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(54) Title: CELLULAR VACCINE PLATFORM AND METHODS OF USE

>YP_009724390.1 surface glycoprotein [Severe acute respiratory
syndrome coronavirus 2]

MEVFLVLLPLVSSQCHLCTATQIFAYNSTTRGVYTPDRVRSSTHSTQSIETPTFSNVYTHA
IHVSGTNGTKKFDNPVITENDGVYFASIAKSNIRGWIFGTITLDSKTQSLITVNNATNVV KYCFPO
TENDPFLGVYIKKNSKSMESRIIVYSSANNCEFLVSPQPLMDLEGKQCKNEKNEKEEVIRNIBOYE
KIYSKMTFINLVRLPQGPSALELVLDPGGINITRPTTLALHRSYLTIPGDSGGWTAGAAAYVVG
VEQPRPFLKYVNGTITDAVDCALDPLSTKSTLRSFTVEKGIYQTSNFRVQPTSTVATHTTNE
TTECKVPLAIFASVYATNPKRSTQVADYGYITRASFTKRYGVSTHLELCTHRYAASFTV
IGDIVAGLAPQCKLADINYLKEDUISCYIWNENNLDEKVGQYNNLYALTKSMLEPILILE
KELTQAGSTSTGYEGTQGYFLQYKSTQINQYKQYKVVYVSTELLSAATVKGKSTTHAKA
KAVENFNGLTGTGVLTESNKKFLPTQQFGRDIADTTDAVRDPQTLEILDI TPCSFGGVSVITPGTN
TSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEHVNNSEYCDIPIGAGI
CASVQTQTNSPRRARSVASQSI IAYTMSLGAENSVAYSNNSIAIPTNFTISVTTTELIPVSMPTKTSVD
CTMYICGDSSTECNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKCQKTFEIKKIGQVPSQ
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KNF
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QYTKAPWZKQVAGATATVILNINLHAGAGAAALQIPFAMQAYRFNGIGVTONVLIYENOKELIANOPNSATGKTQDSI

NTD ESD S1/S2 EP HRI

FIG. 1

(57) Abstract: Cellular vaccine platforms, such as vaccine immune viral opsonization platforms, for eliciting host immune responses are disclosed. Also disclosed are the methods of making and using the cellular vaccine platforms in stimulating host immune responses.



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CELLULAR VACCINE PLATFORM AND METHODS OF USE**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This international PCT application claims priority to, and the benefit of, U.S. Provisional Patent Application Serial No. 63/013,387, filed April 21, 2020, and U.S. Provisional Patent Application Serial No. 63/056,460, filed July 24, 2020, the contents of which are incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in ASCII format, which is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 19, 2021 is named 199827-757601_SL.txt and is 40,840 bytes in size.

BACKGROUND

[0003] Current vaccine development strategies predominantly rely on the delivery of live-attenuated or inactivated forms of microbial pathogens in combination with immune stimulating adjuvants in order to elicit a host immune response and induce the production of lasting antigen-specific antibodies and memory lymphocytes. However, these strategies rely on non-physiological methods of antigen exposure and non-physiological adjuvants, which may not sufficiently generate the immune response desired for a vaccine. Therefore, novel vaccine strategies are needed that more closely mimic and modulate physiological immune responses.

INCORPORATION BY REFERENCE

[0004] All publications, patents, and patent applications herein are incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference. In the event of a conflict between a term herein and a term in an incorporated reference, the term herein controls.

SUMMARY

[0005] Provided herein are, *inter alia*, cellular vaccines, allogeneic universal vaccine generation cells and methods for generating and using the same.

[0006] Some embodiments provide a genetically engineered human cell comprising (a) a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; and (b) an exogenous nucleic acid encoding a cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic

immune cell, or a functional fragment or functional variant of said cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell.

[0007] In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell. In some embodiments, said genomic disruption results in a reduction of HLA or MHC mediated T cell activation and/or proliferation as compared to a comparable cell lacking said genomic disruption. In some embodiments, said genomic disruption results in less HLA or MHC mediated T cell activation and/or proliferation as compared to a comparable cell lacking said genomic disruption. In some embodiments, said comparable cell comprises a human cell lacking said genomic disruption. In some embodiments, said comparable cell comprises a human cell expressing said HLA gene. In some embodiments, said comparable cell comprises said genetically engineered human cell lacking said disruption.

[0008] In some embodiments, said genomic disruption completely inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell.

[0009] In some embodiments, said genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene results in a reduction of HLA or MHC mediated T cell activation or proliferation upon administration of said genetically engineered human cells to a subject as compared to administration of comparable cells without said genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene. In some embodiments, said genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene results in a reduction of HLA or MHC mediated T cell activation or proliferation as compared to comparable cells without said genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene.

[0010] In some embodiments, said genomic disruption is in an HLA class I gene. In some embodiments, said HLA class I gene is an HLA-A gene, HLA-B gene, HLA-C gene, or β -microglobulin gene. In some embodiments, said HLA class I gene is a β -microglobulin gene.

[0011] In some embodiments, said genomic disruption is in an HLA class II gene. In some embodiments, said HLA class II gene is an HLA-DP gene, HLA-DM gene, HLA-DOA gene, HLA-DOB gene, HLA-DQ gene, HLA-DR gene.

[0012] In some embodiments, at least one transcriptional regulator of said HLA gene is a CIITA gene, RFX5 gene, RFXAP gene, or RFXANK gene. In some embodiments, said HLA gene is a CIITA gene.

[0013] In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I gene or said at least one transcriptional regulator of said HLA class I gene and a genomic disruption in at least one HLA class II gene or said at least one transcriptional regulator of said HLA class II gene.

[0014] In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I transcriptional regulator gene and a genomic disruption in at least one HLA class II transcriptional regulator.

[0015] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0016] In some embodiments, said binding results in the activation of cytolytic activity of said NK cell.

[0017] In some embodiments, said cell surface protein is a ligand that specifically binds to a natural killer (NK) cell activating receptor expressed on the surface of an NK cell. In some embodiments, said cell surface protein is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, Necl-2, and immunoglobulin Fc.

[0018] In some embodiments, said cell surface protein is a natural killer (NK) cell activating ligand. In some embodiments, said natural killer cell activating ligand is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, and Necl-2.

[0019] In some embodiments, said cell comprises an exogenous nucleic acid encoding a secretory protein that binds to a receptor expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said secretory protein, wherein said protein attracts said immune cell towards said genetically engineered human cell.

