyadenosines, and > 5 deoxythymidines are interrupted by silent mutation to minimize loss of expression due to frame shift mutations.

In particular, the nucleic acid for insertion can be optimized by codon optimizing the original DNA sequence. For example, the “Invitrogen GeneArt Gene Software” can be used to codon optimize the DNA sequence. To fully optimize the gene sequence, homopolymer sequences (G/C or T/A rich areas) are interrupted via silent mutation(s). To the extent present in the nucleic acid insert sequence, the MVA transcription terminator (T5NT (UUUUUNU)) is interrupted via silent mutation(s). Further optimizations can include, for example, adding a Kozak sequence (GCCACC/ATG), adding a second stop codon, and adding a vaccinia virus transcription terminator, specifically “TTTTTAT”, or variations and/or combinations thereof.

Pharmaceutical Compositions

The recombinant MVA viral vectors of the present invention are readily formulated as pharmaceutical compositions for veterinary or human use, either alone or in combination. The pharmaceutical composition may comprise a pharmaceutically acceptable diluent, excipient, carrier, or adjuvant, or, in an alternative embodiment, one or more antigenic agents, for example an antigen derived from an infectious disease or, in an alternative embodiment, a tumor associated antigen.

In one embodiment, the rMVA is used as an adjuvant effective in enhancing immunogenicity to an infectious agent to protect against and/or treat an infection, the rMVA comprising a polycistronic nucleic acid insert that encodes at least two or more immune checkpoint inhibitor peptides as described herein. In alternative embodiments, the rMVA is used as a vaccine effective in enhancing immunogenicity to an infectious agent to protect against and/or treat an infection, the rMVA comprising a polycistronic nucleic acid insert that encodes at least two or more immune checkpoint inhibitor peptides and one or more antigenic peptides as described herein.

As used herein, the phrase "pharmaceutically acceptable carrier" encompasses any of the standard pharmaceutical carriers, such as those suitable for parenteral administration, such as, for example, by intramuscular, intraarticular (in the joints), intravenous, intradermal, intraperitoneal, and subcutaneous routes. Examples of such formulations include aqueous and non-aqueous, isotonic sterile injection solutions, which contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and nonaqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. One exemplary pharmaceutically acceptable carrier is physiological saline. Carriers include excipients and diluents and must be of sufficiently high purity and sufficiently low toxicity to render them suitable for administration to the patient being treated. The carrier can be inert or it can possess pharmaceutical benefits of its own. The amount of carrier employed in conjunction with the compound is sufficient to provide a practical quantity of material for administration per unit dose of the compound.

Other physiologically acceptable diluents, excipients, carriers, or additional adjuvants and their formulations are known to those skilled in the art.

In some embodiments, additional adjuvants are used as further immune response enhancers. In various embodiments, the additional immune response enhancer is selected from the group consisting of alum-based adjuvants, oil based adjuvants, Specol, RIBI, TiterMax, Montanide ISA50 or Montanide ISA 720, GM-CSF, nonionic block copolymer-based adjuvants, dimethyl dioctadecyl ammoniumbromide (DDA) based adjuvants AS-1 , AS-2, Ribi Adjuvant system based adjuvants, QS21 , Quil A, SAF (Syntex adjuvant in its microfluidized form (SAF-m), dimethyl-dioctadecyl ammonium bromide (DDA), human complement based adjuvants m. vaccae, ISCOMS, MF-59, SBAS-2, SBAS-4, Enhanzyn®, RC-529, AGPs, MPL-SE, QS7, Escin; Digitonin; Gypsophila; and Chenopodium quinoa saponins.

The compositions utilized in the methods described herein can be administered by a route selected from, e.g., parenteral, intramuscular, intraarterial, intravascular, intravenous, intraperitoneal, subcutaneous, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, topical administration, and oral administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered and the severity of the condition being treated). Formulations suitable for oral

administration may consist of liquid solutions, such as an effective amount of the composition dissolved in a diluent (e.g., water, saline, or PEG-400), capsules, sachets or tablets, each containing a predetermined amount of the vaccine. The pharmaceutical composition may also be an aerosol formulation for inhalation, e.g., to the bronchial passageways. Aerosol formulations may be mixed
5 with pressurized, pharmaceutically acceptable propellants (e.g., dichlorodifluoromethane, propane, or nitrogen).

For the purposes of this invention, pharmaceutical compositions suitable for delivering a therapeutic or biologically active agent can include, e.g., tablets, gelcaps, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels, hydrogels, oral gels, pastes, eye drops,
10 ointments, creams, plasters, drenches, delivery devices, suppositories, enemas, injectables, implants, sprays, or aerosols. Any of these formulations can be prepared by well-known and accepted methods of art. See, for example, Remington: The Science and Practice of Pharmacy (21st ed.), ed. A. R. Gennaro, Lippincott Williams & Wilkins, 2005, and Encyclopedia of Pharmaceutical Technology, ed. J. Swarbrick, Informa Healthcare, 2006, each of which is hereby
15 incorporated by reference.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the vaccine dissolved in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the vaccine, as liquids, solids, granules or gelatin; (c) suspensions in an appropriate liquid; (d) suitable emulsions; and (e)
20 polysaccharide polymers such as chitins. The vaccine, alone or in combination with other suitable components, may also be made into aerosol formulations to be administered via inhalation, e.g., to the bronchial passageways. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Suitable formulations for rectal administration include, for example, suppositories, which
25 consist of the vaccine with a suppository base. Suitable suppository bases include natural or synthetic triglycerides or paraffin hydrocarbons. In addition, it is also possible to use gelatin rectal capsules which consist of a combination of the vaccine with a base, including, for example, liquid triglycerides, polyethylene glycols, and paraffin hydrocarbons. The vaccines of the present invention may also be co-administered with cytokines to further enhance immunogenicity. The

cytokines may be administered by methods known to those skilled in the art, e.g., as a nucleic acid molecule in plasmid form or as a protein or fusion protein.

In addition to the active compounds, the pharmaceutical formulations can contain other additives, such as pH-adjusting additives. In particular, useful pH-adjusting agents include acids, such as hydrochloric acid, bases or buffers, such as sodium lactate, sodium acetate, sodium phosphate, sodium citrate, sodium borate, or sodium gluconate. Further, the formulations can contain antimicrobial preservatives. Useful antimicrobial preservatives include methylparaben, propylparaben, and benzyl alcohol. An antimicrobial preservative is typically employed when the formulations is placed in a vial designed for multi-dose use. The pharmaceutical formulations described herein can be lyophilized using techniques well known in the art.

When aqueous suspensions and/or elixirs are desired for oral administration, the compositions of the presently disclosed matter can be combined with various sweetening agents, flavoring agents, coloring agents, emulsifying agents and/or suspending agents, as well as such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

In yet another embodiment, the pharmaceutical composition is provided as an injectable, stable, sterile formulation comprising a rMVA as described herein, in a unit dosage form in a sealed container. The rMVA can be provided in the form of a lyophilizate, which is capable of being reconstituted with a suitable pharmaceutically acceptable carrier to form liquid formulation suitable for injection thereof into a host.

Classes of carriers include, but are not limited to binders, buffering agents, coloring agents, diluents, disintegrants, emulsifiers, flavorants, glidants, lubricants, preservatives, stabilizers, surfactants, tableting agents, and wetting agents. Some carriers may be listed in more than one class, for example vegetable oil may be used as a lubricant in some formulations and a diluent in others. Pharmaceutically acceptable carriers are carriers that do not cause any severe adverse reactions in the human body when dosed in the amount that would be used in the corresponding pharmaceutical composition. Exemplary pharmaceutically acceptable carriers include sugars, starches, celluloses, powdered tragacanth, malt, gelatin; talc, and vegetable oils. Optional active agents may be included in a pharmaceutical composition, which do not substantially interfere with the activity of the morphic form or pharmaceutical composition of the present invention.

Formulations suitable for administration to the lungs can be delivered by a wide range of passive breath driven and active power driven single/-multiple dose dry powder inhalers (DPI). The devices most commonly used for respiratory delivery include nebulizers, metered-dose inhalers, and dry powder inhalers. Several types of nebulizers are available, including jet nebulizers, ultrasonic nebulizers, and vibrating mesh nebulizers. Selection of a suitable lung delivery device depends on parameters, such as nature of the drug and its formulation, the site of action, and pathophysiology of the lung.

In certain embodiments, a pharmaceutical composition comprising a rMVA described herein is administered as a pharmaceutical composition comprising one or more excipients from the Handbook of Pharmaceutical Excipients 9th Edition (or earlier).

Additional-non-limiting examples of pharmaceutically acceptable excipients include vegetable oil, an animal oil, a fish oil or a mineral oil. For example an oil selected from the group consisting of medium chain fatty acid triglyceride, amaranth oil, apricot oil, apple oil, argan oil, artichokes oil, avocado oil, almond oil, acai berry extract, arachis oil, buffalo pumpkin oil, borage seed oil, borage oil, babassu oil, coconut oil, corn oil, cottonseed oil (cotton seed oil), cashew oil, carob oil, Coriander oil, camellia oil (Camellia oil), Cauliflower oil, cape chestnut oil, cassis oil, deer oil, evening primrose oil, grape syrup Oila oil (hibiscus oil), grape seed oil, gourd oil, hazelnut oil, hemp oil, kapok oil, krill oil, linseed oil, macadamia nut oil, Mongolia oil, moringa oil, malula oil, meadowfoam oil, mustard oil, niger seed oil, olive oil, okrao oil Hibiscus oil), palm oil, palm kernel oil, peanut oil, pecan oil, pine oil, pistachio oil, pumpkin oil, papaya oil, perilla oil (perilla oil), poppy seed oil, prune oil, saw palm oil, quinoa oil, rapeseed oil, rice germ oil, rice bran oil, rice oil, rarelman cheer oil, Safflower oil (safflower oil), soybean oil, sesame oil, sunflower oil, thistle oil, tomato oil, wheat germ oil, walnut oil, watermelon oil, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), vitamin A oil, vitamin D oil, vitamin E oil, vitamin K oil, and derivatives thereof; and glycerophospholipids such as lecithin, and any combination thereof.

In certain embodiments, the excipient in the present invention may be a liquid (such as a fat oil) or a solid (a fat or the like) at room temperature.

Methods of Use

The compositions of the invention can be used as adjuvants to enhance, or vaccines for inducing, an immune response.

In exemplary embodiments, the present invention provides an adjuvant for use in a method of preventing an infection in a subject in need thereof (e.g., an unexposed subject), said method comprising administering the composition of the present invention to the subject in combination with an effective amount of an antigenic agent. Alternatively, the present invention provides a vaccine for use in a method of preventing an infection in a subject in need thereof (e.g., an unexposed subject), said method comprising administering the composition of the present invention to the subject. The result of the method is that the subject is partially or completely immunized against the infection.

In other exemplary embodiments, the present invention provides an adjuvant for use in a method of treating a condition such as a cancer in a subject in need thereof, said method comprising administering the composition of the present invention to the subject in combination with an effective amount of an tumor associated antigenic agent. Alternatively, the present invention provides a vaccine for use in a method of treating a condition such as a cancer in a subject in need thereof, said method comprising administering the composition of the present invention to the subject.

In exemplary embodiments, the present invention provides an adjuvant for use in a method of a treating an infectious agent (e.g., an exposed subject, such as a subject who has been recently exposed but is not yet symptomatic, or a subject who has been recently exposed and is only mildly symptomatic), said method comprising administering the composition of the present invention to the subject in combination with a therapeutically effective amount of an antigenic agent targeting the infectious agent. In exemplary embodiments, the present invention provides a vaccine for use in a method of a treating an infectious agent (e.g., an exposed subject, such as a subject who has been recently exposed but is not yet symptomatic, or a subject who has been recently exposed and is only mildly symptomatic), said method comprising administering the composition of the present invention to the subject. The result of treatment is a subject that has an improved therapeutic profile. The result is an improved therapeutic profile. In some instances, as compared with an equivalent untreated control, treatment may ameliorate a disorder or a symptom thereof by, e.g., 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100% as measured by any

standard technique. In some instances, treating can result in the inhibition of infectious agent replication, a decrease in infectious agent titers or load, eradication or clearing of the infectious agent. In other embodiments, treatment may result in amelioration of one or more symptoms of the infection, including any symptom identified above. According to this embodiment, 5 confirmation of treatment can be assessed by detecting an improvement in or the absence of symptoms.

A subject to be treated according to the methods described may be one who has been diagnosed by a medical practitioner as having such a condition. Diagnosis may be performed by any suitable means. A subject in whom the development of an infection is being prevented may or 10 may not have received such a diagnosis. One skilled in the art will understand that a subject to be treated according to the present invention may have been identified using standard tests or may have been identified, without examination, as one at high risk due to the presence of one or more risk factors (e.g., exposure to 2019-nCoV, etc.).

In other embodiments, treatment may result in reduction or elimination of the ability of the 15 subject to transmit the infection to another, uninfected subject. Confirmation of treatment according to this embodiment is generally assessed using the same methods used to determine amelioration of the disorder, but the reduction in viral titer or viral load necessary to prevent transmission may differ from the reduction in viral titer or viral load necessary to ameliorate the disorder.

20 In one embodiment, the present invention is a method of inducing an immune response in a subject (e.g., a human) by administering to the subject a recombinant MVA viral vector described herein encoding two or more immune checkpoint inhibitor peptides in combination with an antigenic agent. The immune response may be a cellular immune response or a humoral immune response, or a combination thereof.

25 The composition may be administered, e.g., by injection (e.g., intramuscular, intraarterial, intravascular, intravenous, intraperitoneal, or subcutaneous).

It will be appreciated that more than one route of administering the vaccines of the present invention may be employed either simultaneously or sequentially (e.g., boosting). In addition, the adjuvants or vaccines of the present invention may be employed in combination with traditional 30 immunization approaches such as employing protein antigens, vaccinia virus and inactivated virus,

as vaccines. Thus, in one embodiment, the vaccines of the present invention are administered to a subject (the subject is "primed" with a vaccine of the present invention) and then a traditional vaccine is administered (the subject is "boosted" with a traditional vaccine). In another embodiment, a traditional vaccine is first administered to the subject followed by administration of the adjuvant or vaccine of the present invention. In yet another embodiment, a traditional vaccine and an adjuvant or vaccine of the present invention are co-administered.

While not to be bound by any specific mechanism, it is believed that upon inoculation with a pharmaceutical composition as described herein, the immune system of the host responds to the adjuvant in combination with an antigenic agent, or vaccine by producing antibodies, both secretory and serum, specific for the infectious agent or tumor associated antigen; and by producing a cell-mediated immune response specific for the targeted agent. As a result of the vaccination, the host becomes at least partially or completely immune to the targeted infection, or resistant to developing moderate or severe disease caused by the targeted infection.

In some embodiments, administration is one time. In some embodiments, administration is repeated at least twice, at least 3 times, at least 4 times, at least 5 times, at least 6 times, at least 7 times, at least 8 times, or more than 8 times.

In one embodiment, administration is repeated twice.

In one embodiment, about 2-8, about 4-8, or about 6-8 administrations are provided.

In one embodiment, about 1-4-week, 2-4 week, 3-4 week, 1 week, 2 week, 3 week, 4 week or more than 4 week intervals are provided between administrations.

In one specific embodiment, a 4-week interval is used between 2 administrations.

Dosage

The adjuvants in combination with an antigenic agent or vaccines are administered in a manner compatible with the dosage formulation, and in such amount as will be therapeutically effective, immunogenic and protective. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the immune system of the individual to synthesize antibodies, and, if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and may be monitored on a patient-by-patient basis. However, suitable dosage ranges

are readily determinable by one skilled in the art and generally range from about 5.0×10^6 TCID₅₀ to about 5.0×10^9 TCID₅₀. The dosage may also depend, without limitation, on the route of administration, the patient's state of health and weight, and the nature of the formulation.

The pharmaceutical compositions of the invention are administered in such an amount as will be therapeutically effective to enhance the immunogenicity of a targeted antigen. The dosage administered depends on the subject to be treated (e.g., the manner of administration and the age, body weight, capacity of the immune system, and general health of the subject being treated). The composition is administered in an amount to provide a sufficient level of expression that enhances or elicits an immune response without undue adverse physiological effects. Preferably, the composition of the invention is administered at a dosage of, e.g., between 1.0×10^4 and 9.9×10^{12} TCID₅₀ of the viral vector, preferably between 1.0×10^5 TCID₅₀ and 1.0×10^{11} TCID₅₀ pfu, more preferably between 1.0×10^6 and 1.0×10^{10} TCID₅₀ pfu, or most preferably between 5.0×10^6 and 5.0×10^9 TCID₅₀. The composition may include, e.g., at least 5.0×10^6 TCID₅₀ of the viral vector (e.g., 1.0×10^8 TCID₅₀ of the viral vector). A physician or researcher can decide the appropriate amount and dosage regimen.

The composition of the method may include, e.g., between 1.0×10^4 and 9.9×10^{12} TCID₅₀ of the viral vector, preferably between 1.0×10^5 TCID₅₀ and 1.0×10^{11} TCID₅₀ pfu, more preferably between 1.0×10^6 and 1.0×10^{10} TCID₅₀ pfu, or most preferably between 5.0×10^6 and 5.0×10^9 TCID₅₀. The composition may include, e.g., at least 5.0×10^6 TCID₅₀ of the viral vector (e.g., 1.0×10^8 TCID₅₀ of the viral vector). The method may include, e.g., administering the composition to the subject two or more times.