[0020] In some embodiments, said genetically engineered human cell comprises a nucleic acid encoding an exogenous protein, an antigenic fragment thereof, or a suicide gene. In some embodiments, said exogenous protein comprises a microbial protein

[0021] In some embodiments, said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

[0022] In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

[0023] In some embodiments, said microbial protein is a viral, bacterial, parasitic, or protozoa protein. In some embodiments, said microbial protein is a viral protein. In some embodiments,

said viral protein is of a virus of order Nidovirales. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of subfamily Orthocoronavirinae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of subgenus Sarbecovirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0024] In some embodiments, said viral protein is of a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0025] In some embodiments, said genetically engineered human cell is differentiated from a stem cell. In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC).

[0026] In some embodiments, said genetically engineered human cell is an epithelial cell or endothelial cell. In some embodiments, said genetically engineered human cell is not a cancer cell.

In some embodiments, said genetically engineered human cell has been irradiated. In some embodiments, said genetically engineered human cell is a stem cell.

[0027] In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC). In some embodiments, said genetically engineered human cell is incapable of proliferation in vitro, in vivo, or both.

[0028] In some embodiments, said genetically engineered human cell is for use in a vaccine.

[0029] In some embodiments, said at least one genomic disruption is mediated by an endonuclease. In some embodiments, said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN).

[0030] In some embodiments, said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA sequence of said at least one HLA gene or at least one transcriptional regulator of said HLA gene.

[0031] Some embodiments provide a genetically engineered human cell comprising: (a) a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; (b) a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and (c) a nucleic acid encoding an exogenous antigenic protein, or an antigenic fragment thereof.

[0032] In some embodiments, said exogenous antigenic protein, or antigenic fragment thereof, is a microbial protein, or an antigenic fragment thereof. In some embodiments, said exogenous antigenic protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

[0033] In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

[0034] In some embodiments, said microbial protein is a viral, bacterial, parasitic, or protozoa protein. In some embodiments, said microbial protein is a viral protein. In some embodiments, said viral protein is of a virus of order Nidovirales. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of subfamily Orthocoronavirinae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of subgenus Sarbecovirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0035] In some embodiments, said viral protein is from a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0036] In some embodiments, said genetically engineered human cell is differentiated from a stem cell. In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC).

[0037] In some embodiments, said genetically engineered human cell is an epithelial cell or endothelial cell. In some embodiments, said genetically engineered human cell is not a cancer cell. In some embodiments, said genetically engineered human cell has been irradiated.

[0038] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0039] Some embodiments provide a population of genetically engineered human cells as disclosed herein.

[0040] Some embodiments provide a pharmaceutical composition comprising said genetically engineered human cell as disclosed herein, and an excipient. Some embodiments provide a unit dosage form comprising a composition or genetically engineered human cell as disclosed herein.

[0041] Some embodiments provide a method of making a population of genetically engineered human stem cells, said method comprising: obtaining a population of human stem cells; inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene; and introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; to thereby produce a population of genetically engineered stem cells.

[0042] In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell. In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA

gene on the surface of said cell for a period of time sufficient to interact with a protein expressed on the surface of an immune cell.

[0043] In some embodiments, said at least one genomic disruption is mediated by an endonuclease. In some embodiments, said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN). In some embodiments, said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA sequence of said at least one HLA gene or at least one transcriptional regulator of an HLA gene.

[0044] In some embodiments, said genomic disruption is a single strand DNA break or a double strand DNA break.

[0045] In some embodiments, said method further comprises introducing a nucleic acid encoding a microbial protein, or an antigenic fragment thereof.

[0046] In some embodiments, said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54. In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

[0047] In some embodiments, said microbial protein is a viral, bacterial, or parasitic protein. In some embodiments, said microbial protein is a viral protein.

[0048] In some embodiments, said stem cells are induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cells are induced pluripotent stem cell (iPSC).

[0049] In some embodiments, said method comprises differentiating said population of genetically engineered human stem cells. In some embodiments, said cells are differentiated into epithelial cells or endothelial cells.

[0050] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0051] Some embodiments provide a method of making a population of terminally differentiated genetically engineered human cells, said method comprising: obtaining a population of human stem cells; inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene; introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell,

or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell, thereby producing a population of genetically engineered human stem cells; and differentiating said population of genetically engineered human stem cells into a population of terminally differentiated genetically engineered human cells.

[0052] In some embodiments, said population of genetically engineered human stem cells are differentiated into epithelial cells or endothelial cells.

[0053] Some embodiments provide a method of immunizing a human subject against a microbe, said method comprising administering to said subject said genetically engineered human cell as disclosed hererin, said composition as disclosed herein, or said pharmaceutical composition as disclosed herein.

[0054] Some embodiments provide a method of immunizing a human subject against a microbe, said method comprising administering to said subject a population of genetically engineered human cells comprising: (a) a genomic disruption in at least one HLA gene or at least one transcriptional regulator of an HLA gene; (b) a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and (c) a nucleic acid encoding a microbial protein, or an antigenic fragment thereof.

[0055] In some embodiments, said binding results in immune cell mediated lysis or phagocytosis of at least a portion of said population of genetically engineered human cells.

[0056] In some embodiments, said administering results in said subject mounting an adaptive immune response against said microbe.

[0057] In some embodiments, said administering results in an increase in activation and/or proliferation of T cells that express a T cell receptor that specifically binds said microbial protein or an antigenic fragment thereof.

[0058] In some embodiments, said administering results in an increase in activation and/or proliferation of B cells that express a B cell receptor that specifically binds said microbial protein or an antigenic fragment thereof.

[0059] In some embodiments, said administering results in an increase in circulating antibodies that specifically bind said microbial protein or antigenic fragment thereof.

[0060] In some embodiments, said microbial protein or antigenic fragment thereof, is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

[0061] In some embodiments, said microbial protein is a viral, bacterial, or parasitic protein. In some embodiments, said microbial protein is a viral protein. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0062] In some embodiments, said viral protein is from a virus selected from the group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, and any combination thereof.

[0063] In some embodiments, said population of genetically engineered human cells are administered intramuscularly or subcutaneously.

[0064] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0065] In some embodiments, said genetically engineered human cells further comprise a suicide gene.

[0066] In some embodiments, said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

[0067] Some embodiments provide a method of immunizing a subject, said method comprising administering to said subject a population of genetically engineered mammalian cells comprising: (a) a genomic disruption in at least one MHC gene or at least one transcriptional regulator of an MHC gene, wherein said disruption results in a reduction of activation of T cell proliferation compared to said genetically engineered human cell without said disruption; and (b) a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant

of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell.