The term "effective amount" is meant the amount of a composition administered to improve, inhibit, or ameliorate a condition of a subject, or a symptom of a disorder, in a clinically relevant manner (e.g., improve, inhibit, or ameliorate infection by arenavirus or provide an effective immune response to infection). Any improvement in the subject is considered sufficient to achieve treatment. Preferably, an amount sufficient to treat is an amount that prevents the occurrence or one or more symptoms of, or is an amount that reduces the severity of, or the length of time during which a subject suffers from, one or more symptoms of a targeted infection or cancer (e.g., by at least 10%, 20%, or 30%, more preferably by at least 50%, 60%, or 70%, and

most preferably by at least 80%, 90%, 95%, 99%, or more, relative to a control subject that is not treated with a composition of the invention).

In some instances, it may be desirable to combine the rMVA of the present invention with immunogenic compositions which induce protective responses to more than one infectious agents, particularly other viruses. For example, the adjuvant compositions of the present invention can be administered simultaneously, separately or sequentially with other genetic immunization vaccines such as those for influenza (Ulmer, J. B. et al., Science 259: 1745-1749 (1993); Raz, E. et al., PNAS (USA) 91 :9519-9523 (1994)), malaria (Doolan, D. L. et al., J. Exp. Med. 183:1739-1746 (1996); Sedegah, M. et al., PNAS (USA) 91 :9866-9870 (1994)), and tuberculosis (Tascon, R. C. et al., Nat. Med. 2:888-892 (1996)).

Administration

As used herein, the term "administering" refers to a method of giving a dosage of a pharmaceutical composition of the invention to a subject. The compositions utilized in the methods described herein can be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, rectal, topical administration, and oral administration. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, intraarterial, intravascular, and intramuscular administration. The preferred method of administration can vary depending on various factors (e.g., the components of the composition being administered, and the severity of the condition being treated).

Administration of the pharmaceutical compositions (e.g., adjuvant or vaccines) of the present invention can be by any of the routes known to one of skill in the art. Administration may be by, e.g., intramuscular injection. The compositions utilized in the methods described herein can also be administered by a route selected from, e.g., parenteral, dermal, transdermal, ocular, inhalation, buccal, sublingual, perilingual, nasal, rectal, topical administration, and oral administration. Parenteral administration includes intravenous, intraperitoneal, subcutaneous, and intramuscular administration. The preferred method of administration can vary depending on various factors, e.g., the components of the composition being administered, and the severity of the condition being treated.

In addition, single or multiple administrations of the compositions of the present invention may be given to a subject. For example, subjects who are particularly susceptible to the targeted antigenic agent may require multiple treatments to establish and/or maintain protection against the virus. Levels of induced immunity provided by the pharmaceutical compositions described herein can be monitored by, e.g., measuring amounts of neutralizing secretory and serum antibodies. The dosages may then be adjusted or repeated as necessary to maintain desired levels of protection against viral infection.

Embodiments

Provided herein are at least the following embodiments:

1. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x, wherein x = 2-10, and M is methionine.
2. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1-10, and M is methionine.
3. The rMVA of embodiments 1 or 2, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-56, or an amino acid sequence at least 95% identical thereto.
4. The rMVA of embodiments 1-3, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS: 1-15, or an amino acid sequence at least 95% identical thereto.
5. The rMVA of embodiments 1-4, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS: 1 or 5, or an amino acid sequence at least 95% identical thereto.
6. The rMVA of embodiments 1-5, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence at least 95% identical thereto.

7. The rMVA of embodiments 1-5, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence at least 95% identical thereto.
8. The rMVA of embodiments 1-7, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS: 57-90, or an amino acid sequence at least 95% identical thereto.
9. The rMVA of embodiments 1-8, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NO: 65, or an amino acid sequence at least 95% identical thereto.
10. The rMVA of embodiments 1-8, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NO: 66, or an amino acid sequence at least 95% identical thereto.
11. The rMVA of embodiments 1-10, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS: 91-127, or an amino acid sequence at least 95% identical thereto.
12. The rMVA of embodiments 1-11, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS: 93, 120, and 123, or an amino acid sequence at least 95% identical thereto.
13. The rMVA of embodiments 1-11, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91).
14. The rMVA of embodiments 1-11, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = R, K, or H (SEQ ID NO: 92).
15. The rMVA of embodiments 1-12, wherein the cleavable peptide is RAKR (SEQ ID NO: 93).
16. The rMVA of embodiments 1-11, wherein the cleavable peptide is RRRR (SEQ ID NO: 94).
17. The rMVA of embodiments 1-11, wherein the cleavable peptide is RKRR (SEQ ID NO: 95).
18. The rMVA of embodiments 1-11, wherein the cleavable peptide is RRKR (SEQ ID NO: 96).

19. The rMVA of embodiments 1-11, wherein the cleavable peptide is RKKR (SEQ ID NO: 97).
20. The rMVA of embodiments 1-11, wherein the cleavable peptide is an amino acid sequence of SEQ ID NOS: 123-127, or an amino acid sequence at least 95% identical thereto.
- 5 21. The rMVA of embodiments 1-12, wherein the cleavable peptide is the amino acid of SEQ ID NOS: 123, or an amino acid sequence at least 95% identical thereto.
22. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 309-324, or an amino acid sequence at least 95% identical thereto.
- 10 23. The rMVA of embodiments 1-22, wherein $x \geq 4$.
24. The rMVA of embodiments 1-22, wherein $x = 3, 4, \text{ or } 5$.
25. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 325-340, or an amino acid sequence at least 95% identical thereto.
- 15 26. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 341-344, or an amino acid sequence at least 95% identical thereto.
27. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 345-348, or an amino acid sequence at least 95% identical thereto.
- 20 28. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 325, or an amino acid sequence at least 95% identical thereto.
29. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 329, or an amino acid sequence at least 95% identical thereto.
- 25 30. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 333, or an amino acid sequence at least 95% identical thereto.
31. The rMVA of embodiments 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 337, or an amino acid sequence at least 95% identical thereto.
32. The rMVA of embodiments 1-31, wherein the polycistronic nucleic acid further encodes an antigenic peptide.
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33. The rMVA of embodiment 32, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
34. The rMVA of embodiment 33, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
- 5 35. The rMVA of embodiment 34, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a
10 Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.
36. The rMVA of embodiment 32, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the
15 Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2);
20 Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.
37. The rMVA of embodiment 32, wherein the antigenic peptide is derived from the group
25 consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with
30 K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein

Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); the MUC-1 MARV GPTM amino acid sequence; the Marburg virus VP40 amino acid sequence; and the MUC-1-ECD-MARV-TM-ICD sequence; or fragment thereof.

38. The rMVA of embodiment 33, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.
39. The rMVA of embodiment 33, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
40. The rMVA of embodiment 32, wherein the antigenic peptide is derived from mucin 1, or fragment thereof.
41. The rMVA of embodiment 40, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.

42. The method of embodiment 40, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
43. The rMVA of embodiment 40, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
- 5 44. The rMVA of embodiment 40, wherein the mucin 1 comprises an extracellular domain fragment of human mucin 1.
45. The rMVA of embodiment 44, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
- 10 46. The rMVA of embodiment 40, wherein the mucin 1 comprises an intracellular domain fragment of human mucin 1.
47. The rMVA of embodiment 46, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
- 15 48. The method of embodiment 40, wherein the mucin 1 is selected from SEQ ID NO: 363-364, or an amino acid sequence at least 95% identical thereto.
49. The method of embodiment 48, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
50. The method of embodiment 48, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
- 20 51. The rMVA of embodiment 32, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
52. The rMVA of embodiment 32, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
- 25 53. The rMVA of embodiments 51-52, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 350, 354, 356, 365, 366, 367, 368, 369, 377, 379, or an amino acid sequence at least 95% identical thereto.
- 30 54. The rMVA of embodiments 32-53, wherein the antigenic peptide includes a secretion signal.

55. The rMVA of embodiment 54, wherein the secretion signal is fused to the N-terminus of the antigenic peptide.
56. The rMVA of embodiment 55, wherein the secretion signal is selected from an amino acid sequence of SEQ ID NOS: 57-90, or an amino acid sequence at least 95% identical thereto.
- 5 57. The rMVA of embodiment 56, wherein the secretion signal comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
58. The rMVA of embodiment 56, wherein the secretion signal comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.
59. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted between
10 two essential and highly conserved MVA genes.
60. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into a natural deletion site.
61. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the MVA at a site selected from between MVA genes I8R and G1L, between MVA genes A50R
15 and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
62. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes I8R and G1L.
63. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the
20 rMVA at a site selected from between MVA genes A50R and B1R in a restructured and modified deletion site III.
64. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A5 and A6L.
65. The rMVA of embodiments 32-64, wherein the nucleic acid encoding the antigenic peptide
25 amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.
66. A method of increasing an immune response to a target antigen in a patient comprising administering to the patient an effective amount of an rMVA viral vector of embodiments 1-65, wherein the patient has been or is being administered an effective amount of the target
30 antigen.

67. The method of embodiment 66, wherein the rMVA viral vector is administered concomitantly with or subsequent to the administration of the target antigen.
68. The method of embodiments 66-67, wherein the target antigen is selected from the group consisting of an infectious agent and tumor associated antigen.
- 5 69. The method of embodiment 68, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
70. The method of embodiment 69, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.
- 10 71. The method of embodiments 66-67, wherein the target antigen is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR, or fragment thereof.
- 15 72. The method of embodiments 66-67, wherein the target antigen is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; 20 the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2
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full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.

73. The method of embodiment 68, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.
74. The method of embodiment 68, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
75. The method of embodiments 66-67, wherein the target antigen is derived from mucin 1, or fragment thereof.
76. The method of embodiment 75, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.

77. The method of embodiment 75, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
78. The method of embodiment 75, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
- 5 79. The method of embodiment 75, wherein the mucin 1 comprises an extracellular domain fragment of human mucin 1.
80. The method of embodiment 79, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
- 10 81. The method of embodiment 75, wherein the mucin 1 comprises an intracellular domain fragment of human mucin 1.
82. The method of embodiment 81, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
- 15 83. The method of embodiment 75, wherein the mucin 1 is selected from SEQ ID NO: 363-364, or an amino acid sequence at least 95% identical thereto.
84. The method of embodiment 83, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
85. The method of embodiment 83, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
- 20 86. The method of embodiments 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
87. The method of embodiments 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
- 25 88. The method of embodiments 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 350, 354, 356, 365, 366, 367, 368, 369, 377, 379, or an amino acid sequence at least 95% identical thereto.

89. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide), wherein x = 1-10, and M is methionine.
- 5 90. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Peptide), wherein x = 1-10, and M is methionine.
- 10 91. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Peptide-Cleavable Peptide)(Viral Matrix Protein), wherein x = 1-10, and M is methionine.
- 15 92. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Antigenic Peptide)), wherein x = 1-10, and M is methionine.
- 20 93. The rMVA of embodiments 89-92, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-56, or an amino acid sequence at least 95% identical thereto.
94. The rMVA of embodiments 89-93, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-15, or an amino acid sequence at least 95% identical thereto.
- 25 95. The rMVA of embodiments 89-94, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1 or 5, or an amino acid sequence at least 95% identical thereto.
96. The rMVA of embodiments 89-95, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO. 1, or an amino acid sequence at least 95% identical thereto.

97. The rMVA of embodiments 89-95, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO. 5, or an amino acid sequence at least 95% identical thereto.
- 5 98. The rMVA of embodiments 89-97, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS. 57-90, or an amino acid sequence at least 95% identical thereto.
99. The rMVA of embodiments 89-98, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
- 10 100. The rMVA of embodiments 89-98, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.
101. The rMVA of embodiments 89-100 wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 91-126, or an amino acid sequence at least 95% identical thereto.
- 15 102. The rMVA of embodiments 89-101, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 93, 120, and 123.
103. The rMVA of embodiments 89-101, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91).
- 20 104. The rMVA of embodiments 89-101, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = R, K, or H (SEQ ID NO: 92).
105. The rMVA of embodiments 89-102, wherein the cleavable peptide is RAKR (SEQ ID NO: 93).
106. The rMVA of embodiments 89-101, wherein the cleavable peptide is RRRR (SEQ ID NO: 94).
- 25 107. The rMVA of embodiments 89-101, wherein the cleavable peptide is RKRR (SEQ ID NO: 95).
108. The rMVA of embodiments 89-101, wherein the cleavable peptide is RRKR (SEQ ID NO: 96).

109. The rMVA of embodiments 89-101, wherein the cleavable peptide is RKKR (SEQ ID NO: 97).
110. The rMVA of embodiments 89-101, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 123-127, or an amino acid sequence at least 95% identical thereto.
111. The rMVA of embodiments 89-102, wherein the cleavable peptide comprises the amino acid sequence of SEQ ID NO. 123, or an amino acid sequence at least 95% identical thereto.
112. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
113. The rMVA of embodiment 112, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
114. The rMVA of embodiment 113, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.
115. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.

116. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.
117. The rMVA of embodiment 112, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.
118. The rMVA of embodiment 112, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.

119. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from mucin 1, or fragment thereof.
120. The rMVA of embodiment 119, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.
- 5 121. The method of embodiment 119, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
122. The rMVA of embodiment 119, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
123. The rMVA of embodiment 119, wherein the mucin 1 comprises an extracellular domain
10 fragment of human mucin 1.
124. The rMVA of embodiment 123, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
125. The rMVA of embodiment 119, wherein the mucin 1 comprises an intracellular domain
15 fragment of human mucin 1.
126. The rMVA of embodiment 125, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
127. The method of embodiment 119, wherein the mucin 1 is selected from SEQ ID NO: 363-
20 364, or an amino acid sequence at least 95% identical thereto.
128. The method of embodiment 127, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
129. The method of embodiment 127, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
- 25 130. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
131. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95%
30 identical thereto.

132. The rMVA of embodiments 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 403, or an amino acid sequence at least 95% identical thereto.
133. The rMVA of embodiments 89-132, wherein the glycoprotein signal peptide is derived from a *Filoviridae*.
134. The rMVA of embodiments 89-133, wherein the glycoprotein signal peptide comprises the amino acid sequence of SEQ ID NO. 396, or an amino acid sequence at least 95% identical thereto.
135. The rMVA of embodiments 89-133, wherein the glycoprotein transmembrane peptide comprises the amino acid sequence of SEQ ID NO. 398, or an amino acid sequence at least 95% identical thereto.
136. The rMVA of embodiments 89-135, wherein the viral matrix protein comprises the amino acid sequence of SEQ ID NO. 400, or an amino acid sequence at least 95% identical thereto.
137. The rMVA of embodiments 89-136, wherein $x \geq 4$.
138. The rMVA of embodiments 89-136, wherein x is 3, 4, or 5.
139. The rMVA of embodiments 89-138, wherein the polycistronic nucleic acid is inserted between two essential and highly conserved MVA genes.
140. The rMVA of embodiments 89-138, wherein the polycistronic nucleic acid is inserted into a natural deletion site.
141. The rMVA of embodiments 89-138, wherein the polycistronic nucleic acid is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
142. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes I8R and G1L.
143. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A50R and B1R in a restructured and modified deletion site III.
144. The rMVA of embodiments 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A5 and A6L.

145. The rMVA of embodiments 89-144, wherein the nucleic acid encoding the antigenic peptide amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.

146. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:

- 5 a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $x = 1-10$, and M is methionine; and
- b) a second nucleic acid sequence encoding an antigenic peptide;
- wherein the Immune Checkpoint Inhibitor Peptide is selected from an amino acid having
- 10 the sequence of SEQ ID NO:1-57; and,
- wherein the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more vaccinia virus promoters.

147. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:

- 15 a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $x = 1-10$, and M is methionine; and
- b) a second nucleic acid sequence encoding an antigenic peptide;
- wherein the Immune Checkpoint Inhibitor Peptide is SEQ ID NO:1, and the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more
- 20 vaccinia virus promoters.

148. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:

- a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $x = 1-10$, and M is methionine; and
- 25 b) a second nucleic acid sequence encoding an antigenic peptide;
- wherein the Immune Checkpoint Inhibitor Peptide is SEQ ID NO:5, and the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more vaccinia virus promoters.

149. The rMVA of embodiments 146-148, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS. 57-90, or an amino acid sequence at least 95% identical thereto.
150. The rMVA of embodiments 146-149, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
151. The rMVA of embodiments 146-149, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.
152. The rMVA of embodiments 146-151, wherein the vaccinia virus promoter is selected from the nucleic acid sequence of SEQ ID NO:128-308.
153. The rMVA of embodiment 152, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
154. The rMVA of embodiment 153, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
155. The rMVA of embodiment 154, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.
156. The rMVA of embodiment 152, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2);

Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.

- 5 157. The rMVA of embodiment 152, wherein the antigenic peptide is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.
- 10 158. The rMVA of embodiment 152, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
159. The rMVA of embodiments 146-158, wherein the first nucleic acid sequence and the second nucleic acid sequence are inserted into the MVA between essential MVA genes.
- 25 160. The rMVA of embodiments 146-158, wherein the first nucleic acid sequence is inserted into the MVA between essential MVA genes.
161. The rMVA of embodiments 146-160, wherein the second nucleic acid sequence is inserted into the MVA between essential MVA genes.
162. The rMVA of embodiments 146-158, wherein the first nucleic acid sequence and the second nucleic acid sequence are inserted into the MVA at sites selected from between MVA genes
- 30

I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.

163. The rMVA of embodiments 146-158, wherein the first nucleic acid sequence is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.

164. The rMVA of embodiments 146-158, wherein the second nucleic acid sequence is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.

165. The rMVA of embodiments 146-164, wherein the vaccinia virus promoter is a nucleic acid sequence of SEQ ID NOS:128-130, or a nucleic acid sequence at least 95% identical thereto.