[0068] In some embodiments, said immunizing is specific for an antigen, and wherein said genetically engineered mammalian cells further comprise a nucleic acid encoding the antigen or a fragment thereof.

[0069] In some embodiments, said immunizing is specific for an antigen, and wherein said genetically engineered mammalian cells further comprise the antigen or a fragment thereof.

[0070] In some embodiments, said activation results in immune cell mediated lysis or phagocytosis of at least a portion of said population of genetically engineered mammalian cells.

[0071] In some embodiments, said administration results in said subject mounting an adaptive immune response against said antigen. In some embodiments, said administration results in an increase in activation and/or proliferation of T cells that express a T cell receptor that specifically binds a peptide of said antigen. In some embodiments, said administration results in an increase in activation and/or proliferation of B cells that express a B cell receptor that specifically binds a peptide of said antigen. In some embodiments, said administration results in an increase in circulating antibodies that specifically bind said antigen.

[0072] In some embodiments, said antigen is secreted by said genetically engineered mammalian cell, expressed on the surface of said genetically engineered mammalian cell, or expressed within the cytoplasm of said genetically engineered mammalian cell.

[0073] In some embodiments, said antigen is a viral, bacterial, fungal, or parasitic protein. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, or Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus.

[0074] In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0075] In some embodiments, said viral protein is from a virus selected from the group that comprises at least one of influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus,

MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0076] In some embodiments, said antigen comprises a protein or peptide associated with a cancer or a tumor.

[0077] In some embodiments, said antigen comprises a neoantigen.

[0078] In some embodiments, said population of genetically engineered cells are administered intramuscularly or subcutaneously.

[0079] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell. In some embodiments, said genetically engineered mammalian cells further comprise a suicide gene.

[0080] In some embodiments, said genetically engineered mammalian cells comprise genetically engineered human cells, and said MHC gene comprises an HLA gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[0081] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0082] **FIG. 1** shows the amino acid sequence SARS-CoV-2 Spike (S) protein, and individual domains therein (SEQ ID NO: 1).

[0083] **FIG. 2** is a flow chart showing an exemplary workflow of the cellular vaccine platform described herein.

[0084] **FIG. 3** is a representation of a vaccine cell described herein.

[0085] **FIG. 4A** is an exemplary illustration of an immune response to a viral infection. **FIG. 4B** shows an exemplary response resulting from vaccination using composition provided herein. A Universal Vaccine Cell (UVC) delivers an antigen-loaded living cell in-vivo, genetically engineered to elicit a natural physiologic, and potent activation of the immune system for production of neutralizing antibodies and lasting cellular immunity. The UVC can possess self-adjuvating properties via robust lysis by innate immune cells, activating the cellular and antibody immune response similar to a native host response to viral infection thus recapitulating natural physiologic immunity.

[0086] **FIG. 5** shows exemplary inhibitory and activating receptors on NK cells and their cognate ligands on target cells. Any one of these receptors can be ectopically or endogenously expressed by a vaccine cell described herein.

[0087] **FIG. 6** is an exemplary schematic showing that NK cells recognize platform cells, for instance cells missing MHC-I components as either foreign, virally infected or pathogenic, and target them for cytolysis.

[0088] **FIG. 7** shows that platform cells, for instance CRISPR Knockout B2M (a component of the MHC class I complex) iPSC cells demonstrate an abolished expression of MHCI even after IFN γ stimulation.

[0089] **FIG. 8A** shows that B2M deficient platform cells described herein fail to activate the proliferation of MHC-mismatched T cells compared to control iPS cells, demonstrating the potency of cells described herein. **FIG. 8B** shows a flow cytometry plot on Day 7 of platform cells differentiated into CD31+CD144+ endothelial cells.

[0090] **FIG. 9** shows flow cytometry plots, acquired 48 hours post transfection, of platform cell-derived endothelial cells described herein, overexpressing NK-activating ligands of **Table 5**.

[0091] **FIG. 10** shows that upon lysis of endothelial cells expressing variants of the SARS-CoV-2 spike protein (full length and spike S1 subunit), both protein antigen variants could be detected abundantly and showed a dose-dependent increase with vaccine cell number.

[0092] **FIG. 11** is a schematic of the SARS-CoV-2 virus and spike protein structure.

[0093] **FIG. 12** shows a natural killer (NK) cell killing assay. Shown is percent of dead target cells either K562 or iPSC-derived endothelial cells (differentiated from platform cells) at increasing effector-to-target (E:T) ratios.

[0094] **FIG. 13** is a schematic of a Universal Vaccine Cell (UVC). The UVC comprises a deletion in the B2M locus (KO-B2M), rendering it MHC-I deficient. The UVC also comprises two knock-in (KI) constructs. One KI construct expresses a NK ligand MICA on the cell surface of the UVC, and another KI construct expresses SARS-CoV-2 spike protein and nucleocapsid phosphoprotein intracellularly.

[0095] **FIG. 14A** shows the full-length amino acid sequence of the SARS-CoV-2 nucleocapsid phosphoprotein (**SEQ ID NO: 54**).

[0096] **FIG. 14B** shows a schematic of the expression cassette of the SARS-CoV-2 Spike (SPIKE) protein and nucleocapsid phosphoprotein (N) in the UVC, connected by a T2A peptide cleavage sequence. The construct is driven by a EF1 α promoter.

[0097] **FIG. 14C** shows that Nucleocapsid phosphoprotein has the highest density of epitopes across the SARS-CoV-2 genome. The distribution of functional epitopes across the SARS-CoV-2 genome is plotted. Each bar represents one validated epitope. The X-axis shows its position in the SARS-CoV-2 ORFome (open reading frame-ome). The bar fill indicates its MHC restriction, and the height of the bar indicates the fraction of MHC-matched patients recognizing the epitope. Patients were considered positive for an epitope if the aggregate performance of the epitope in

the screen data exceeded a threshold (mean + 2SD of the enrichment of all SARS-CoV-2 fragments in the healthy controls). For clarity, overlapping epitopes are plotted as adjacent bars. [0098] **FIG. 14D** shows that ORF1ab has the most epitopes among all the SARS-CoV-2 ORFs. The number of epitopes for each SARS-CoV-2 ORF is plotted. The stacked bar graphs show the number of immunodominant epitopes per ORF, with the bar fills indicating the MHC restriction of each epitope. The MHC fill-coding is the same as that of **FIG. 14C**.

[0099] **FIG. 15** shows that UVCs express a high level of NK ligand MICA but do not express any MHC-I. Panel A shows a flow cytometry analysis of NK ligand MICA in the UVC and a parent induced pluripotent stem cell (iPSC). The X-axis shows the fluorescent intensity of the MICA protein. The Y-axis shows the number of cells. The area fill indicating the type of cells is shown on the right of the plot. Panel B shows a flow cytometry analysis of expression of MHC-I in the UVC and the parental iPSC. The X-axis shows the fluorescent intensity of MHC-1 (HLA subtype A, B, or C). The Y-axis shows the number of cells.

[0100] **FIG. 16** shows that MHC-I deficient UVCs trigger robust cell lysis by monkey NK cells *in vitro*. A flow cytometry-based natural killer cytotoxicity assay involving Calcein AM (CAM) staining was used to measure the amount of cytotoxicity in the UVCs in the presence of the NK cells. The X-axis shows the effector-to-target (E:T) ratios. The Y-axis shows the percentage (%) of the NK cell cytotoxic activity. MHC-I deficient endothelial cells with (KO EC) showed higher cytotoxicity when mixed with the Macaque NK cells, when compared to that of the wildtype ECs (WT EC). Both KO EC and WT EC were differentiated from the UVC iPSCs.

[0101] **FIG. 17A** and **FIG. 17B** show that additional NK ligands increase the NK cell response to MHC-I deficient UVCs. **FIG. 17A** shows that the additional NK ligands could increase the expression of cytokines in the NK cells in response to the MHC-I deficient UVCs. Intracellular cytokine staining assays was used to measure the expression of CD107a, MIP1- β , IFN- γ , or TNF- α in the NK cells. The summary of all responding NK cells is also shown on the far-right. The X-axis lists the KO-UVCs expressing no ligands (KO), MICA (KO-MICA), MICB, (KO-MICB), or ULBP1 (KO-ULBP1). The Y-axis shows the percentage (%) of NK cells responsive to the KO-UVC. Each point represents an individual animal tested. **FIG. 17B** shows that the additional NK ligands could induce the expression of multiple cytokines in the NK cells. Simplified Presentation of Incredibly Complex Evaluations (SPICE) was used to analyze the multidimensional cytokine responses of the NK cells. The pie arc and pie chart fill legends are shown at the bottom.

[0102] **FIG. 18A** and **FIG. 18B** show robust expression of the SARS-CoV-2 spike protein in the UVC iPSCs. **FIG. 18A** shows that about half of the UVC iPSCs expressed the spike protein. A flow cytometry analysis was used to measure the expression of the SARS-CoV-2 spike protein.

The X- and Y- axis show the fluorescent staining intensity of the spike protein and the forward scatter height FSC-H, respectively. About 48.5 % of MHC-I deficient (B2M^{-/-}) iPSCs engineered to express both MICA (MICA⁺) and the SARS-CoV-2 spike protein (Spike⁺) had a high level expression of the spike protein, as compared to only about 0.41 % of the control iPSCs engineered to only express MICA. **FIG. 18B** shows that the expression level of the spike protein in the UVC iPSCs was comparable to that in HEK293 cells transiently transfected with a spike protein expression plasmid. A cell surface flow analysis was used to measure the expression of the SARS-CoV-2 spike protein in HEK293 cells and UVC iPSCs. The X- and Y-axis show the fluorescent staining intensity of the spike protein and the number of cells, respectively.

[0103] FIG. 19A and FIG. 19B show the results of an antibody ELISA at weeks 0, 2, 6, and 8 post vaccination with UVC expressing a SARS-CoV-2 spike protein or receptor binding domain thereof, for 6 monkeys, for both the receptor binding domain of the SARS-CoV-2 spike protein (**FIG. 19A**) or full length spike protein (**FIG. 19B**), which demonstrates functional testing of the UVC in an NHP model.

DETAILED DESCRIPTION

[0104] The following description and examples illustrate embodiments of the invention in detail. It is to be understood that this invention is not limited to the particular embodiments described herein and as such can vary. Those of skill in the art will recognize that there are numerous variations and modifications of this invention, which are encompassed within its scope.

Definitions

[0105] The term "about" and its grammatical equivalents in relation to a reference numerical value and its grammatical equivalents as used herein can include a range of values plus or minus 10% from that value. For example, the amount "about 10" includes amounts from 9 to 11. The term "about" in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[0106] The term "vaccine" and its grammatical equivalents as used herein refer to an agent that elicits a host immune response to an infectious disease.

[0107] The term "cellular vaccine" and its grammatical equivalents as used herein refer to a vaccine agent that utilizes cells to expose antigens to the host immune system.

[0108] The term "target cell" or "target cell line" and their grammatical equivalents as used herein refer to a selected cell line described herein as the carrier of a certain type of pathogen antigens.

[0109] The term "activation" or "activating" and its grammatical equivalents as used herein can refer to a process whereby a cell transitions from a resting state to an active state.

[0110] The term "antigen" and its grammatical equivalents as used herein refer to a molecule that contains one or more epitopes or binding sites capable of being bound by one or more receptors or antibodies. For example, an antigen can stimulate a host's immune system to elicit a cellular antigen-specific immune response or a humoral antibody response when the antigen is presented. An antigen can also have the ability to elicit a cellular and/or humoral response by itself or when present in combination with another molecule or other molecules.

[0111] An "engineered cell" and its grammatical equivalents as used herein refer to a cell that comprises an exogenous nucleic acid or amino acid sequence; or contains an alteration, addition, or deletion in an endogenous nucleic acid sequence.

[0112] The "innate immune system" as discussed herein refers to the first line of defense against non-self pathogens is the innate, or non-specific, immune response of a subject. The innate immune response consists of physical, chemical and cellular defenses against pathogens. "Innate immune cell" as described herein refers generally to a phagocytic or cytolytic immune cell involved in the innate immune response. Specifically, these phagocytic or cytolytic immune cells include monocytes (which develop into macrophages), macrophages, neutrophils, eosinophils, basophils, and Natural killer (NK) cells, and mast cells.

[0113] The term "construct" and its grammatical equivalents as used herein refer to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either *in vitro* or *in vivo*.

[0114] The term "vector" and its grammatical equivalents as used herein refer to any nucleic acid construct capable of directing the delivery or transfer of a foreign genetic material to target cells, where it can be replicated and/or expressed. The term "vector" as used herein comprises the construct to be delivered. A vector can be a linear or a circular molecule. A vector can be integrating or nonintegrating.

[0115] The term "integration" and its grammatical equivalents as used herein refer to one or more nucleotides of a construct that is stably inserted into the cellular genome, *i.e.*, covalently linked to the nucleic acid sequence within the cell's chromosomal DNA.