166. The rMVA of embodiments 146-165, wherein the vaccinia virus promoter is SEQ ID NO:130, or a nucleic acid sequence at least 95% identical thereto.

167. The rMVA of embodiments 146-166, wherein the nucleic acid encoding the antigenic peptide amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.

168. The rMVA of embodiments 146-167, wherein $x \geq 4$.

169. The rMVA of embodiments 146-167, wherein x is 3, 4, or 5.

170. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

i) a first nucleic acid sequence encoding an amino acid sequence comprising (Mucin 1 Extracellular Fragment Peptide-Glycoprotein Transmembrane Peptide-Mucin 1 Intracellular Fragment Peptide); and

ii) a second nucleic acid sequence encoding an amino acid sequence comprising a Marburg virus (MARV) VP40 Protein; and

iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $x = 1-10$, and M is methionine;

wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

5 171. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

i) a first nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 402 encoding a chimeric amino acid sequence;

ii) a second nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 404;

10 iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein x = 1-10, and M is methionine;

15 wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

172. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

20 i) a first nucleic acid sequence encoding a chimeric amino acid sequence comprising the amino acid sequence of SEQ ID NO: 403; and

ii) a second nucleic acid sequence encoding a MARV VP40 matrix protein comprising the amino acid sequence of SEQ ID NO: 405; and

25 iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein x = 1-10, and M is methionine;

30 wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

173. The rMVA of embodiments 170-172, wherein the third nucleic acid sequence comprises the nucleic sequence of SEQ ID NO: 408, or a nucleic acid sequence at least 95% identical thereto.
- 5 174. The rMVA of embodiments 170-172, wherein the third nucleic acid sequence comprises the nucleic sequence of SEQ ID NO: 409, or a nucleic acid sequence at least 95% identical thereto.
175. The rMVA of embodiments 170-172, wherein the third nucleic acid sequence is an amino acid sequence selected from SEQ ID NOS: 1, 5, or 309-348, or an amino acid at least 95% identical thereto.
- 10 176. The rMVA of embodiment 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 325, or an amino acid sequence at least 95% identical thereto.
177. The rMVA of embodiment 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 329, or
15 an amino acid sequence at least 95% identical thereto.
178. The rMVA of embodiment 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 333, or an amino acid sequence at least 95% identical thereto.
179. The rMVA of embodiment 175, wherein the third nucleic acid sequence encodes an immune
20 checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 337, or an amino acid sequence at least 95% identical thereto.
180. The rMVA of embodiments 170-179, wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted between two essential and highly conserved MVA genes.
- 25 181. The rMVA of embodiments 170-179, wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into the rMVA at a site selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
182. The rMVA of embodiments 170-179, wherein the first nucleic acid sequence is inserted
30 between MVA genes I8R and G1L.

183. The rMVA of embodiments 170-179, wherein the second nucleic acid sequence is inserted between MVA genes A50R and B1R in the restructured and modified deletion site III.
184. The rMVA of embodiments 170-179, wherein the third nucleic acid sequence is inserted between the two essential MVA genes A5R and A6L.
- 5 185. The rMVA of embodiments 170-179, wherein the first nucleic acid sequence is inserted between MVA genes I8R and G1L, the second nucleic acid sequence is inserted between MVA genes A50R and B1R in the restructured and modified deletion site III, and the third nucleic acid sequence is inserted between the two essential MVA genes A5R and A6L.
186. The rMVA of embodiments 170-185, wherein the vaccinia virus promoter is a nucleic acid
10 sequence selected from SEQ ID NOS: 128-308.
187. The rMVA of embodiment 170-186, wherein the vaccinia virus promoter is SEQ ID NO:130, or a nucleic acid sequence at least 95% identical thereto.
188. A pharmaceutical composition comprising at least one rMVA of embodiments 89-187 and a pharmaceutically acceptable carrier.
- 15 189. A method of preventing, treating, or inducing an immune response against, a target antigen in a patient in need thereof, said method comprising administering an effective amount of the pharmaceutical composition of embodiment 188, wherein the pharmaceutical composition enhances immunity directed against the target antigen.
190. The method of embodiment 189, wherein the target antigen is selected from the group
20 consisting of a tumor associated antigen and an infectious agent.
191. The method of embodiment 190, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic
25 tumor associate antigen, or fragment thereof.
192. The method of embodiment 190, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma
30 antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2,

melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.

193. The method of embodiments 189-192, wherein the patient is a human having a cancer.

194. The method of embodiment 193, wherein the cancer is selected from bowel cancer, ovarian cancer, breast cancer, malignant melanoma, hepatoma, testicular cancer, prostate cancer, multiple myeloma, lymphoma, colorectal cancer, bile duct cancer, pancreatic cancer, lung cancer, melanoma, soft tissue sarcoma, or colon cancer.

195. The method of embodiment 190, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.

196. The method of embodiment 195, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

197. The method of embodiment 190, wherein the infectious agent is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H;

HIV IN; and HIV PR; SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.

198. The method of embodiments 195-197, wherein the patient is a human exposed to the infectious agent.

199. The method of embodiment 198, wherein the exposed human is symptomatic.

200. The method of embodiment 198, wherein the exposed human is asymptomatic.

201. The method of embodiments 195-197, wherein the patient is a human unexposed to the infectious agent.

202. The method of embodiments 188-201, wherein the rMVA administration is selected from intramuscular, intraarterial, intravascular, intravenous, intraperitoneal, or subcutaneous injection.

203. The method of embodiments 188-202, wherein the rMVA comprises an adjuvant for enhancing an immune response.

204. The method of embodiments 188-202, wherein the rMVA comprises a vaccine for inducing an immune response.

205. The method of embodiments 192-204, wherein the patient is administered the pharmaceutical composition at least 2 or more times.

206. The method of embodiment 205, wherein the administrations are separated by at least a 4-week interval.

207. A method of enhancing an immune response in a patient comprising administering to the patient an effective amount of an rMVA of embodiments 89-187.

5 208. A method of inducing an immune response to a MUC1 antigen in a patient comprising administering to the patient an effective amount of an rMVA of embodiments 119-145 or 170-187.

209. The method of embodiments 207-208, wherein the patient is human.

10 EXAMPLES

The claimed invention is further described by way of the following non-limiting examples. Further aspects and embodiments of the present invention will be apparent to those of ordinary skill in the art, in view of the above disclosure and following experimental exemplification, 15 included by way of illustration and not limitation, and with reference to the attached figures.

EXAMPLE 1. Mice

All animal experiments were carried out in strict accordance with the Policy on Humane Care and Use of Laboratory Animals of the United States Public Health Service. The protocol was 20 approved by the Institutional Animal Care and Use Committee (IACUC) at The Rockefeller University. Mice were euthanized using CO₂, and every effort was made to minimize suffering. Human fetal liver samples were obtained via a non-profit partner (Advanced Bioscience Resources, Alameda, CA). As no information was obtained that would identify the subjects from whom the samples were derived, Institutional Review Board approval for their use was not 25 required. (See Huang J. et al., "An AAV vector-mediated gene delivery approach facilitates reconstitution of functional human CD8⁺ T cells in mice", PLoS One, 2014 Feb 6, 9(2), e88205. doi: 10.1371/journal.pone.0088205. eCollection 2014.PMID:24516613)

Six to eight-week-old female BALB/c mice were purchased from The Jackson Laboratory (Bar Harbor, ME). NOD.CgtmlUnc Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice exhibiting features 30 of both severe combined immunodeficiency mutations and interleukin (IL)-2 receptor gamma-

chain deficiency were also purchased from Jackson Laboratories and maintained under specific pathogen-free conditions in the animal facilities at The Rockefeller University Comparative Bioscience Center. All mice were maintained under standard conditions in the Laboratory Animal Research Center of The Rockefeller University and the protocol was approved by the Institutional Animal Care and Use Committee at The Rockefeller University (Assurance no. A3081-01).

EXAMPLE 2. Generation of HIS-CD8 Mice

Preparation of the recombinant AAV9 (rAAV9) vectors encoding human IL-3, IL-15, GM-CSF, and HLA-A*0201 were constructed. (See Huang J., et al., “An AAV vector-mediated gene delivery approach facilitates reconstitution of functional human CD8⁺ T cells in mice”, PLoS One, 2014 Feb 6, 9(2), e88205. doi: 10.1371/journal.pone.0088205. eCollection 2014.PMID:24516613)

Four-week-old NSG mice were transduced with rAAV9 encoding HLA-A*0201 by perithoracic injection and with rAAV9 encoding HLA-A*0201 and AAV9 encoding human IL-3, IL-15, and GM-CSF, by IV injection. (See Huang J., et al., “An AAV vector-mediated gene delivery approach facilitates reconstitution of functional human CD8⁺ T cells in mice”, PLoS One, 2014 Feb 6, 9(2), e88205. doi: 10.1371/journal.pone.0088205. eCollection 2014.PMID:24516613)

Two weeks later, mice were subjected to 150-Gy total body sub-lethal irradiation for myeloablation, and several hours later, each transduced, irradiated mouse was engrafted intravenously with 1×10^5 HLA-A*0201+ matched, CD34⁺ human hematopoietic stem cells (HSCs). CD34⁺ HSCs among lymphocytes derived from HLA-A*0201+ fetal liver samples were isolated using a Human CD34 Positive Selection kit (Stem Cell Technologies Inc. Vancouver, BC, Canada; See Lepus CM, et al., “Comparison of human fetal liver, umbilical cord blood, and adult blood hematopoietic stem cell engraftment in NOD-scid/gammac^{-/-}, Balb/c-Rag1^{-/-}-gammac^{-/-}, and C.B-17-scid/bg immunodeficient mice”, Hum Immunol., 2009 Oct, 70(10), 790-802. doi: 10.1016/j.humimm.2009.06.005. Epub 2009 Jun 12. PMID: 19524633). At 14 weeks after HSC engraftment, the reconstitution status of human CD45⁺ cells in the blood of HIS-CD8 mice was determined by flow cytometric analysis. (See Huang J., et al., “An AAV vector-mediated gene delivery approach facilitates reconstitution of functional human CD8⁺ T cells in mice”, PLoS One, 2014 Feb 6, 9(2), e88205. doi: 10.1371/journal.pone.0088205. eCollection 2014.PMID:24516613)

EXAMPLE 3. AdPyCS and AdPfCS Vaccines

Preparation of the recombinant serotype 5 adenovirus that expressed *P. yoelii* circumsporozoite protein (PyCS), AdPyCS, was constructed. (See Rodrigues EG, et al., “Single immunizing dose of recombinant adenovirus efficiently induces CD8⁺ T cell-mediated protective immunity against malaria”, *J Immunol.*, 1997 Feb 1, 158(3), 1268-74. PMID: 9013969).

EXAMPLE 4. ELISpot Assay and Flow Cytometry to Measure Antigen-Specific CD8⁺ T cells

The relative numbers of splenic PyCS-specific, IFN- γ -secreting CD8⁺ T cells of AdPyCS-immunized mice were determined by an ELISpot assay, using a mouse IFN- γ ELISpot kit (Abcam, Cambridge, MA) and a synthetic 9-mer peptide, SYVPSAEQI (SEQ ID NO: 406) (Peptide 2.0 Inc., Chantilly, VA) corresponding to the immunodominant CD8⁺ T cell epitope within PyCS. (See Li X, et al., “Human CD8⁺ T cells mediate protective immunity induced by a human malaria vaccine in human immune system mice”, *Vaccine*, 2016 Aug 31, 34(38), 4501-4506. doi: 10.1016/j.vaccine.2016.08.006. Epub 2016 Aug 5.; PMID: 27502569). After the collection of splenocytes from mice 12 days after AdPyCS immunization, 5×10^5 splenocytes were placed on each well of the 96-well ELISpot plates were pre-coated with IFN- γ antibody and incubated with the SYVPSAEQI (SEQ ID NO: 406) peptide at 5 μ g/mL for 24 h at 37°C, in a CO₂ incubator. After the ELISpot plates were washed, they were incubated with biotinylated anti-mouse IFN- γ antibody for 2–3 h at RT, followed by incubation with avidin-conjugated with horseradish peroxidase for 45 min at RT in the dark. Finally, the spots were developed after the addition of the ELISpot substrate (Abcam). To identify the number of IFN- γ -secreting CD8⁺ T cells in each well, the mean number of spots (for duplicates) counted in the wells incubated with splenocytes in the presence of the peptide was subtracted by the mean number of spots (for duplicates) counted in the wells that were incubated with splenocytes only. The percentage of IFN- γ ⁺ T cells among splenocytes of immunized mice were determined by a flow cytometry. After isolating splenocytes the cells were washed twice and blocked for 5 min on ice using inactivated normal mouse serum supplemented with anti-CD16/CD32 (clone 93 – BioLegend, San Diego, CA, USA).

EXAMPLE 5. Staining with HLA-A*0201 tetramer loaded with YLNKIQNSL peptide

The Allophycocyanin (APC)-labeled human HLA-A*0201 tetramer loaded with the peptide YLNKIQNSL (SEQ ID NO: 407), corresponding to the PfCSP CD8⁺ T-cell epitope, was provided by the NIH Tetramer Core Facility (See Blum-Tirouvanziam U, et al., “Localization of HLA-A2.1-restricted T cell epitopes in the circumsporozoite protein of Plasmodium falciparum”, J Immunol., 1995 Apr 15, 154(8), 3922-31; PMID: 7535817; 43; Bonelo A, et al., “Generation and characterization of malaria-specific human CD8⁺ lymphocyte clones: effect of natural polymorphism on T cell recognition and endogenous cognate antigen presentation by liver cells”, Eur J Immunol., 2000 Nov, 30(11), 3079-88; doi: 10.1002/1521-4141(200011)30:11<3079::AID-IMMU3079>3.0.CO;2-7. PMID: 11093122) (Table 12).

Table 12 – Synthetic 9-mer Peptide Sequences

SEQ ID NO:	Peptide Sequence:
406	SYVPSAEQI
407	YLNKIQNSL

Twelve days after immunization of HIS-CD8 mice with AdPfCS, the spleens were harvested from the mice, and splenocytes were stained with APC-labeled human HLA-A*0201 tetramer loaded with YLNKIQNSL (SEQ ID NO: 407) and PE-labeled anti-human CD8 antibody (BioLegend, San Diego, CA). The percentage of HLA-A*0201-restricted, PfCSP-specific CD8⁺ T cells among the total human CD8⁺ T-cell population was determined using a BD LSR II flow cytometer (Franklin Lakes, NJ). (See Li X, et al., “Human CD8⁺ T cells mediate protective immunity induced by a human malaria vaccine in human immune system mice”, Vaccine, 2016 Aug 31, 34(38), 4501-4506; doi: 10.1016/j.vaccine.2016.08.006. Epub 2016 Aug 5. PMID: 27502569)

EXAMPLE 6. MVA construction, seed stock preparation, VLP formation, and protein expression

Two recombinant MVAs, MVA-5x.LD01 and MVA-5x.LD10, were constructed that encode an optimized nucleic acid sequence of five repeats of LD01 (SEQ ID NO: 408) or LD10 (SEQ ID NO: 409) in polycistronic format (Table 13). A signal sequence (SEQ ID NO: 66) was added prior to LD01 or LD10 to route the peptides for secretion from the cell and a dual cleavage site (SEQ ID NO: 123) was added following the sequences to facilitate production of monomer

peptides from the polycistronic design. The resultant LD01 insert encoded for the amino acid sequence of SEQ ID NO:332. The resultant LD10 insert encoded for the amino acid sequence of SEQ ID NO: 337. The starting material for recombinant virus production was parental MVA that had been harvested in 1974, before the appearance of Bovine Spongiform Encephalopathy /Transmissible Spongiform Encephalopathy (BSE/TSE) and plaque purified 3 times using certified reagents from sources free of BSE. A shuttle vector was used to insert the LD01 or LD10 sequences between two essential genes I8R/G1L of MVA by means of homologous recombination. The chosen insertion site has been identified as supporting high expression and insert stability. All inserted sequences were codon optimized for MVA as below:

Table 13 - Sequence Optimization

SEQ NO:	ID	Identifier	Nucleic Acid Sequence
408	5xLD01		ATGGACGCCATGAAGAGAGGACTTTGTTGCGTCCTACTACTATGCGGAG CGGTATTCGTATCTCCGTCGCAAGAAATTCACGCGAGATTTCAGAAGAGG TGCCAGATGCAGAAGAACATCTACCGGACAGATCTCCACCTTGAGAGT AAATATCACAGCGCCGCTATCTCAGAGAGCCAAGAGAGGATCGGGAGC GACAACTTCTCGCTATTGAAACAAGCGGGAGATGTCGAAGAGAACCC AGGACCAGATGCTATGAAGAGAGGACTTTGCTGCGTATTGCTATTGTGC GGAGCCGCTTTCGTCTCACCATCTCAAGAAATCCATGCCAGATTCAGAA GAGGTGCTAGATGTAGAAGAACCTCCACGGGACAAATCAGTACCCTAA GAGTTAACATCACCGCGCCGTTGAGTCAAAGAGCTAAGAGAGGTTCCG GAGCCACCAACTTCAGTTTGCTAAAGCAGGCGGGAGATGTGGAAGAGA ATCCTGGTCCTGACGCAATGAAGAGAGGACTTTGCTGCGTTCTATTGCT ATGCGGTGCCGTCTTTGTTTCTCCGAGTCAAGAGATACACGCTAGATTC AGAAGAGGTGCAAGATGTAGAAGAACCTCGACCGGTCAAATCTCGACG CTTAGAGTCAATATTACCGCGCCATTGTGTCGAGAGAGCGAAGAGAGGA TCGGGAGCCACTAATTTTCAGTCTACTTAAGCAAGCGGGAGATGTAGAG GAGAATCCTGGACCGGATGCCATGAAGAGAGGACTTTGTTGCGTTCTGT TGCTTTGCGGAGCTGTGTTTCGTTCAGTCCTTCTCAAGAGATTCATGCAAG ATTCAGAAGAGGTGCAAGATGCAGAAGAACCAGTACGGGACAGATTTTC GACATTAAGAGTGAACATTACTGCGCCTTTGTCTCAAAGAGCGAAGAG AGGTTCCGGAGCGACGAATTTCTCGTTGCTCAAGCAAGCGGGAGATGT AGAAGAGAACCCAGGACCTGATGCAATGAAGAGAGGACTTTGTTGCGT ATTACTTCTTTGCGGTGCAGTGTGTTGTCTCGCCGTCACAAGAGATCCAC GCAAGATTTCAGAAGAGGTGCCAGATGTAGAAGAACTAGTACAGGACAA ATCTCCACGCTAAGAGTAAACATAACGGCACCACTATCTCAATAA
409	5xLD10		ATGGACGCCATGAAGAGAGGACTTTGTTGCGTCCTACTACTATGCGGAG CGGTATTCGTATCTCCGTCGCAAGAAATTCACGCGAGATTTCAGAAGAGG TGCCAGATCTACAGGACAGATCTCTACCCTAAGAGTCAATATCACAGCG CCGCTATCTCAGAGAGCGAAGAGAGGATCGGGAGCGACAACTTCTCG CTATTGAAACAAGCGGGAGATGTGAGGAGAACCCAGGACCAGATGCT ATGAAGAGAGGACTTTGCTGCGTATTGCTATTGTGCGGAGCCGTGTTTCG TCTCGCCATCTCAAGAAATCCATGCCAGATTTCAGAAGAGGTGCTAGAA

		GTACCGGACAAATCTCCACGTTGAGAGTAAACATTACCGCGCCGTTGTC GCAAAGAGCTAAGAGAGGTTCCGGAGCCACTAAGTTTCTAGTTTGCTAAA GCAGGCGGGAGATGTGGAAGAGAATCCTGGTCCTGACGCAATGAAGAG AGGACTTTGCTGCGTTCTATTGCTATGCGGTGCCGTTCTTTGTTTCTCCGA GTCAAGAGATACACGCTAGATTTCAGAAGAGGTGCTAGATCCACGGGAC AAATCAGTACCCTTAGAGTGAACATCACGGCGCCACTTTCTCAAAGAGC CAAGAGAGGTTCCGGAGCGACCAATTTCTCGTTGCTAAAGCAAGCGGG AGATGTAGAAGAGAATCCCGGACCGGATGCCATGAAGAGAGGACTTTG TTGCGTGCTGTTGCTTTGCGGAGCTGTGTTTCGTCAGTCCTTCTCAAGAGA TTCATGCAAGATTTCAGAAGAGGTGCAAGATCGACCGGTCAAATTTCTGA CGCTAAGAGTTAACATAACGGCGCCCTTGAGTCAGAGAGCCAAGAGAG GATCGGGAGCCACTAAGTTCTCGTTGTTGAAGCAGGCGGGAGATGTAG AAGAGAATCCGGGTCCAGATGCAATGAAGAGAGGACTTTGTTGCGTAT TACTTCTTTGCGGTGCAGTGTTTGTCTCGCCGTCACAAGAGATCCACGC AAGATTTCAGAAGAGGTGCCAGAAGTACGGGTCAAATTAGTACCTTGAG AGTCAATATTACGGCGCCTTTGTACAGTAATGA
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Silent mutations were introduced to interrupt homo-polymer sequences (>4G/C and >4A/T), which reduce RNA polymerase errors that possibly lead to frameshift mutations. All vaccine inserts were placed under control of the modified H5 early/late vaccinia promoter (SEQ ID NO: 130). Vectors, Research Seed Virus (RSV), and Research Stocks (RS) were prepared in a dedicated room with full traceability and complete documentation of all steps using BSE/TSE-free raw materials, and therefore can be directly used for production of cGMP Master Seed Virus (MSV). For production of RSV for animal studies, a chicken embryo fibroblast cell line, DF-1 cells (ATCC, CRL-12203), were seeded into sterile tissue culture flasks and infected with MVA-5x.LD01 or MVA-5x.LD10 at an MOI of 0.01. Cells were recovered 3 days post-infection, disrupted by sonication, and bulk harvest material clarified by low-speed centrifugation. The clarified viral harvest was purified using sucrose cushion ultracentrifugation twice. The purified viruses were titrated by limiting dilution in DF1 cells, diluted to 1×10^8 TCID₅₀/mL in sterile PBS + 7% sucrose, dispensed into sterile vials, and stored at -80°C.

EXAMPLE 7. Production of anti-LD01/LD10 mAb

KLH conjugated LD01 peptide formulated in Sigma adjuvant system (Cat No. S6322) was used to immunize SJL/J mice intramuscularly. Following two similar intramuscular boosts at 2-week intervals, the mice were culled and spleens and lymph nodes were collected. Splenocytes and lymphocytes were isolated and fused to HL-1 mouse myeloma cells and cultured for 13 days. On day 13, colonies were picked manually and transferred to selection media. Culture supernatants

were screened for specificity by ELISA using plate coated BSA conjugated peptides. Supernatants were screened against BSA-conjugated LD01 peptide as well as LD10. Two clones (3F11 and 7G10) were selected based on their high level of binding to both peptides as well as the high concentration of supernatant antibody. Monoclonal cultures of these two clones were expanded and the supernatants were used to purify the antibodies. Cell suspensions, containing at least 8.0×10^7 cells in 2xT-75 flasks, were aseptically transferred to 2x50 mL centrifuge tubes and centrifuged at 1000 rpm for 5 minutes. The resulting cell pellet was re-suspended in 25 mL of HyClone HYQSFMMAB media + 5% FBS and slowly added to 250 mL bag containing 225 mL of HyClone HYQSFMMAB media + 5% FBS. The bag was placed in an incubator set at 5% CO₂, 37°C for 10-14 days. After 10-14 days of growth, the contents of the 250 mL bag were transferred to a 250 mL centrifuge bottle, 10 mL of Neutralization Buffer (1M TRIS, 1.5M NaCl, pH 8.5) was added to it, and centrifuged at 8600 rpm for 10 min using a Sorvall GSA rotor. The supernatant was filtered using a 0.45 µm bottle top filter. A 5 mL protein A column connected to a FPLC Purification System was washed with 25 mL of ultra-pure water followed by 25 mL of 50 mM TRIS, 250 mM NaCl, pH 8.0. The filtered supernatant was loaded onto the column at a flow rate of 7 mL/minute. The column was further washed with 15 mL of 50 mM TRIS, 250 mM NaCl, pH 8.0. Elution fractions were collected in 15 mL tubes containing 800 µL of Neutralization Buffer (1M Tris Base, 1.5M NaCl, pH 7.4). The antibody was eluted with 20 mL of 50 mM Glycine, pH 3.0 and dialyzed against 1-2L of 1xPBS pH 7.4 (depending on volume of purified Ab) on a stirrer at 4°C overnight. The dialyzed antibody was sterile filtered and aliquoted for storage.

EXAMPLE 8. Dot blot assay

DF-1 cells were infected at a multiplicity of infection of 0.5 with parental MVA, MVA-5X.LD01 or MVA-5X.LD10 and 48 hours later the supernatant was collected. In order to concentrate secreted peptide, supernatant was passed through Pierce C-18 tips (ThermoFisher, Cat. No. 87782). Twenty microliters from each sample and 125 ng of synthetic LD01 peptide were spotted onto a PVDF membrane, allowed to dry at room temperature, then blocked with Intercept blocking buffer (Li-Cor, Cat. No. 927-70001) for 30 mins at room temperature. The membrane was incubated overnight at 4°C in primary antibody (Leidos, clone: 7G10) diluted in blocking buffer at 1:1000. Three washes with PBST (PBS with 0.05% Tween-20) were performed, and the

membrane was probed for 1 h with anti-mouse-680RD (Invitrogen, Cat. No. A-21058) (1:10,000). The membrane was then washed again and imaged using Odyssey imager.

EXAMPLE 9. Immunocytochemistry assay

5 DF-1 cells were infected at a multiplicity of infection of 0.5 with parental MVA, MVA-5X.LD01 or MVA-5X.LD10 for 48 hours, subsequently cells were fixed in 1:1 methanol:acetone and washed with water. Cells were then probed with a mouse anti-LD01/LD10 antibody (Leidos, clone: 3F11) at room temperature for 1 hour. Three washes with water were performed and the cells were stained for 1 hour with anti-mouse-HRP at 1:1000 dilution (VWR, Cat. No. 10150-400).
10 The cells were then washed again and developed with AEP substrate kit (Abcam Cat. No. ab64252). Images of stained cells were captured at 20x magnification using light microscopy.

EXAMPLE 10. Data Analysis

 Statistical analyses were performed using GraphPad Prism (GraphPad Software, Inc., La
15 Jolla, CA). The two-tailed Unpaired t-test was used to determine between two groups. Data are expressed as the mean $\pm$ SEM and $P < 0.05$ was considered statistically significant.

EXAMPLE 11. MVA vector construction

 To establish whether LD10 could be expressed by a viral vector, a recombinant MVA virus
20 that encodes five repeats of the LD10 sequence in polycistronic format (MVA-5x.LD10) (Fig. 7) and a similar recombinant MVA virus expressing five repeats of the LD01 sequence was constructed (MVA-5x.LD01) (Fig. 7) according to Example 6. To facilitate peptide secretion, a signal sequence was added prior to LD01 or LD10, and a dual cleavage site was added following the sequences in order to facilitate production of the monomer LD01 or LD10 from the
25 polycistronic design.

 Immunohistochemistry on infected cells was performed using a mAb cross reactive to LD01 and LD10; to initially determine whether the recombinant MVA vectors express LD01 or LD10. Cells were fixed and permeabilized with 50:50 methanol/acetone.

EXAMPLE 12. LD01 and LD10 are produced by MVA-infected cells

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A dot blot was performed on infected cell supernatants to establish that LD01 or LD10 is being secreted by the recombinant MVA vector. The parental MVA vector showed negligible signal as shown in FIG 8B. Liquid chromatography tandem mass spectrometry of the cell supernatants identified LD01 and LD10 fragments corroborated the dot blot results.

5 Cells infected with the parental MVA vector showed no specific staining, however, cells infected with either MVA-5X.LD01 or MVA-5X.LD10 vectors showed positive staining as shown in FIG 8A; indicating the intracellular expression of the peptides. Both MVA-5X.LD01 and MVA-5X.LD10 vector samples demonstrated positive staining, arguing for secretion of LD01 and LD10. LD01 and LD10 are expressed and secreted by the recombinant MVA vectors. The above
10 technique was used to generate the image in FIG. 8.

EXAMPLE 13. Delivery of LD01 or LD10 via a viral vector enhances expansion of vaccine-induced, antigen-specific CD8⁺ T cells

Having confirmed that LD01 and LD10 are expressed in and secreted from cells infected
15 with peptide-encoding MVA constructs (Fig. 8A and Fig. 8B), AdPyCS-specific CD8⁺ T cell expansion following treatment with MVA-encoding LD01 or LD10 was assessed. A parental MVA vector was included as a negative control, while synthetic LD01 and LD10da served as positive controls. As shown in Fig. 9, treatment with 100 µg of LD01 or LD10da directly following vaccination significantly increased antigen-specific CD8⁺ T cell numbers relative to AdPyCS
20 alone. Similarly, injection of 10⁸ TCID₅₀ of MVA-5X.LD01 or MVA-5X.LD10 enhanced antigen-specific CD8⁺ T cell expansion, which contrasted the treatment with the parental MVA vector (Fig. 9). Taken together, these *in vivo* results indicate that the delivery of LD01 or LD10 via the MVA vector results in increased activation of immune effector cells and immunomodulatory activity that is likely due to their expression *in vivo*. As such, these results corroborate that peptide-
25 based immunomodulators can be successfully delivered by viral vector and induce significantly enhanced immune response.

EXAMPLE 14. MVA-VLP-MUC-1-LD10 construction and validation of insert integrity

Starting with parental MVA virus, shuttle vectors were used to insert the optimized MUC-
30 1 and Marburg virus (MARV) transmembrane glycoprotein (GP) transmembrane domain (TM)

chimeric nucleic acid sequence (SEQ ID NO: 402) encoding a MUC-1-MARV GPTM amino acid sequence (SEQ ID NO: 403) between MVA genes I8R and G1L, the MARV VP40 nucleic acid sequence (SEQ ID NO: 404) encoding a MARV VP40 amino acid sequence (SEQ ID NO: 405) between MVA genes A50R and B1R in the restructured and modified deletion site III, and the 5xLD10 (SEQ ID NO: 409) nucleic acid sequence encoding a 5xLD10 amino acid sequence (SEQ ID NO: 337) between the two essential MVA genes A5R and A6L by means of homologous recombination. These insertion sites were previously demonstrated to support high expression and stability of transgenes. Silent mutations were introduced to interrupt homo-polymer sequences (>4G/C and >4A/T), which reduce RNA polymerase errors that possibly lead to frameshift mutations. The inserted sequences were codon optimized for expression under control of the modified H5 early/late vaccinia promoter (SEQ ID NO: 130) by the MVA virus.

Viral vectors, Research Seed Virus (RSV), and Research Stocks (RS) were prepared in a dedicated room with full traceability and complete documentation of all steps using BSE/TSE-free raw materials capable of production of cGMP Master Seed Virus (MSV), as described previously (Example 6). The chicken embryo fibroblast cell line, DF-1 cells (ATCC, CRL-12203), was seeded in sterile tissue culture flasks and infected with either MVA parental or MVA-VLP-MUC-1-LD10 recombinant virus at a multiplicity of infection of 0.01. Viral DNA samples harvested from these cells were analyzed by PCR to examine transgene insert integrity (Fig. 10), using specific primers upstream and downstream of each insert (Table 14). MVA parental viral DNA use used as a negative control and the DNA from three different plasmids, containing the Muc1, VP40 or LD10 genes, was used as a positive control. The bands identified matched the expected sizes (Fig. 11).

Table 14 - Primer Sequences

SEQ ID NO:	Sequence Description	Nucleic Acid Sequence
410	p55 LD10 F	AGATCGGAGATGACTGCGATG
411	p54 LD10R/ GFP R	CGATGGATGGTCAGATTGTCC
412	p35 MUC-1 F	GAGAGGACGGGAGAATTAAC TA
413	p36 MUC-1 R	TGGTAGGAATACCAGATACGAC
414	p44 VP40 F	GGAGCAGAGTTTACATCTTCCAA
415	p10 VP40 R	CTCCGTGAGAATATCCTTGCTC

EXAMPLE 15. Validation of recombinant protein production by MVA-VLP-MUC-1-LD10 infected DF-1 cells

To establish the expression of MUC-1 and VP40 protein from the recombinant MVA-VLP-MUC-1-LD10 viral vector, DF1 cells were cultured in 6-well plates and infected with either parental modified vaccinia Ankara (pMVA) or recombinant MVA virus encoding VLP-MUC-1-LD10. Cellular supernatant and lysate were harvested and analyzed by SDS-PAGE on a Mini-Protean TGX gel and transferred to a PVDF membrane. The membranes were then probed with MUC1 antibody (mouse monoclonal VU4H5, Santa Cruz #sc-7313, 1:200). The expected size of MUC-1 protein is 63 kDa. Robust expression of MUC-1 protein was observed only in MVA-VLP-MUC-1-LD10 lysate and not in the supernatant fraction of cells infected with the recombinant MVA virus encoding VLP-MUC-1-LD10 (Fig. 12). Negligible signal was observed in all other negative control samples.

Transferred membranes were similarly probed with VP40 antibody (rabbit polyclonal, IBT Bioservices #0303-001, 1:1000). The expected size of recombinant VP40 protein is 32 kDa. Robust expression of VP40 protein was observed in MVA-VLP-MUC-1-LD10 cellular supernatant and lysate, suggesting that VP40 is expressed and also secreted in cells infected with the recombinant MVA virus encoding VLP-MUC-1-LD10 (Fig. 13).

To confirm expression of LD10 peptide, a dot blot was performed on infected cell lysates. As a positive control, 20 ng of a Leidos LD10 peptide was included. The membrane was probed with LD10 antibody (mouse, Leidos 014, 7G10). Labeling of peptide and the MVA-VLP-MUC_1-LD10 sample confirmed LD10 expression in MVA-VLP-MUC-1-LD10-infected cells (Fig. 14).

EXAMPLE 16. Establishing MVA vaccine purity of DF-1 cells infected with MVA-VLP-MUC-1-LD10

DF1 cells were infected in technical triplicate with 30 plaque forming units (PFU) of virus, and separately, in technical triplicate with 60 PFU of virus in a 6-well plate. All wells were probed with MUC-1 antibody (mouse monoclonal VU4H5, Santa Cruz #sc-7313, 1:200) and the number of plaques were counted (Fig. 15). The wells were washed before being probed again with MVA antibody and MVA positive plaques were counted. The percentage of MUC1 plaques versus the

number of MVA plaques was calculated to observe purity of the vaccine. Approximately 95% or greater MVA-positive plaques were also positive for MUC-1 expression at both infection quantities.

5 DF1 cells were infected in technical triplicate with 30PFU of virus, and separately, in technical triplicate with 60 PFU of virus in a 6-well plate. All wells were probed with VP40 antibody (rabbit polyclonal, IBT Bioservices #0303-001, 1:1000) and the number of plaques were counted (Fig. 16). The wells were washed before being probed again with MVA antibody and MVA positive plaques were counted. The percentage of VP40 plaques vs the number of MVA
10 positive plaques were also positive for VP40 expression at both infection quantities.