[0116] The term "transgene" and its grammatical equivalents as used herein refer to a gene or genetic material that is transferred into a cell. For example, a transgene can be a stretch or segment of DNA containing a gene that is introduced into a cell. A transgene can retain its ability to produce RNA or polypeptides (*e.g.*, proteins) in an engineered cell. A transgene can be composed of different nucleic acids, for example RNA or DNA. A transgene can comprise recombination arms. A transgene can comprise engineered sites.

[0117] The term "CRISPR", "CRISPR system," or "CRISPR nuclease system" and their grammatical equivalents can include a non-coding RNA molecule (*e.g.*, guide RNA) that binds to DNA and Cas proteins (*e.g.*, Cas9) with nuclease functionality (*e.g.*, two nuclease domains). *See, e.g.*, Sander, J.D., et. al, "CRISPR-Cas systems for editing, regulating and targeting genomes," Nature Biotechnology, 32:347-355 (2014); *see also e.g.*, Hsu, P.D., et al., "Development and applications of CRISPR-Cas9 for genome engineering," Cell 157(6): 1262-1278 (2014).

[0118] The term "sequence" and its grammatical equivalents as used herein refer to a nucleotide sequence, which can be DNA or RNA; can be linear, circular or branched; and can be either single stranded or double stranded. A sequence can be mutated. A sequence can be of any length, for example, between 2 and 1,000,000 or more nucleotides in length (or any integer value there between or there above), *e.g.*, between about 100 and about 10,000 nucleotides or between about 200 and about 500 nucleotides.

[0119] As used herein, the terms "reprogramming" or "dedifferentiation" or "increasing cell potency" or "increasing developmental potency" refers to a method of increasing the potency of a cell or dedifferentiating the cell to a less differentiated state. For example, a cell that has an increased cell potency has more developmental plasticity (*i.e.*, can differentiate into more cell types) compared to the same cell in the non-reprogrammed state. In other words, a reprogrammed cell is one that is in a less differentiated state than the same cell in a non-reprogrammed state.

[0120] As used herein, the term "differentiation" is the process by which an unspecialized ("uncommitted") or less specialized cell acquires the features of a specialized cell such as, for example, a blood cell or a muscle cell. A differentiated or differentiation- induced cell is one that has taken on a more specialized ("committed") position within the lineage of a cell. The term "committed", when applied to the process of differentiation, refers to a cell that has proceeded in the differentiation pathway to a point where, under normal circumstances, it will continue to differentiate into a specific cell type or subset of cell types, and cannot, under normal circumstances, differentiate into a different cell type or revert to a less differentiated cell type. As used herein, the term "pluripotent" refers to the ability of a cell to form all lineages of the body or soma (*i.e.*, the embryo proper). For example, embryonic stem cells are a type of pluripotent stem cells that are able to form cells from each of the three germs layers, the ectoderm, the mesoderm, and the endoderm. Pluripotency is a continuum of developmental potencies ranging from the incompletely or partially pluripotent cell (*e.g.*, an epiblast stem cell or EpiSC), which is unable to give rise to a complete organism to the more primitive, more pluripotent cell, which is able to give rise to a complete organism (*e.g.*, an embryonic stem cell).

[0121] As used herein, the term "induced pluripotent stem cells" or, iPSCs, means that the stem cells are produced from differentiated adult, neonatal or fetal cells that have been induced or changed, *i.e.*, reprogrammed into cells capable of differentiating into tissues of all three germ or dermal layers: mesoderm, endoderm, and ectoderm. The iPSCs produced do not refer to cells as they are found in nature.

[0122] As used herein, the term "Universal Vaccine Cell" "UVC" refers to a vaccine composition described herein. A vaccine composition can comprise a cell provided herein.

[0123] As used herein, the term "embryonic stem cell" refers to naturally occurring pluripotent stem cells of the inner cell mass of the embryonic blastocyst. Embryonic stem cells are pluripotent and give rise during development to all derivatives of the three primary germ layers: ectoderm, endoderm and mesoderm. They do not contribute to the extraembryonic membranes or the placenta, *i.e.*, are not totipotent.

[0124] As used herein, the term "multipotent stem cell" refers to a cell that has the developmental potential to differentiate into cells of one or more germ layers (ectoderm, mesoderm and endoderm), but not all three. Thus, a multipotent cell can also be termed a "partially differentiated cell." Multipotent cells are well known in the art, and examples of multipotent cells include adult stem cells, such as for example, hematopoietic stem cells and neural stem cells. "Multipotent" indicates that a cell may form many types of cells in a given lineage, but not cells of other lineages. For example, a multipotent hematopoietic cell can form the many different types of blood cells (red, white, platelets, etc.), but it cannot form neurons. Accordingly, the term "multipotency" refers to a state of a cell with a degree of developmental potential that is less than totipotent and pluripotent.

[0125] Pluripotency can be determined, in part, by assessing pluripotency characteristics of the cells. Pluripotency characteristics include, but are not limited to: (i) pluripotent stem cell morphology; (ii) the potential for unlimited self-renewal; (iii) expression of pluripotent stem cell markers including, but not limited to SSEA1 (mouse only), SSEA3/4, SSEA5, TRA1-60/81, TRA1-85, TRA2-54, GCTM-2, TG343, TG30, CD9, CD29, CD133/prominin, CD140a, CD56, CD73, CD90, CD105, OCT4, NANOQ SOX2, CD30 and/or CD50; (iv) ability to differentiate to all three somatic lineages (ectoderm, mesoderm and endoderm); (v) teratoma formation consisting of the three somatic lineages; and (vi) formation of embryoid bodies consisting of cells from the three somatic lineages.

[0126] Two types of pluripotency have previously been described: the "primed" or "metastable" state of pluripotency akin to the epiblast stem cells (EpiSC) of the late blastocyst, and the "Naive" or "Ground" state of pluripotency akin to the inner cell mass of the early /preimplantation blastocyst. While both pluripotent states exhibit the characteristics as described

above, the naive or ground state further exhibits: (i) pre-inactivation or reactivation of the X-chromosome in female cells; (ii) improved clonality and survival during single-cell culturing; (iii) global reduction in DNA methylation; (iv) reduction of H3K27me3 repressive chromatin mark deposition on developmental regulatory gene promoters; and (v) reduced expression of differentiation markers relative to primed state pluripotent cells. Standard methodologies of cellular reprogramming in which exogenous pluripotency genes are introduced to a somatic cell, expressed, and then either silenced or removed from the resulting pluripotent cells are generally seen to have characteristics of the primed-state of pluripotency. Under standard pluripotent cell culture conditions such cells remain in the primed state unless the exogenous transgene expression is maintained, wherein characteristics of the ground-state are observed.

[0127] A "pluripotency factor," or "reprogramming factor," refers to an agent capable of increasing the developmental potency of a cell, either alone or in combination with other agents. Pluripotency factors include, without limitation, polynucleotides, polypeptides, and small molecules capable of increasing the developmental potency of a cell. Exemplary pluripotency factors include, for example, transcription factors and small molecule reprogramming agents.

[0128] As used herein, the term "pluripotent stem cell morphology" refers to the classical morphological features of an embryonic stem cell. Normal embryonic stem cell morphology is characterized by being round and small in shape, with a high nucleus-to- cytoplasm ratio, the notable presence of nucleoli, and typical inter-cell spacing.

Overview

[0129] The present disclosure provides, *inter alia*, a novel cellular vaccine platform that offers distinct advantages over current systems that enable the development of robust, safe, and highly scalable cellular vaccines for any pathogen. Standard vaccines that are used to vaccinate against microbes utilize viruses, lipid nanoparticles, or nucleic acids. Unlike these standard vaccines, cellular vaccines provides the distinct advantage of delivering the immunogenic antigen in a physiologically relevant way, enabling the host immune cell to engage with the antigen as it would if the subject was naturally infected. In addition, the cellular component is likely to act as an intrinsic adjuvant via the in vivo creation of apoptotic bodies that will stimulate, attract and recruit cells of the innate system to facilitate a robust immune response and development of immunological memory. The cellular vaccine actually "mimics" the natural process of immune cell lysis of infected cells, and therefore the antigen is delivered to the immune system in the exact same way as it would be via a naturally acquired immunity to invading pathogen. Thus, some embodiments are self-adjuvanting.

[0130] Cancer cell line based cellular vaccines are currently in development. However, unlike these other cellular vaccines, the present disclosure provides novel cellular vaccines that are genetically engineered, which enables the precise creation of the “ideal” target cell that is designed to be killed; as opposed to a natural quirk of a cancer cell line biology. The cell surface receptors expressed by the target cell are specifically designed for a “targeted” lysis by defined cells of the host innate immune system (*i.e.*, absence of MHC and gain of the “missing-self” signal, and targeted expression of “kill-me” signals for cytolytic and phagocytic cells).

[0131] Embodiments of the present disclosure provide a cellular vaccine platform that utilizes a stem cell (*e.g.*, an induced pluripotent stem cell) that can be differentiated *in vitro*. The use of stem cells (*e.g.*, iPSCs) is beneficial, as it avoids having to use any type of transformed cancer cell, while retaining the ability to perpetually grow a stock of engineered vaccine to massive scale production of a stable and consistent cell product. Differentiation into a defined, terminally differentiated and stable cell lineage (such as epithelial cells or skin dendritic “Langerhans” cells) allows the vaccine to move even further away from a cancer-type cell to engineer the same cell type as that from the recipient tissue where it will be delivered.

[0132] Further provided herein are vaccines comprising genetically engineered cells differentiated into an epithelial (dendritic) Antigen Presenting Cell (APC) from a stem cell (*e.g.*, an iPSC) such that there is “APC mimicry.” Some embodiments of the vaccine comprise an MHC null and NK/Mo Innate Immunity+ APC being presented to the host patients’ native MHC specific APC/innate immune system. As such, the vaccine should produce a superior and safer immune antigenic response and naturalizing Ab production to confer a lasting immunity post intradermal/SQ injection in to the skin and the frontline site of APC in the body post the vaccine injection of our Universal Vaccine Cell (UVC).

[0133] Table 1 provides a further comparison between cellular vaccine and viral vector based vaccine, and exemplary benefits of cell based vaccines.

Table 1. Cellular Vaccine vs Viral Vector Based Vaccine

	Universal Vaccine Cell	Viral Vector Based Vaccine	mRNA Vaccine
Vaccine Delivery Vehicle	Live physiologic Antigen Presenting Cell; Live mammalian cell	Non-replicating adenovirus; <i>e.g.</i> , AD26	Synthetic non-physiological nanoparticle
Immunogenic Antigen	Full length proteins (knowledge of precise immunogenic epitope not needed)	Epitope fragments and predetermined immunogenic sequences required	mRNA expressing viral antigen protein
Antigen Presentation Method	Mimics physiological lytic stage of viral infection	Presentation via decoy viral infection	Antigen expression and presentation limited by Nanoparticle immunogenicity

Antigen Density	Multiple Copies of Antigenic Viral Epitope per Cell	Multiple Copies of Antigenic Viral Epitope per Cell	Multiple Copies of Antigenic Viral Epitope per Cell
Adjuvant Immunogenicity	Self-Adjuvanting; highly efficient physiologic antigen presentation to enhance immunogenicity Engineered NK Target and HLA null phenotype to enhance cell lysis and immunogenicity	Viral Vector Immunogenicity, potential for NAB's to Viral Vector	Theoretically less immunogenic and efficient antigen presentation to host APC
Commercial Scale	Stable cell line, single stage large scale manufacturing	Two-stage bioreactor manufacturing	Efficient low cost, large volume scale

[0134] In some embodiments, provided herein is a genetically engineered human cell that comprises: (a) a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; and (b) an exogenous nucleic acid encoding a cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell.

[0135] In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell. In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell for a period of time sufficient to interact with a protein expressed on the surface of an NK cell.

[0136] In some embodiments, said genomic disruption is in an HLA class I gene. In some embodiments, said HLA class I gene is an HLA-A gene, HLA-B gene, HLA-C gene, or β -microglobulin gene. In some embodiments, said HLA class I gene is a β -microglobulin gene.

[0137] In some embodiments, said genomic disruption is in an HLA class II gene. In some embodiments, said HLA class II gene is an HLA-DP gene, HLA-DM gene, HLA-DOA gene, HLA-DOB gene, HLA-DQ gene, HLA-DR gene.

[0138] In some embodiments, said at least one transcriptional regulator of said HLA gene is a CIITA gene, RFX5 gene, RFXAP gene, or RFXANK gene. In some embodiments, said HLA gene is a CIITA gene.

[0139] In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I gene or said at least one transcriptional regulator of said

HLA class I gene and a genomic disruption in at least one HLA class II gene or said at least one transcriptional regulator of said HLA class II gene.

[0140] In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I transcriptional regulator gene and a genomic disruption in at least one HLA class II transcriptional regulator.