Claims**We Claim:**

1. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x, wherein x = 2-10, and M is methionine.
2. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide)), wherein x = 1-10, and M is methionine.
3. The rMVA of claims 1 or 2, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-56, or an amino acid sequence at least 95% identical thereto.
4. The rMVA of claims 1-3, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS: 1-15, or an amino acid sequence at least 95% identical thereto.
5. The rMVA of claims 1-4, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS: 1 or 5, or an amino acid sequence at least 95% identical thereto.
6. The rMVA of claims 1-5, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO: 1, or an amino acid sequence at least 95% identical thereto.
7. The rMVA of claims 1-5, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence at least 95% identical thereto.
8. The rMVA of claims 1-7, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS: 57-90, or an amino acid sequence at least 95% identical thereto.

9. The rMVA of claims 1-8, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NO: 65, or an amino acid sequence at least 95% identical thereto.
10. The rMVA of claims 1-8, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NO: 66, or an amino acid sequence at least 95% identical thereto.
11. The rMVA of claims 1-10, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS: 91-127, or an amino acid sequence at least 95% identical thereto.
12. The rMVA of claims 1-11, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS: 93, 120, and 123, or an amino acid sequence at least 95% identical thereto.
13. The rMVA of claims 1-11, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91).
14. The rMVA of claims 1-11, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = R, K, or H (SEQ ID NO: 92).
15. The rMVA of claims 1-12, wherein the cleavable peptide is RAKR (SEQ ID NO: 93).
16. The rMVA of claims 1-11, wherein the cleavable peptide is RRRR (SEQ ID NO: 94).
17. The rMVA of claims 1-11, wherein the cleavable peptide is RKRR (SEQ ID NO: 95).
18. The rMVA of claims 1-11, wherein the cleavable peptide is RRKR (SEQ ID NO: 96).
19. The rMVA of claims 1-11, wherein the cleavable peptide is RKKR (SEQ ID NO: 97).
20. The rMVA of claims 1-11, wherein the cleavable peptide is an amino acid sequence of SEQ ID NOS: 123-127, or an amino acid sequence at least 95% identical thereto.
21. The rMVA of claims 1-12, wherein the cleavable peptide is the amino acid of SEQ ID NOS: 123, or an amino acid sequence at least 95% identical thereto.
22. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 309-324, or an amino acid sequence at least 95% identical thereto.
23. The rMVA of claims 1-22, wherein $x \geq 4$.
24. The rMVA of claims 1-22, wherein $x = 3, 4, \text{ or } 5$.

25. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 325-340, or an amino acid sequence at least 95% identical thereto.
26. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 341-344, or an amino acid sequence at least 95% identical thereto.
27. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes an amino acid sequence selected from SEQ ID NOS: 345-348, or an amino acid sequence at least 95% identical thereto.
28. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 325, or an amino acid sequence at least 95% identical thereto.
29. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 329, or an amino acid sequence at least 95% identical thereto.
30. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 333, or an amino acid sequence at least 95% identical thereto.
31. The rMVA of claims 1-2, wherein the polycistronic nucleic acid encodes the amino acid sequence of SEQ ID NO: 337, or an amino acid sequence at least 95% identical thereto.
32. The rMVA of claims 1-31, wherein the polycistronic nucleic acid further encodes an antigenic peptide.
33. The rMVA of claim 32, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
34. The rMVA of claim 33, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
35. The rMVA of claim 34, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus;

Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

36. The rMVA of claim 32, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.
37. The rMVA of claim 32, wherein the antigenic peptide is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); the MUC-1 MARV GPTM amino acid sequence; the Marburg virus VP40 amino acid sequence; and the MUC-1-ECD-MARVTM-ICD sequence; or fragment thereof.

38. The rMVA of claim 33, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.
39. The rMVA of claim 33, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
40. The rMVA of claim 32, wherein the antigenic peptide is derived from mucin 1, or fragment thereof.
41. The rMVA of claim 40, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.
42. The method of claim 40, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
43. The rMVA of claim 40, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
44. The rMVA of claim 40, wherein the mucin 1 comprises an extracellular domain fragment of human mucin 1.
45. The rMVA of claim 44, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
46. The rMVA of claim 40, wherein the mucin 1 comprises an intracellular domain fragment of human mucin 1.

47. The rMVA of claim 46, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
48. The method of claim 40, wherein the mucin 1 is selected from SEQ ID NO: 363-364, or an amino acid sequence at least 95% identical thereto.
49. The method of claim 48, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
50. The method of claim 48, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
51. The rMVA of claim 32, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
52. The rMVA of claim 32, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
53. The rMVA of claims 51-52, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 350, 354, 356, 365, 366, 367, 368, 369, 377, 379, or an amino acid sequence at least 95% identical thereto.
54. The rMVA of claims 32-53, wherein the antigenic peptide includes a secretion signal.
55. The rMVA of claim 54, wherein the secretion signal is fused to the N-terminus of the antigenic peptide.
56. The rMVA of claim 55, wherein the secretion signal is selected from an amino acid sequence of SEQ ID NOS: 57-90, or an amino acid sequence at least 95% identical thereto.
57. The rMVA of claim 56, wherein the secretion signal comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
58. The rMVA of claim 56, wherein the secretion signal comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.
59. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted between two essential and highly conserved MVA genes.

60. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into a natural deletion site.
61. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the MVA at a site selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
62. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes I8R and G1L.
63. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A50R and B1R in a restructured and modified deletion site III.
64. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A5 and A6L.
65. The rMVA of claims 32-64, wherein the nucleic acid encoding the antigenic peptide amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.
66. A method of increasing an immune response to a target antigen in a patient comprising administering to the patient an effective amount of an rMVA viral vector of claims 1-65, wherein the patient has been or is being administered an effective amount of the target antigen.
67. The method of claim 66, wherein the rMVA viral vector is administered concomitantly with or subsequent to the administration of the target antigen.
68. The method of claims 66-67, wherein the target antigen is selected from the group consisting of an infectious agent and tumor associated antigen.
69. The method of claim 68, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
70. The method of claim 69, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus;

Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

71. The method of claims 66-67, wherein the target antigen is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR, or fragment thereof.
72. The method of claims 66-67, wherein the target antigen is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.
73. The method of claim 68, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated

tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.

74. The method of claim 68, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
75. The method of claims 66-67, wherein the target antigen is derived from mucin 1, or fragment thereof.
76. The method of claim 75, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.
77. The method of claim 75, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
78. The method of claim 75, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
79. The method of claim 75, wherein the mucin 1 comprises an extracellular domain fragment of human mucin 1.
80. The method of claim 79, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
81. The method of claim 75, wherein the mucin 1 comprises an intracellular domain fragment of human mucin 1.

82. The method of claim 81, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
83. The method of claim 75, wherein the mucin 1 is selected from SEQ ID NO: 363-364, or an amino acid sequence at least 95% identical thereto.
84. The method of claim 83, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
85. The method of claim 83, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
86. The method of claims 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
87. The method of claims 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
88. The method of claims 66-67, wherein the target antigen is derived from an amino acid sequence selected from SEQ ID NOS: 350, 354, 356, 365, 366, 367, 368, 369, 377, 379, or an amino acid sequence at least 95% identical thereto.
89. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Secretion Signal Peptide-Antigenic Peptide), wherein $x = 1-10$, and M is methionine.
90. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-Glycoprotein Transmembrane Peptide), wherein $x = 1-10$, and M is methionine.
91. An rMVA viral vector comprising a heterologous, polycistronic nucleic acid, wherein the polycistronic nucleic acid encodes (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Glycoprotein Signal Peptide-Antigenic Peptide-

Glycoprotein Transmembrane Peptide-Cleavable Peptide)(Viral Matrix Protein), wherein x = 1-10, and M is methionine.

92. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising a heterologous polycistronic nucleic acid insert encoding a polypeptide wherein the polypeptide comprises ((M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavable Peptide)_x(Antigenic Peptide)), wherein x = 1-10, and M is methionine.
93. The rMVA of claims 89-92, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-56, or an amino acid sequence at least 95% identical thereto.
94. The rMVA of claims 89-93, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1-15, or an amino acid sequence at least 95% identical thereto.
95. The rMVA of claims 89-94, wherein the immune checkpoint inhibitor peptide comprises an amino acid sequence selected from SEQ ID NOS. 1 or 5, or an amino acid sequence at least 95% identical thereto.
96. The rMVA of claims 89-95, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO. 1, or an amino acid sequence at least 95% identical thereto.
97. The rMVA of claims 89-95, wherein the immune checkpoint inhibitor peptide comprises the amino acid sequence of SEQ ID NO. 5, or an amino acid sequence at least 95% identical thereto.
98. The rMVA of claims 89-97, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS. 57-90, or an amino acid sequence at least 95% identical thereto.
99. The rMVA of claims 89-98, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
100. The rMVA of claims 89-98, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.

101. The rMVA of claims 89-100 wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 91-126, or an amino acid sequence at least 95% identical thereto.
102. The rMVA of claims 89-101, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 93, 120, and 123.
103. The rMVA of claims 89-101, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = any amino acid (SEQ ID NO: 91).
104. The rMVA of claims 89-101, wherein the cleavable peptide comprises an amino acid sequence RX(R/K)R, wherein X = R, K, or H (SEQ ID NO: 92).
105. The rMVA of claims 89-102, wherein the cleavable peptide is RAKR (SEQ ID NO: 93).
106. The rMVA of claims 89-101, wherein the cleavable peptide is RRRR (SEQ ID NO: 94).
107. The rMVA of claims 89-101, wherein the cleavable peptide is RKRR (SEQ ID NO: 95).
108. The rMVA of claims 89-101, wherein the cleavable peptide is RRKR (SEQ ID NO: 96).
109. The rMVA of claims 89-101, wherein the cleavable peptide is RKKR (SEQ ID NO: 97).
110. The rMVA of claims 89-101, wherein the cleavable peptide comprises an amino acid sequence selected from SEQ ID NOS. 123-127, or an amino acid sequence at least 95% identical thereto.
111. The rMVA of claims 89-102, wherein the cleavable peptide comprises the amino acid sequence of SEQ ID NO. 123, or an amino acid sequence at least 95% identical thereto.
112. The rMVA of claims 89-111, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
113. The rMVA of claim 112, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
114. The rMVA of claim 113, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus;

Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

115. The rMVA of claims 89-111, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.
116. The rMVA of claims 89-111, wherein the antigenic peptide is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.
117. The rMVA of claim 112, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated

tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.

118. The rMVA of claim 112, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
119. The rMVA of claims 89-111, wherein the antigenic peptide is derived from mucin 1, or fragment thereof.
120. The rMVA of claim 119, wherein the mucin 1 is encoded by the nucleic acid sequence of SEQ ID NO: 402, or a nucleic acid sequence at least 95% identical thereto.
121. The method of claim 119, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 349, or an amino acid sequence at least 95% identical thereto.
122. The rMVA of claim 119, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 403, or an amino acid sequence at least 95% identical thereto.
123. The rMVA of claim 119, wherein the mucin 1 comprises an extracellular domain fragment of human mucin 1.
124. The rMVA of claim 123, wherein the extracellular domain fragment of human mucin 1 is selected from SEQ ID NO: 358-361, or an amino acid sequence at least 95% identical thereto.
125. The rMVA of claim 119, wherein the mucin 1 comprises an intracellular domain fragment of human mucin 1.

126. The rMVA of claim 125, wherein the intracellular domain fragment of human mucin 1 comprises the amino acid sequence of SEQ ID NO: 362, or an amino acid sequence at least 95% identical thereto.
127. The method of claim 119, wherein the mucin 1 is selected from SEQ ID NO: 363-364, or an amino acid sequence at least 95% identical thereto.
128. The method of claim 127, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 363, or an amino acid sequence at least 95% identical thereto.
129. The method of claim 127, wherein the mucin 1 comprises the amino acid sequence of SEQ ID NO: 364, or an amino acid sequence at least 95% identical thereto.
130. The rMVA of claims 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 349-357, or an amino acid sequence at least 95% identical thereto.
131. The rMVA of claims 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
132. The rMVA of claims 89-111, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 403, or an amino acid sequence at least 95% identical thereto.
133. The rMVA of claims 89-132, wherein the glycoprotein signal peptide is derived from a *Filoviridae*.
134. The rMVA of claims 89-133, wherein the glycoprotein signal peptide comprises the amino acid sequence of SEQ ID NO. 396, or an amino acid sequence at least 95% identical thereto.
135. The rMVA of claims 89-133, wherein the glycoprotein transmembrane peptide comprises the amino acid sequence of SEQ ID NO. 398, or an amino acid sequence at least 95% identical thereto.
136. The rMVA of claims 89-135, wherein the viral matrix protein comprises the amino acid sequence of SEQ ID NO. 400, or an amino acid sequence at least 95% identical thereto.
137. The rMVA of claims 89-136, wherein $x \geq 4$.
138. The rMVA of claims 89-136, wherein x is 3, 4, or 5.

139. The rMVA of claims 89-138, wherein the polycistronic nucleic acid is inserted between two essential and highly conserved MVA genes.
140. The rMVA of claims 89-138, wherein the polycistronic nucleic acid is inserted into a natural deletion site.
141. The rMVA of claims 89-138, wherein the polycistronic nucleic acid is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
142. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes I8R and G1L.
143. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A50R and B1R in a restructured and modified deletion site III.
144. The rMVA of claims 1-58, wherein the polycistronic nucleic acid is inserted into the rMVA at a site selected from between MVA genes A5 and A6L.
145. The rMVA of claims 89-144, wherein the nucleic acid encoding the antigenic peptide amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.
146. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:
a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $\times = 1-10$, and M is methionine; and
b) a second nucleic acid sequence encoding an antigenic peptide;
wherein the Immune Checkpoint Inhibitor Peptide is selected from an amino acid having the sequence of SEQ ID NO: 1-57; and,
wherein the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more vaccinia virus promoters.
147. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:
a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $\times = 1-10$, and M is methionine; and

- b) a second nucleic acid sequence encoding an antigenic peptide;
wherein the Immune Checkpoint Inhibitor Peptide is SEQ ID NO:1, and the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more vaccinia virus promoters.
148. A recombinant modified vaccinia Ankara (rMVA) viral vector comprising:
a) a first nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide) $\times$ (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein $\times = 1-10$, and M is methionine; and
b) a second nucleic acid sequence encoding an antigenic peptide;
wherein the Immune Checkpoint Inhibitor Peptide is SEQ ID NO:5, and the first nucleic acid sequence and the second nucleic acid sequence are under the control of one or more vaccinia virus promoters.
149. The rMVA of claims 146-148, wherein the secretion signal peptide comprises an amino acid sequence selected from SEQ ID NOS. 57-90, or an amino acid sequence at least 95% identical thereto.
150. The rMVA of claims 146-149, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 65, or an amino acid sequence at least 95% identical thereto.
151. The rMVA of claims 146-149, wherein the secretion signal peptide comprises the amino acid sequence of SEQ ID NO. 66, or an amino acid sequence at least 95% identical thereto.
152. The rMVA of claims 146-151, wherein the vaccinia virus promoter is selected from the nucleic acid sequence of SEQ ID NO:128-308.
153. The rMVA of claim 152, wherein the antigenic peptide is derived from the group consisting of an infectious agent and tumor associated antigen.
154. The rMVA of claim 153, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
155. The rMVA of claim 154, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a

Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus; Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

156. The rMVA of claim 152, wherein the antigenic peptide is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; or fragment thereof.
157. The rMVA of claim 152, wherein the antigenic peptide is derived from the group consisting of the SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.

158. The rMVA of claim 152, wherein the antigenic peptide is derived from an amino acid sequence selected from SEQ ID NOS: 358-394, or an amino acid sequence at least 95% identical thereto.
159. The rMVA of claims 146-158, wherein the first nucleic acid sequence and the second nucleic acid sequence are inserted into the MVA between essential MVA genes.
160. The rMVA of claims 146-158, wherein the first nucleic acid sequence is inserted into the MVA between essential MVA genes.
161. The rMVA of claims 146-160, wherein the second nucleic acid sequence is inserted into the MVA between essential MVA genes.
162. The rMVA of claims 146-158, wherein the first nucleic acid sequence and the second nucleic acid sequence are inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
163. The rMVA of claims 146-158, wherein the first nucleic acid sequence is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
164. The rMVA of claims 146-158, wherein the second nucleic acid sequence is inserted into the MVA at sites selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
165. The rMVA of claims 146-164, wherein the vaccinia virus promoter is a nucleic acid sequence of SEQ ID NOS:128-130, or a nucleic acid sequence at least 95% identical thereto.
166. The rMVA of claims 146-165, wherein the vaccinia virus promoter is SEQ ID NO:130, or a nucleic acid sequence at least 95% identical thereto.
167. The rMVA of claims 146-166, wherein the nucleic acid encoding the antigenic peptide amino acid sequence is in an open reading frame downstream of a Methionine (M) start codon.
168. The rMVA of claims 146-167, wherein $x \geq 4$.
169. The rMVA of claims 146-167, wherein x is 3, 4, or 5.

170. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

- i) a first nucleic acid sequence encoding an amino acid sequence comprising (Mucin 1 Extracellular Fragment Peptide-Glycoprotein Transmembrane Peptide-Mucin 1 Intracellular Fragment Peptide); and
- ii) a second nucleic acid sequence encoding an amino acid sequence comprising a Marburg virus (MARV) VP40 Protein; and
- iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein x = 1-10, and M is methionine;

wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

171. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

- i) a first nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 402 encoding a chimeric amino acid sequence;
- ii) a second nucleic acid sequence comprising the nucleic acid sequence of SEQ ID NO: 404;
- iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein x = 1-10, and M is methionine;

wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

172. A recombinant modified vaccinia ankara (rMVA) viral vector comprising:

- i) a first nucleic acid sequence encoding a chimeric amino acid sequence comprising the amino acid sequence of SEQ ID NO: 403; and

ii) a second nucleic acid sequence encoding a MARV VP40 matrix protein comprising the amino acid sequence of SEQ ID NO: 405; and

iii) a third nucleic acid sequence encoding an amino acid sequence comprising (M)(Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide-Cleavage Peptide)_x (Secretion Signal Peptide-Immune Checkpoint Inhibitor Peptide), wherein x = 1-10, and M is methionine;

wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are under the control of a vaccinia virus promoter; and wherein upon expression, the chimeric amino acid sequence and VP40 matrix protein are capable of assembling together to form virus-like particles (VLPs).

173. The rMVA of claims 170-172, wherein the third nucleic acid sequence comprises the nucleic sequence of SEQ ID NO: 408, or a nucleic acid sequence at least 95% identical thereto.

174. The rMVA of claims 170-172, wherein the third nucleic acid sequence comprises the nucleic sequence of SEQ ID NO: 409, or a nucleic acid sequence at least 95% identical thereto.

175. The rMVA of claims 170-172, wherein the third nucleic acid sequence is an amino acid sequence selected from SEQ ID NOS: 1, 5, or 309-348, or an amino acid at least 95% identical thereto.

176. The rMVA of claim 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 325, or an amino acid sequence at least 95% identical thereto.

177. The rMVA of claim 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 329, or an amino acid sequence at least 95% identical thereto.

178. The rMVA of claim 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 333, or an amino acid sequence at least 95% identical thereto.

179. The rMVA of claim 175, wherein the third nucleic acid sequence encodes an immune checkpoint inhibitor peptide comprising the amino acid sequence of SEQ ID NOS: 337, or an amino acid sequence at least 95% identical thereto.
180. The rMVA of claims 170-179, wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted between two essential and highly conserved MVA genes.
181. The rMVA of claims 170-179, wherein the first nucleic acid sequence, the second nucleic acid sequence, and the third nucleic acid sequence are inserted into the rMVA at a site selected from between MVA genes I8R and G1L, between MVA genes A50R and B1R in a restructured and modified deletion site III, or between MVA genes A5 and A6L.
182. The rMVA of claims 170-179, wherein the first nucleic acid sequence is inserted between MVA genes I8R and G1L.
183. The rMVA of claims 170-179, wherein the second nucleic acid sequence is inserted between MVA genes A50R and B1R in the restructured and modified deletion site III.
184. The rMVA of claims 170-179, wherein the third nucleic acid sequence is inserted between the two essential MVA genes A5R and A6L.
185. The rMVA of claims 170-179, wherein the first nucleic acid sequence is inserted between MVA genes I8R and G1L, the second nucleic acid sequence is inserted between MVA genes A50R and B1R in the restructured and modified deletion site III, and the third nucleic acid sequence is inserted between the two essential MVA genes A5R and A6L.
186. The rMVA of claims 170-185, wherein the vaccinia virus promoter is a nucleic acid sequence selected from SEQ ID NOS: 128-308.
187. The rMVA of claim 170-186, wherein the vaccinia virus promoter is SEQ ID NO:130, or a nucleic acid sequence at least 95% identical thereto.
188. A pharmaceutical composition comprising at least one rMVA of claims 89-187 and a pharmaceutically acceptable carrier.
189. A method of preventing, treating, or inducing an immune response against, a target antigen in a patient in need thereof, said method comprising administering an effective amount of the pharmaceutical composition of claim 188, wherein the pharmaceutical composition enhances immunity directed against the target antigen.

190. The method of claim 189, wherein the target antigen is selected from the group consisting of a tumor associated antigen and an infectious agent.
191. The method of claim 190, wherein the tumor associated antigen is derived from an oncofetal tumor associate antigen, an oncoviral tumor associate antigen, overexpressed/accumulated tumor associate antigen, cancer-testis tumor associate antigen, lineage-restricted tumor associate antigen, mutated tumor associate antigen, or idiotypic tumor associate antigen, or fragment thereof.
192. The method of claim 190, wherein the tumor associated antigen is derived from the b melanoma antigen (BAGE) family, cancer-associated gene (CAGE) family, G antigen (GAGE) family, melanoma antigen (MAGE) family, sarcoma antigen (SAGE) family and X antigen (XAGE) family, CT9, CT10, NY-ESO-1, L antigen (LAGE) 1, Melanoma antigen preferentially expressed in tumors (PRAME), and synovial sarcoma X (SSX) 2, melanoma antigen recognized by T cells-1/2 (Melan-A/MART-1/2), Gp100/pmel17, tyrosine-related protein (TRP) 1 and 2, P. polypeptide, melanocortin 1 receptor (MC1R), and prostate-specific antigen, β -catenin, breast cancer antigen (BRCA) 1/2, cyclin-dependent kinase (CDK) 4, chronic myelogenous leukemia antigen (CML) 66, fibronectin, p53, Ras, or TGF- β RII, or fragment thereof.
193. The method of claims 189-192, wherein the patient is a human having a cancer.
194. The method of claim 193, wherein the cancer is selected from bowel cancer, ovarian cancer, breast cancer, malignant melanoma, hepatoma, testicular cancer, prostate cancer, multiple myeloma, lymphoma, colorectal cancer, bile duct cancer, pancreatic cancer, lung cancer, melanoma, soft tissue sarcoma, or colon cancer.
195. The method of claim 190, wherein the infectious agent is a virus, bacterium, fungi, parasite, or amoeba.
196. The method of claim 195, wherein the virus is selected from the group consisting of Adenovirus; Herpesvirus; a Poxvirus; a single stranded DNA; a Parvovirus; a double stranded RNA virus; Reovirus; a positive-single stranded RNA virus; Coronavirus; Picornavirus; Togavirus; a negative-single stranded RNA virus; a Orthomyxovirus; a Rhabdovirus; a single-stranded RNA-Retrovirus; a double-stranded DNA-Retrovirus; a Flaviviridae virus; Alphavirus virus, Filoviridae virus; a Paramyxoviridae virus;

Rhabdoviridae virus; a Nyamiviridae virus; an Arenaviridae virus; a Bunyaviridae virus; or Ophioviridae virus; and Orthomyxoviridae virus.

197. The method of claim 190, wherein the infectious agent is derived from the Ebola virus, the envelope glycoprotein of Ebola virus, the matrix protein VP40 of Ebola virus; the Lassa virus, Lassa virus protein Z; the Zika virus, Zika virus non-structural protein 1 (NSP-1); the Marburg virus; the Marburg virus glycoprotein; the Marburg VP40 matrix protein; the Plasmodium sp. parasite; Plasmodium falciparum; Plasmodium sp. circumsporozoite protein (CSP); Plasmodium sp. male gametocyte surface protein P230p (Pfs230 antigen); Plasmodium sp. sporozoite micronemal protein essential for cell traversal (SPECT2); Plasmodium sp. GTP-binding protein; putative antigen; the human immunodeficiency virus; HIV Env protein; HIV gp41; HIV gp120; HIV gp160; HIV Gag protein; HIV MA; HIV CA; HIV SP1; HIV NC; HIV SP2; HIV P6; HIV Pol protein; HIV RT; HIV RNase H; HIV IN; and HIV PR; SARS-CoV2; the SARS-CoV2 full-length S protein Wuhan Strain, the SARS-CoV2 S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length S protein Delta variant; the SARS-CoV2 full-length S protein Delta variant plus; the SARS-CoV2 full-length S protein stabilized by 2 proline substitutions; the SARS-CoV2 full-length stabilized S protein; the SARS-CoV2 full-length stabilized S protein with K417T, E484K, and N501Y substitutions; the SARS-CoV2 full-length stabilized S protein Delta variant; the SARS-CoV2 full-length stabilized S protein Delta variant plus; the SARS-CoV2 E protein; the SARS-CoV2 M protein; the SARS-CoV2 PP1ab polyprotein amino acid sequence; the SARS-CoV2 PP1a polyprotein amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP1-3 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP4-11 amino acid sequence (Wuhan Hu1); the SARS-CoV2 ORF1b polyprotein NSP12-16 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP12 amino acid sequence (Wuhan Hu1); the SARS-CoV2 NSP13-14 amino acid sequence (Wuhan Hu1); and the SARS-CoV2 NSP15-16 amino acid sequence (Wuhan Hu1); or fragment thereof.
198. The method of claims 195-197, wherein the patient is a human exposed to the infectious agent.
199. The method of claim 198, wherein the exposed human is symptomatic.
200. The method of claim 198, wherein the exposed human is asymptomatic.

201. The method of claims 195-197, wherein the patient is a human unexposed to the infectious agent.
202. The method of claims 188-201, wherein the rMVA administration is selected from intramuscular, intraarterial, intravascular, intravenous, intraperitoneal, or subcutaneous injection.
203. The method of claims 188-202, wherein the rMVA comprises an adjuvant for enhancing an immune response.
204. The method of claims 188-202, wherein the rMVA comprises a vaccine for inducing an immune response.
205. The method of claims 192-204, wherein the patient is administered the pharmaceutical composition at least 2 or more times.
206. The method of claim 205, wherein the administrations are separated by at least a 4-week interval.
207. A method of enhancing an immune response in a patient comprising administering to the patient an effective amount of an rMVA of claims 89-187.
208. A method of inducing an immune response to a MUC1 antigen in a patient comprising administering to the patient an effective amount of an rMVA of claims 119-145 or 170-187.
209. The method of claims 207-208, wherein the patient is human.

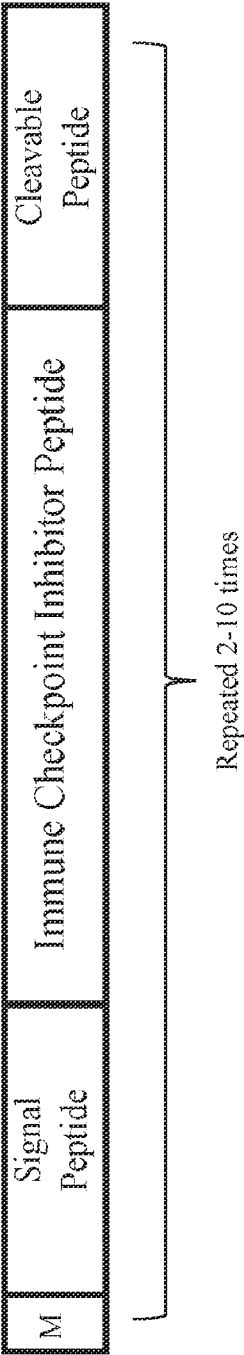


Fig. 1A

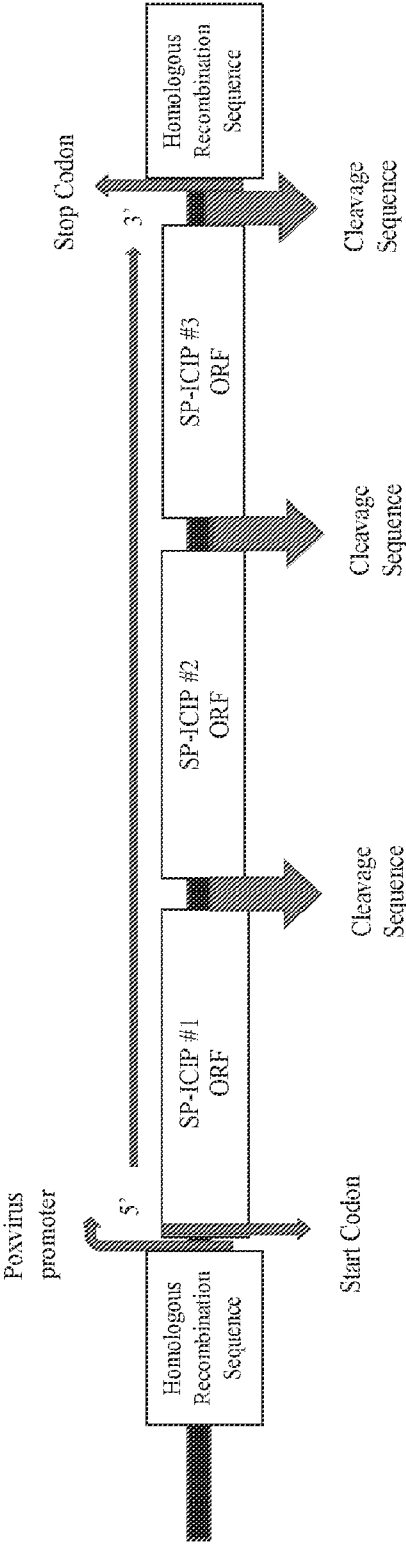


Fig. 1B

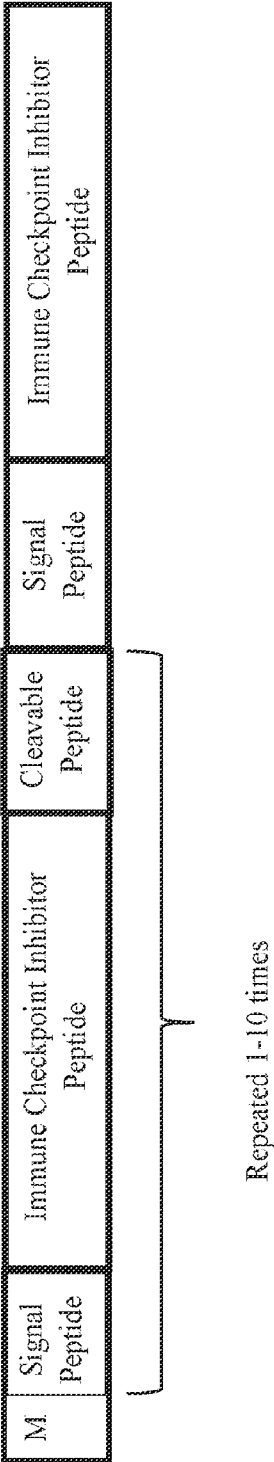


Fig 2A

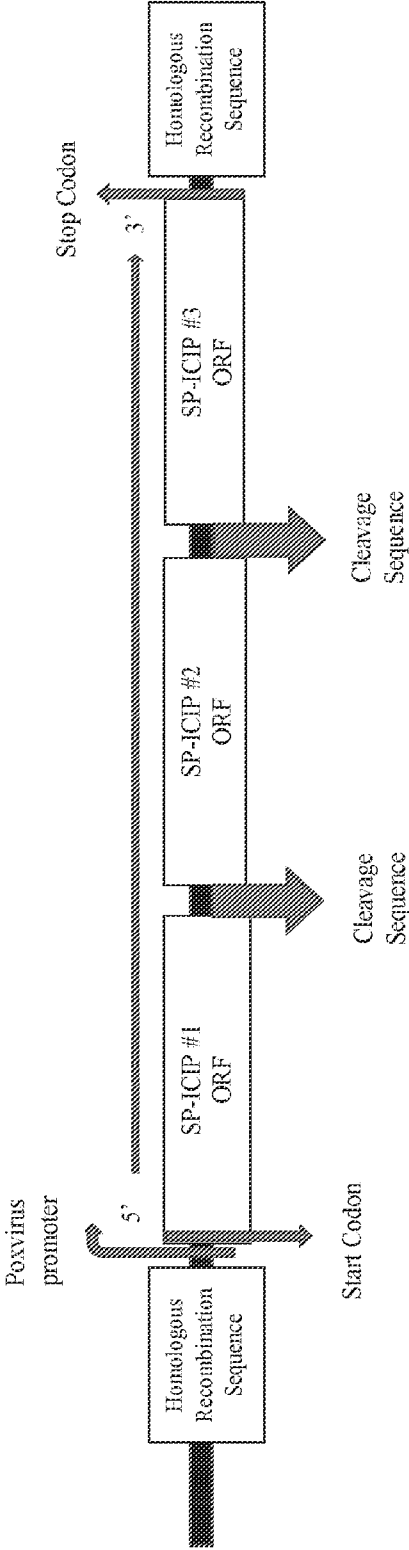
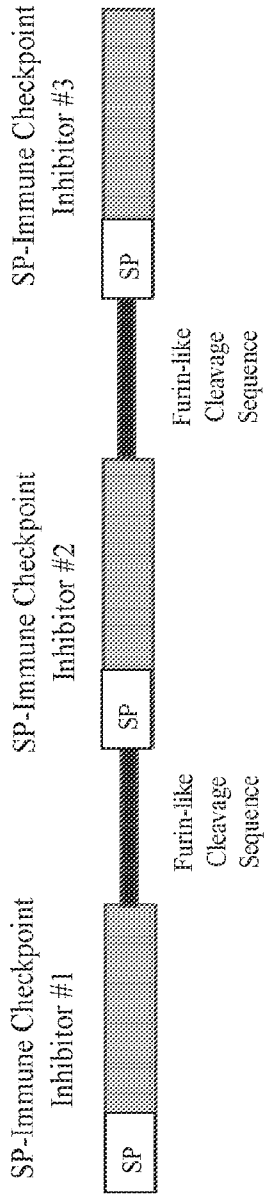