[0141] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0142] In some embodiments, said binding results in the activation of cytolytic activity of said NK cell. In some embodiments, said cell surface protein is a ligand that specifically binds to a natural killer (NK) cell activating receptor expressed on the surface of an NK cell. In some embodiments, said cell surface protein is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, Necl-2, and immunoglobulin Fc. In some embodiments, said cell surface protein is a natural killer (NK) cell activating ligand. In some embodiments, said natural killer cell activating ligand is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, and Necl-2.

[0143] In some embodiments, said cell comprises an exogenous nucleic acid encoding a secretory protein that binds to a receptor expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said secretory protein, wherein said protein attracts said immune cell towards said genetically engineered human cell.

[0144] In some embodiments, said genetically engineered human cell further comprises a nucleic acid encoding an exogenous protein, an antigenic fragment thereof, or a suicide gene. In some embodiments, said exogenous protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54. In some embodiments, said exogenous protein comprises an exogenous antigenic protein.

[0145] In some embodiments, said genetically engineered human cell further comprises a nucleic acid encoding a microbial protein, or an antigenic fragment thereof. In some embodiments, said genetically engineered human cell further comprises a cancer or tumor related protein or an antigenic fragment thereof. In some embodiments, said cancer or tumor related protein comprises a neoantigen or an antigenic fragment thereof.

[0146] In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

[0147] In some embodiments, said microbial protein is a viral, bacterial, parasitic, or protozoa protein. In some embodiments, said microbial protein is a viral protein.

[0148] In some embodiments, said viral protein is of a virus of order Nidovirales. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of subfamily Orthocoronavirinae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of subgenus Sarbecovirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0149] In some embodiments, said viral protein is of a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0150] In some embodiments, said genetically engineered human cell is differentiated from a stem cell. In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC).

[0151] In some embodiments, said genetically engineered human cell is an epithelial cell or endothelial cell. In some embodiments, said genetically engineered human cell is not a cancer cell. In some embodiments, said genetically engineered human cell has been irradiated. In some embodiments, said genetically engineered human cell is a stem cell. In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC). In some embodiments, said genetically engineered human cell is incapable of proliferation in vitro, in vivo, or both.

[0152] In some embodiments, said at least one genomic disruption is mediated by an endonuclease. In some embodiments, said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN). In some embodiments, said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA sequence of said at least one HLA gene or at least one transcriptional regulator of said HLA gene.

[0153] In some embodiments, provided herein is a genetically engineered human cell that comprises: (a) a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; (b) a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and (c) a nucleic acid encoding an exogenous protein, or an antigenic fragment thereof.

[0154] In some embodiments, said exogenous protein, or antigenic fragment thereof, is a microbial protein, or an antigenic fragment thereof.

[0155] In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell. In some embodiments, said microbial protein is microinjected, electroporated, or otherwise inserted into said genetically engineered human cell using a technique known in the art.

[0156] In some embodiments, said microbial protein is a viral, bacterial, parasitic, or protozoa protein.

[0157] In some embodiments, said microbial protein is a viral protein. In some embodiments, said viral protein is of a virus of order Nidovirales. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of subfamily Orthocoronavirinae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of subgenus Sarbecovirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein

of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0158] In some embodiments, said viral protein is of a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0159] In some embodiments, said genetically engineered human cell is differentiated from a stem cell. In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cell is an induced pluripotent stem cell (iPSC). In some embodiments, said genetically engineered human cell is an epithelial cell or endothelial cell. In some embodiments, said genetically engineered human cell is not a cancer cell. In some embodiments, said genetically engineered human cell has been irradiated.

[0160] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0161] In some embodiments, provided herein is a composition comprising a population of genetically engineered human cells described herein.

[0162] In some embodiments, provided herein is a pharmaceutical composition comprising a genetically engineered human cell described herein.

[0163] In some embodiments, provided herein is a unit dosage form comprising a pharmaceutical composition comprising a genetically engineered human cell described herein.

[0164] In some embodiments, provided herein is a method of making a population of genetically engineered human stem cells, said method comprising: obtaining a population of human stem cells; inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene; and introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; to thereby produce a population of genetically engineered stem cells.

[0165] In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell. In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell for a period of time sufficient to interact with a protein expressed on the surface of an immune cell.

[0166] In some embodiments, said at least one genomic disruption is mediated by an endonuclease. In some embodiments, said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN). In some embodiments, said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA sequence of said at least one HLA gene or at least one transcriptional regulator of an HLA gene. In some embodiments, said genomic disruption is a single strand DNA break or a double strand DNA break.

[0167] In some embodiments, said method further comprises introducing a nucleic acid encoding a microbial protein, or an antigenic fragment thereof. In some embodiments, said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54. In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell. In some embodiments, said microbial protein is a viral, bacterial, or parasitic protein. In some embodiments, said microbial protein is a viral protein.

[0168] In some embodiments, said method further comprises introducing a nucleic acid encoding a cancer or tumor related protein, a neoantigen, or an antigenic fragment thereof.

[0169] In some embodiments, said stem cells are induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, said stem cells are induced pluripotent stem cell (iPSC). In some embodiments, said method further comprises differentiating said population of genetically engineered human stem cells. In some embodiments, said cells are differentiated into epithelial cells or endothelial cells.

[0170] In some embodiments, said genetically engineered human cell of any one of claims 79-93, wherein said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0171] In some embodiments, provided herein is a method of making a population of genetically engineered human differentiated cells, said method comprising: obtaining a population of human

stem cells; inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene; introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and to thereby produce a population of genetically engineered human stem cells; and differentiating said population of genetically engineered human stem cells into a population of terminally differentiated genetically engineered human cells.

[0172] In some embodiments, said population of genetically engineered human stem cells are differentiated into epithelial cells or endothelial cells.

[0173] In some embodiments, provided herein is a method of immunizing a human subject against a microbe, said method comprising administering to said subject said genetically engineered human cell described herein, a composition comprising a genetically engineered human cell described herein, or said a pharmaceutical composition comprising a genetically engineered human cell described herein.

[0174] In some embodiments, provided herein is a method of immunizing a human subject against a microbe, said method comprising administering to said subject a population of genetically engineered human cells that comprise: (a) a genomic disruption in at least one HLA gene or at least one transcriptional regulator of an HLA gene; (b) a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and (c) a nucleic acid encoding a microbial protein, or an antigenic fragment thereof.

[0175] In some embodiments, said binding results in immune cell mediated lysis or phagocytosis of at least a portion of said population of genetically engineered human cells.