Fig. 2B

Translation



Post-Translation Processing

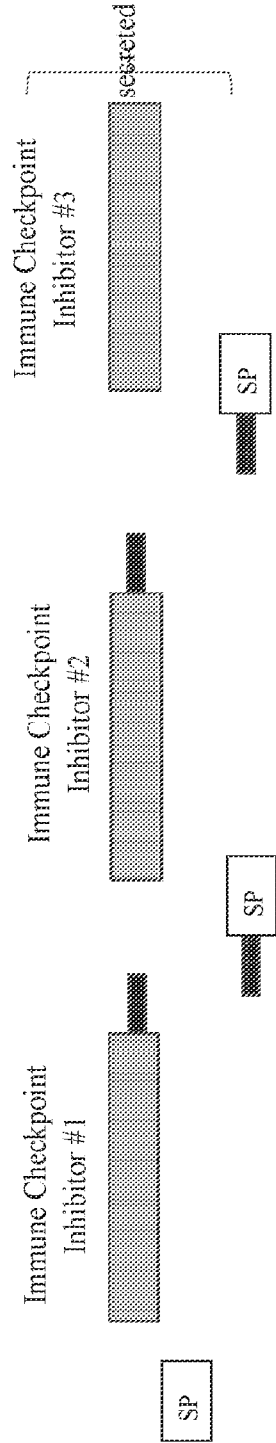
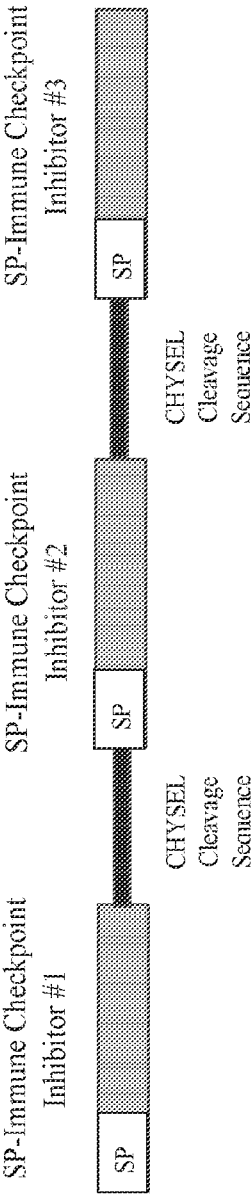


FIG. 3A

Translation



Co & Post-Translation Processing

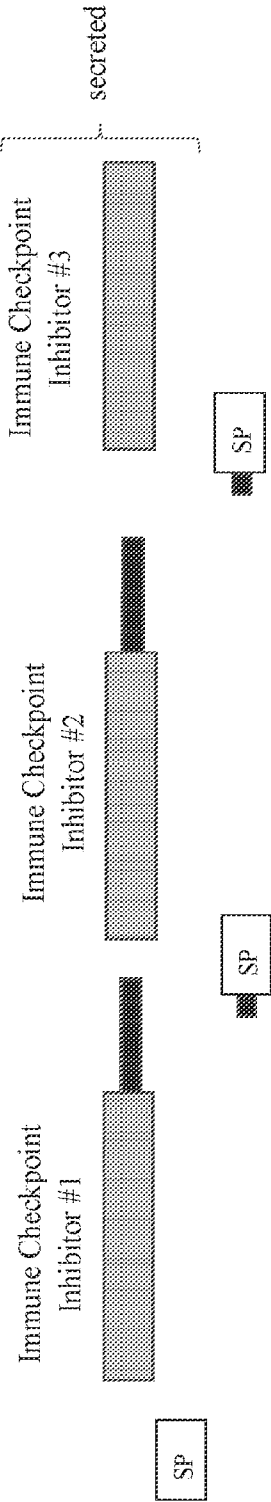
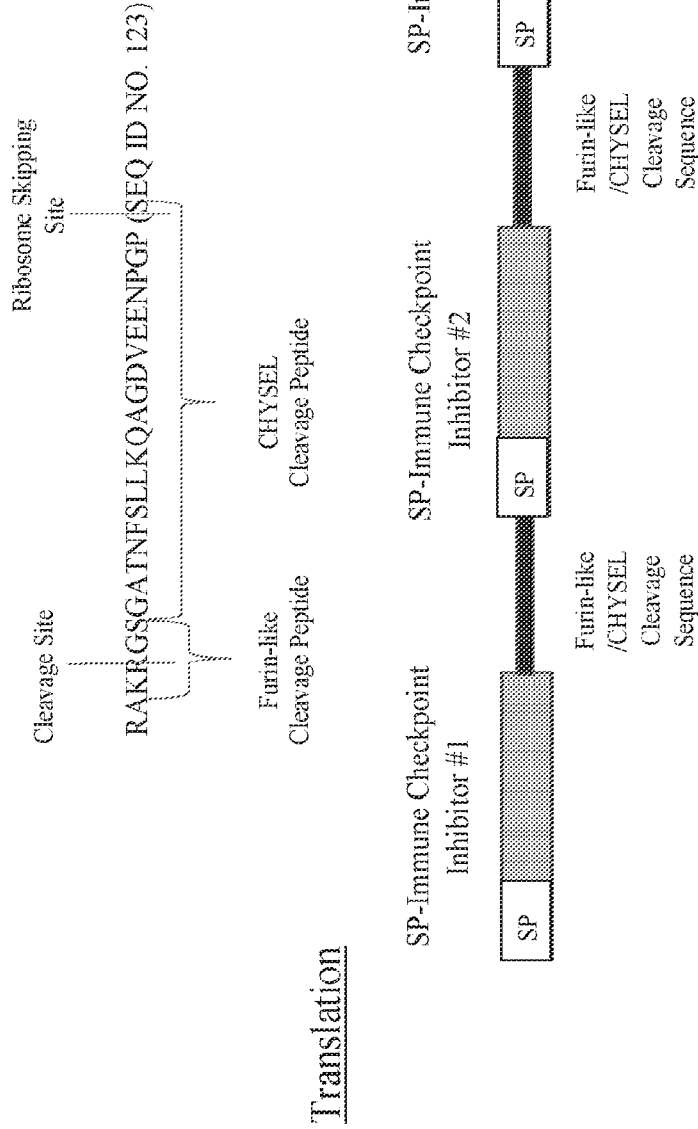


FIG. 3B



Co & Post-Translation Processing

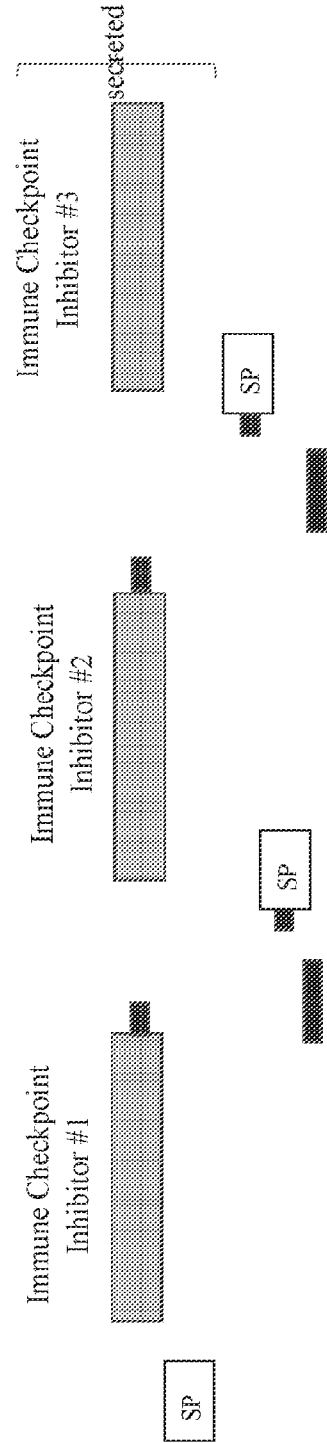


Fig. 3C

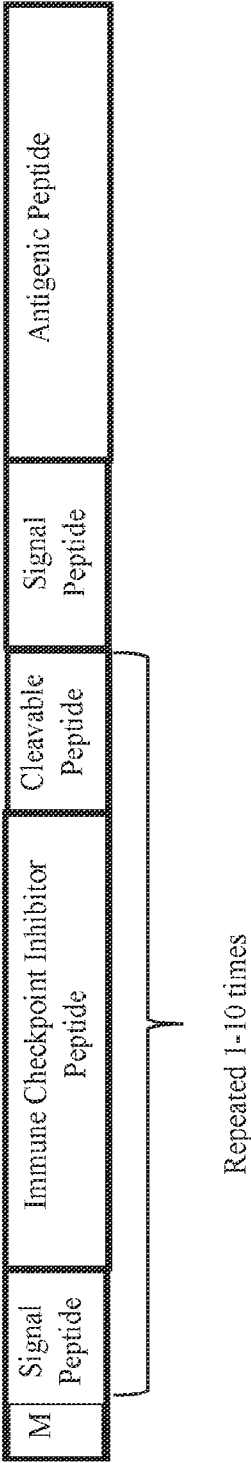


Fig. 4A

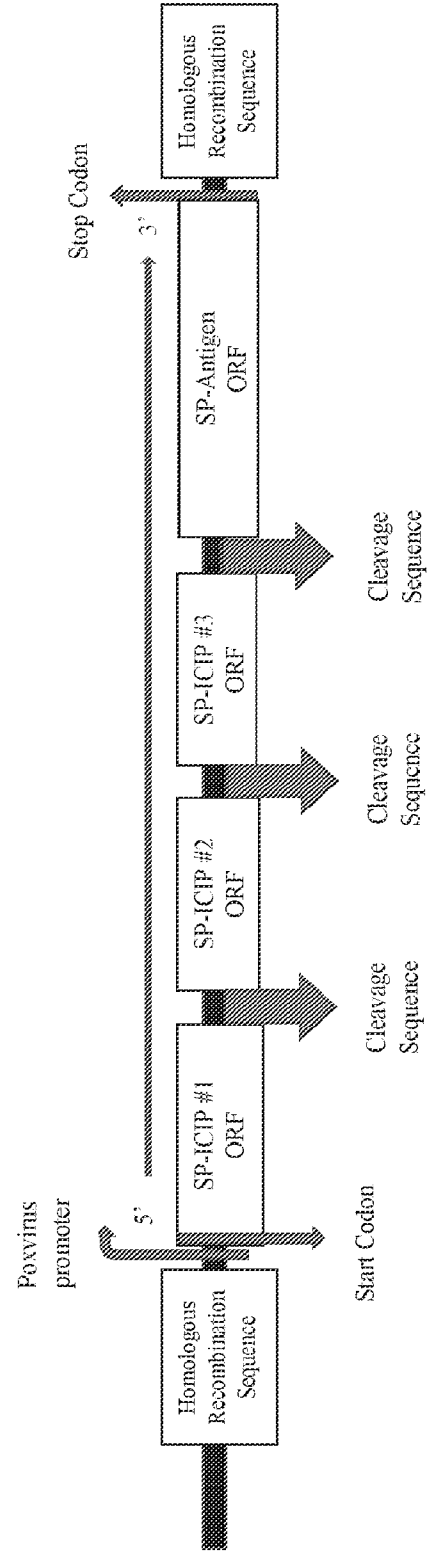


Fig. 4B

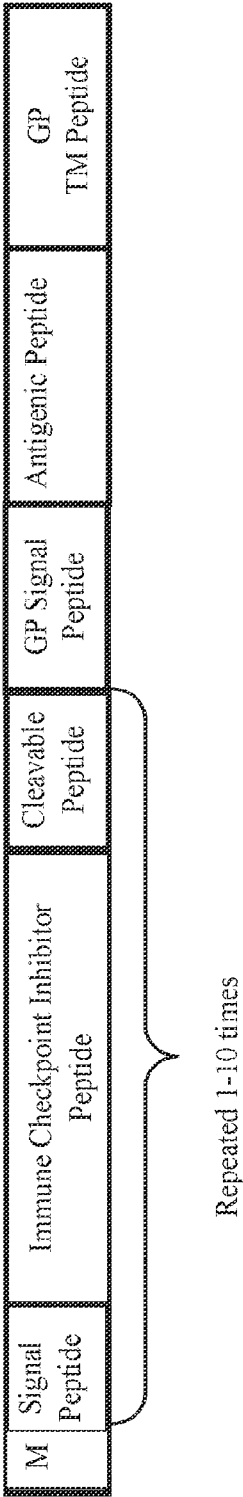


Fig. 5A

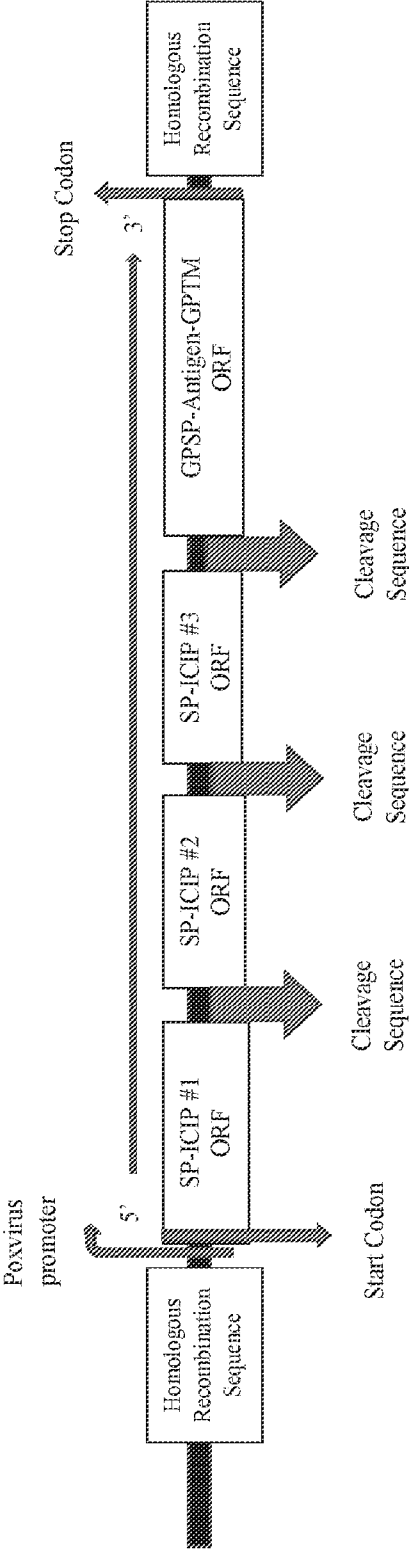


Fig. 5B

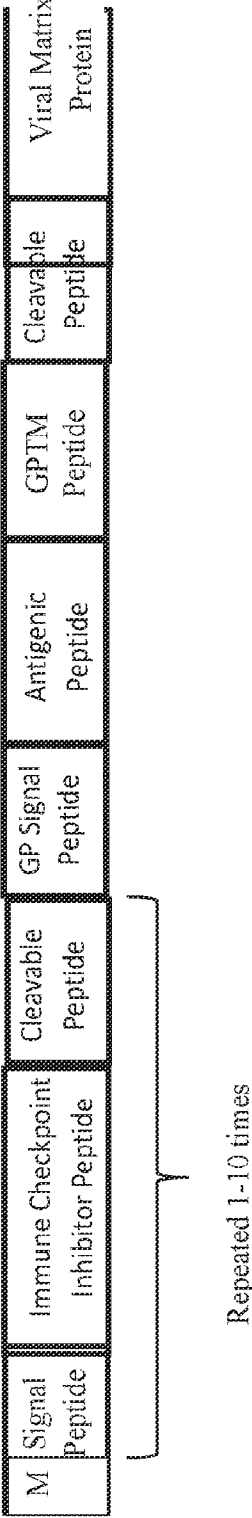


Fig. 6A

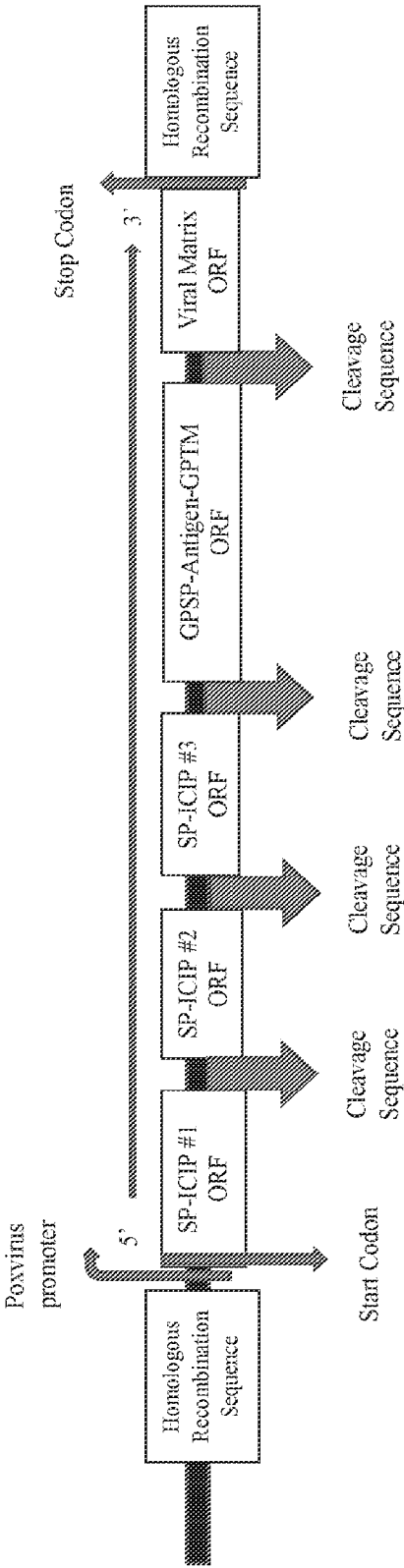


Fig. 6B

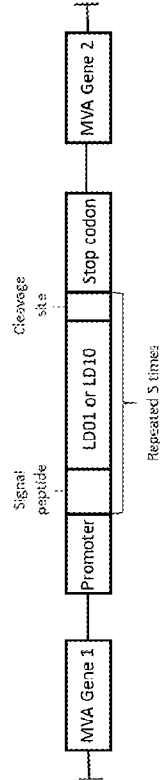
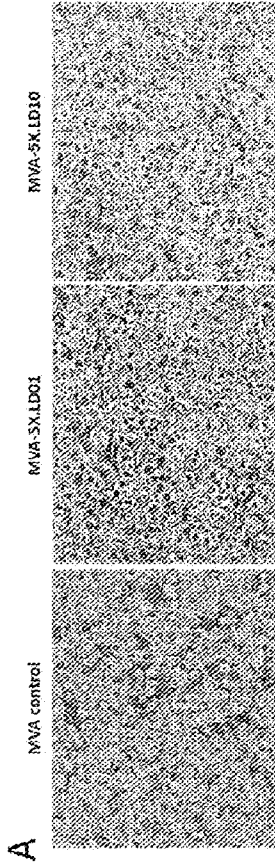


FIG. 7



A

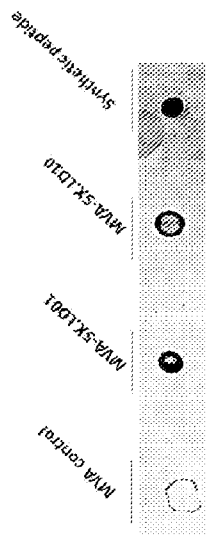


FIG. 8

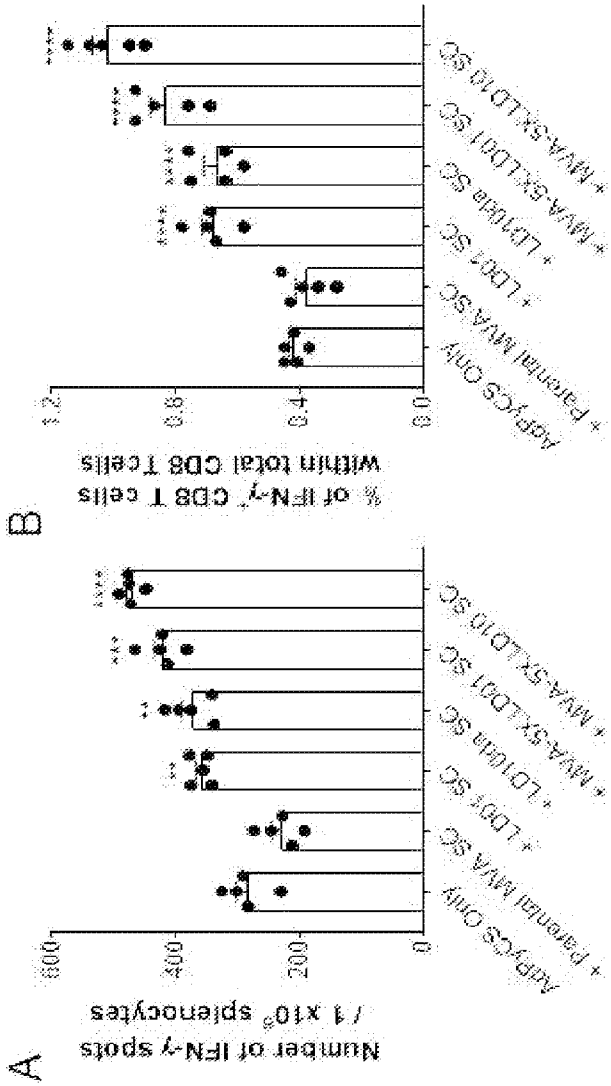


FIG. 9

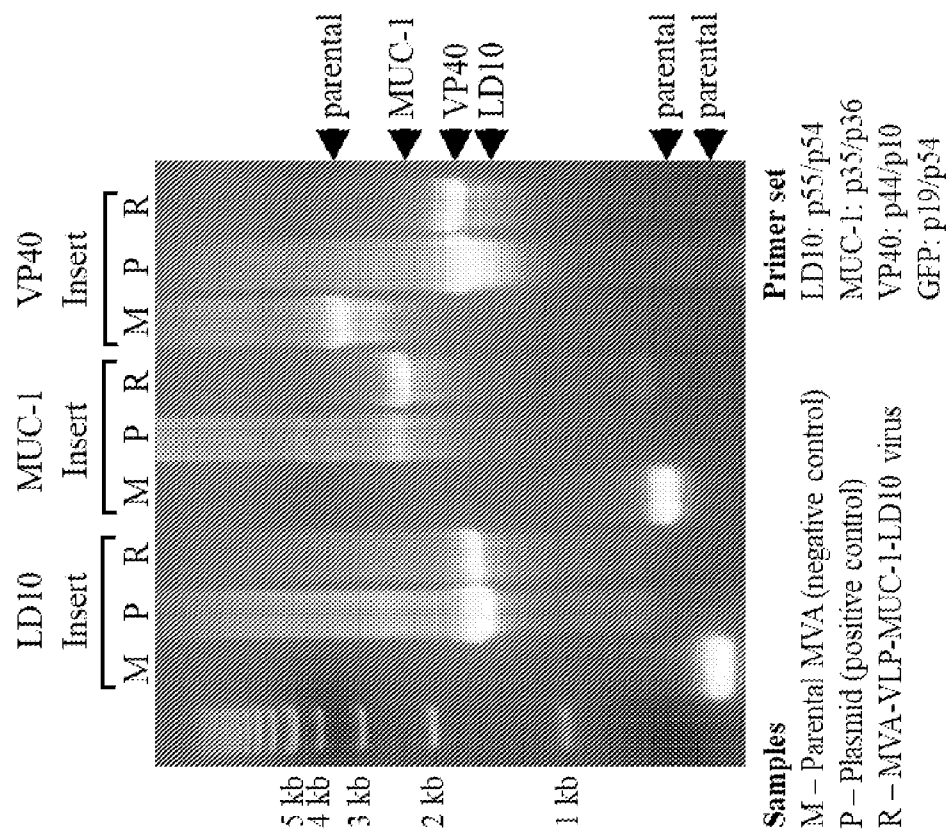


FIG. 10

PCR expected fragments			
Insert (antigen)	LD10	Muc1	VP40
MVA parental (M)	337 bp	553 bp	3302 bp
pGeo-5xLD10 (P)	1631 bp	2273 bp	1700 bp
Muc1-5xLD10 (R)	1631 bp	2273 bp	1700 bp

FIG. 11

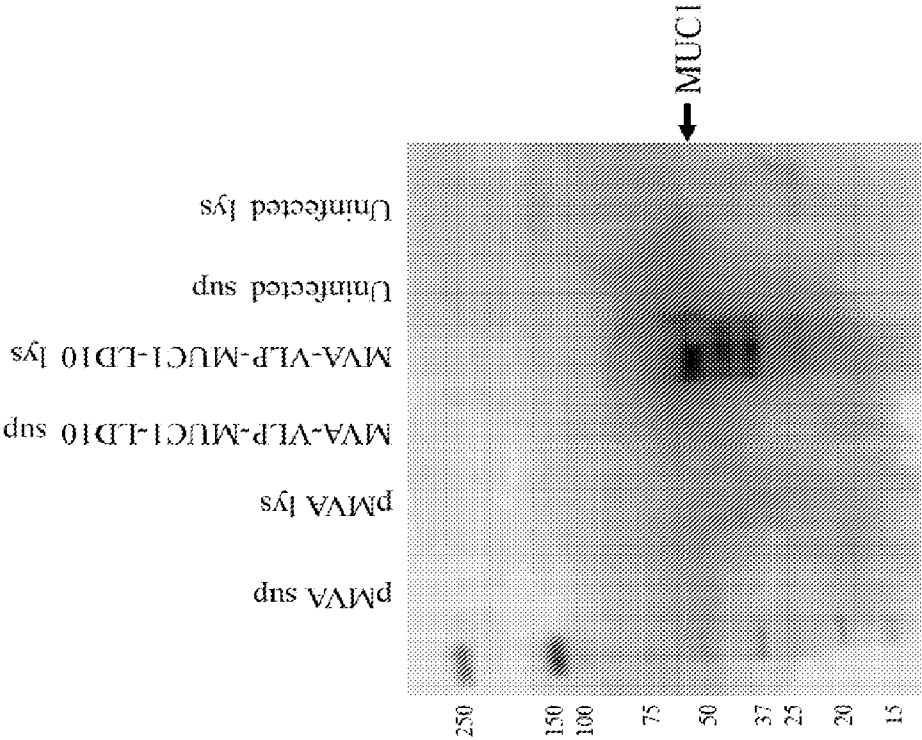


FIG. 12

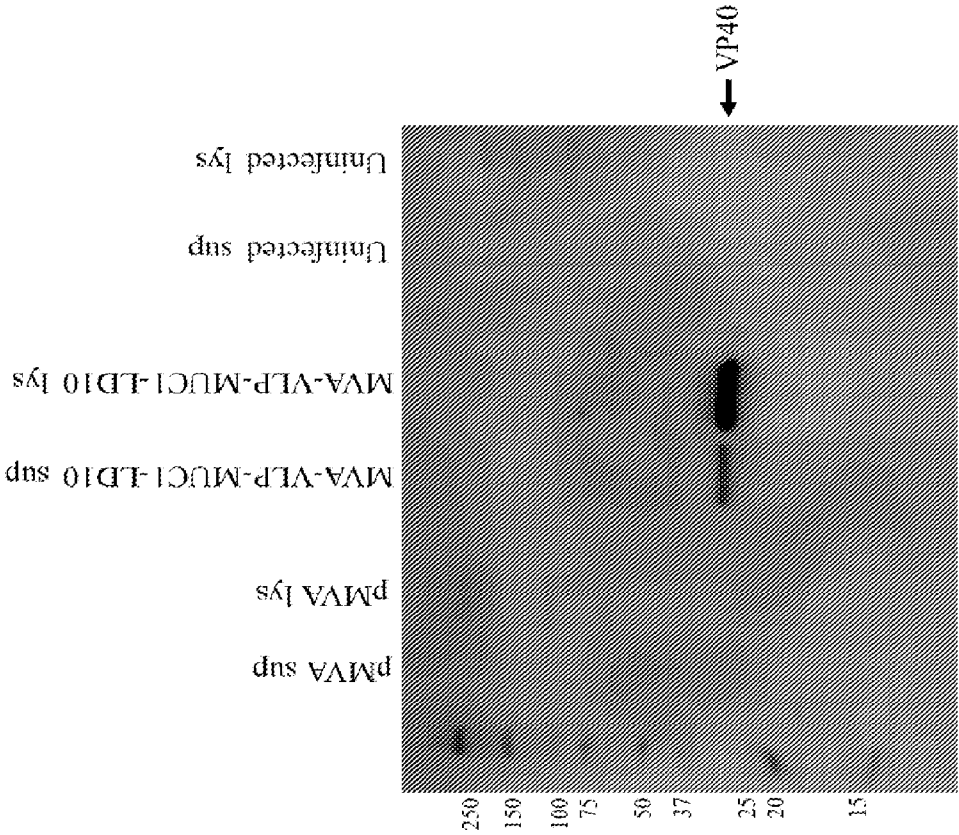


FIG. 13

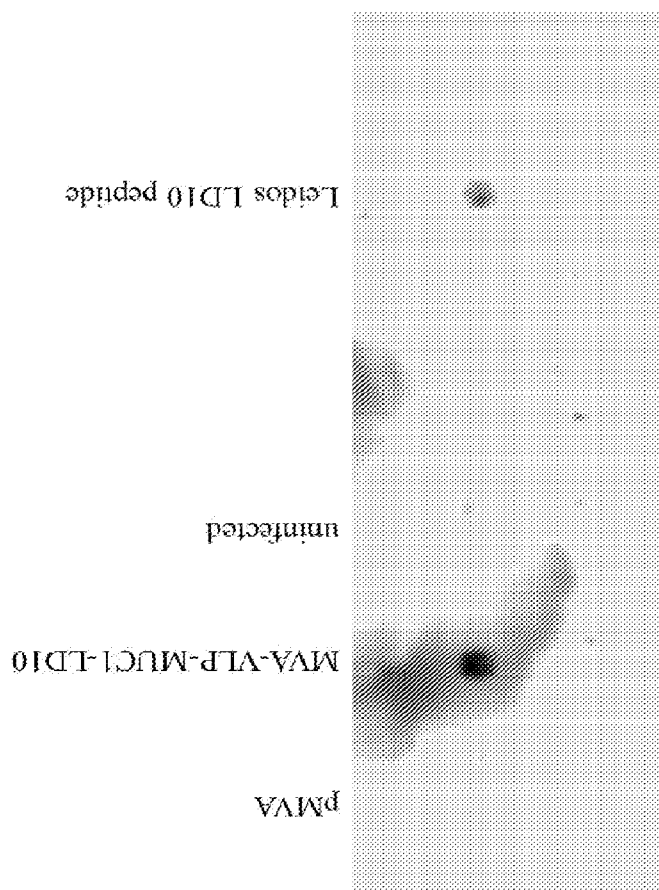


FIG. 14

	MUCI (PFU)	pMVA (PFU)	% Purity
30 PFU	16.7	17	98.24
60 PFU	43.7	46.3	94.38

	MUCI (PFU)	pMVA (PFU)
30 PFU	16	17
30 PFU	14	14
30 PFU	20	20
60 PFU	52	55
60 PFU	40	42
60 PFU	39	42

FIG. 15

	VP40 (PFU)	pMVA (PFU)	% Purity
30 PFU	21	22	95.45
60 PFU	35.7	37.7	94.69

	VP40 (PFU)	pMVA (PFU)
30 PFU	23	23
30 PFU	19	20
30 PFU	21	23
60 PFU	39	41
60 PFU	43	45
60 PFU	25	27

FIG. 16

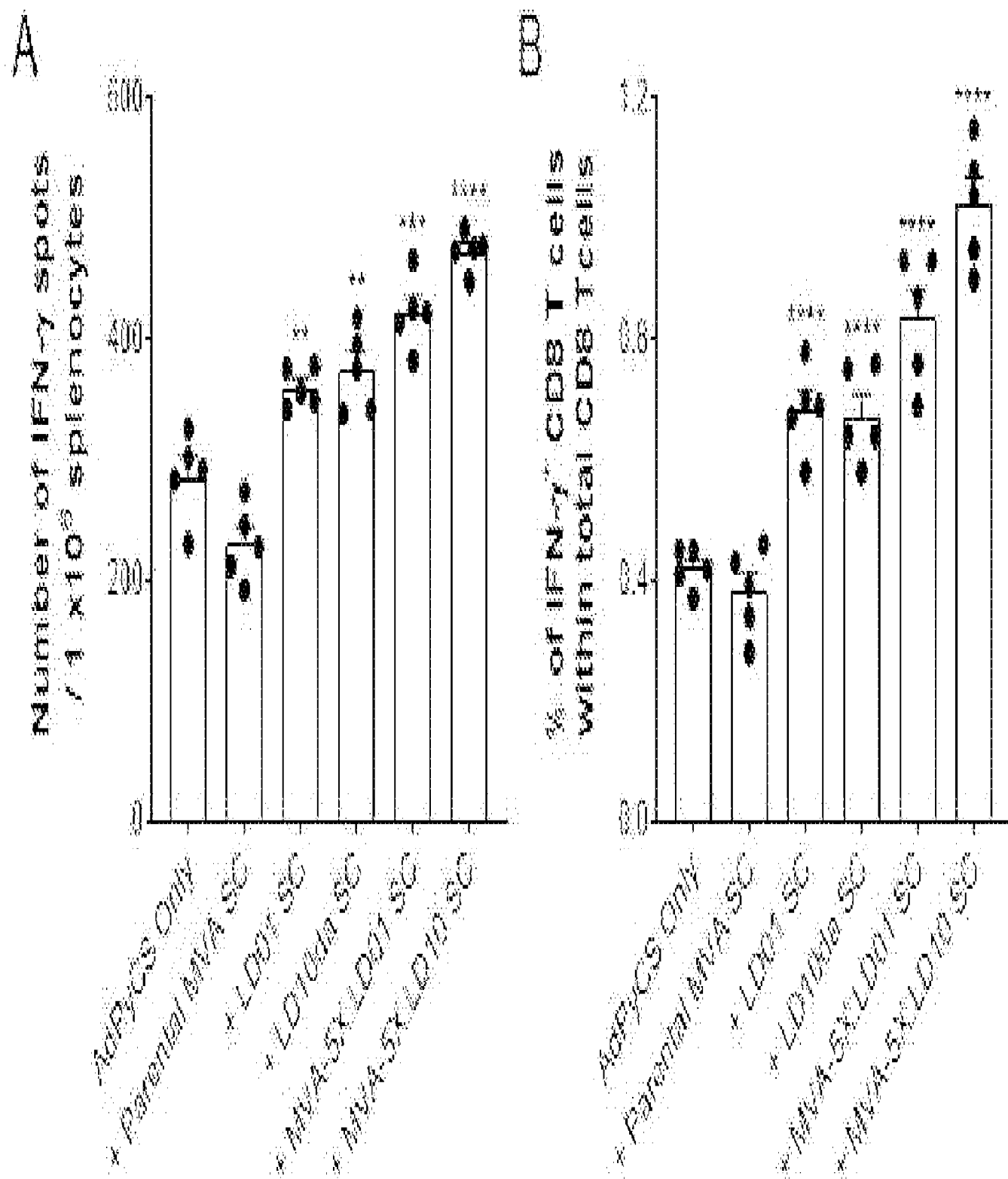


FIG. 9