[0176] In some embodiments, said administering results in said subject mounting an adaptive immune response against said microbe. In some embodiments, said administering results in an increase in activation and/or proliferation of T cells that express a T cell receptor that specifically binds a peptide of said microbial protein. In some embodiments, said administering results in an increase in activation and/or proliferation of B cells that express a B cell receptor that specifically binds a peptide of said microbial protein. In some embodiments, said administering results in an increase in circulating antibodies that specifically bind said microbial protein. In some embodiments, said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the

cytoplasm of said genetically engineered human cell. In some embodiments, said microbial protein is a viral, bacterial, or parasitic protein.

[0177] In some embodiments, said microbial protein is a viral protein. In some embodiments, said viral protein is of a virus of family Coronaviridae. In some embodiments, said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said viral protein is of a virus of genus Betacoronavirus. In some embodiments, said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2. In some embodiments, said viral protein is a spike protein of SEQ ID NO: 1. In some embodiments, said viral protein is a spike protein encoded by SEQ ID NO: 53.

[0178] In some embodiments, said viral protein is of a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

[0179] In some embodiments, said population of genetically engineered cells are administered intramuscularly or subcutaneously.

[0180] In some embodiments, said immune cell is an innate immune cell. In some embodiments, said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil. In some embodiments, said innate immune cell is an NK cell.

[0181] In some embodiments, said human cells further comprise a suicide gene, for instance a truncated EGFR or a truncated HER2 gene that is devoid of or exhibits low levels of intracellular activity, but can be targeted by administering an agent such as an EGFR or HER2 binding antibody. In some embodiment, said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

Platform

[0182] Provided herein is, *inter alia*, a vaccine platform that comprises genetically modified platform cells for use in vaccines. Specifically, platform cells described herein are stem cells such as embryonic stem cells or pluripotent stem cells that are genetically modified by disruption of one or more MHC genes (or specifically in the case of human cells, HLA genes) to facilitate

use as an allogeneic vaccine platform. The platform cell described herein can be modified to express an exogenous protein or antigenic fragment thereof relevant for a specific vaccine tailored to specific antigens such as viral antigens.

[0183] In some embodiments, the platform cell comprises a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; and expresses an exogenous protein that binds to a phagocytic or cytolytic immune cell, for instance an innate immune cell, and stimulates activity (*e.g.*, phagocytosis, cytolytic activity, proinflammatory cytokine secretion) of the immune cell. In some embodiments, the platform cell expresses a secretory exogenous protein that attracts a phagocytic or cytolytic immune cell to the platform cell (or vaccine cell designed from the platform cell). In some embodiments, the platform cell expresses and secretes or presents on its surface an exogenous cell surface protein that binds to a phagocytic or cytolytic immune cell.

Platform Cells

[0184] In some embodiments, the platform cells described herein are engineered stem cells. In some embodiments, the stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC). In some embodiments, the stem cell is an induced pluripotent stem cell (iPSC). In some embodiments, the cells are mammalian. In some embodiments, the cells are human. In some embodiments, the cells are murine or non-human primate cells. In some cases, a cell such as an iPS can be differentiated into an Epithelial (Ectoderm derives iPS), APC (Langerhans, dendritic cell) or combinations thereof.

HLA Modification:

[0185] In some embodiments, a platform cell described herein comprises a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene. In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell. In some embodiments, said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell for a period of time sufficient to interact with a protein expressed on the surface of an innate immune cell.

[0186] In some embodiments, said genomic disruption results in a reduction in HLA or MHC mediated T cell activation and/or proliferation, for instance, in a subject that is administered an engineered cell described herein or in an ex vivo assay, as compared to another cell expressing said HLA gene. In some embodiments, said genomic disruption results in less HLA or MHC

mediated T cell response, for instance, in a subject that is administered an engineered cell described herein or in an ex vivo assay, as compared to a comparable cell lacking said genomic disruption.

[0187] In some cases, a platform cell can be a stem cell that is engineered to be HLA deficient. An HLA deficient cell can be HLA-class I deficient, or HLA-class II deficient, or both. In certain embodiments, an HLA deficient cell refers to cells that either lack, or no longer maintain, or have reduced level of surface expression of a complete MHC complex comprising a HLA class I protein heterodimer and/or a HLA class II heterodimer, such that the diminished or reduced level is less than the level naturally detectable by other cells or by synthetic methods.

[0188] HLA class I deficiency can be achieved by functional deletion or genomic disruption of any region of the HLA class I locus (chromosome 6p21), or deletion, disruption, or reducing the expression level of HLA class-I associated genes including, not being limited to, beta-2 microglobulin (B2M) gene, TAP 1 gene, TAP 2 gene and Tapasin. In some embodiments, the HLA class I gene disrupted is an HLA-A gene, HLA-B gene, HLA-C gene.

[0189] HLA class II deficiency can be achieved by functional deletion, disruption or reduction of HLA-II associated genes including, not being limited to, RFXANK, CIITA, RFX5 and RFXAP. In some embodiments, the HLA class II gene disrupted is an HLA-DP gene, HLA-DM gene, HLA-DOA gene, HLA-DOB gene, HLA-DQ gene, HLA-DR gene.

[0190] In some embodiments, provided herein are platform cells that are HLA deficient stem cells such as iPSC that are further modified by introducing genes expressing proteins related but not limited to improved differentiation potential, antigen targeting, antigen presentation, antibody recognition, persistence, immune evasion, resistance to suppression, proliferation, costimulation, cytokine stimulation, cytokine production (autocrine or paracrine), chemotaxis, and cellular cytotoxicity, such as non-classical HLA class I proteins (*e.g.*, HLA-E and HLA-G), chimeric antigen receptor (CAR), T cell receptor (TCR), CD 16 Fc Receptor, BCL11b, NOTCH, RUNX1, IL15, 41BB, DAPIO, DAP12, CD24, CD3z, 41BBL, CD47, CD 113, and PDL1.

[0191] In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I gene or said at least one transcriptional regulator of said HLA class I gene and a genomic disruption in at least one HLA class II gene or said at least one transcriptional regulator of said HLA class II gene. In some embodiments, said genetically engineered human cell comprises a genomic disruption in at least one HLA class I transcriptional regulator gene and a genomic disruption in at least one HLA class II transcriptional regulator.

[0192] In some embodiments, a platform cell does not express any HLA I proteins on the cell surface. In some embodiments, the cell does not express any HLA II proteins on the cell surface. In some embodiments, the cell does not express any HLA I or HLA II proteins on the cell

surface. In some embodiments, a platform cell described herein does not express enough HLA I protein on the cell surface for an immune response to be mounted by a subject if administered to a non-HLA matched subject. In some embodiments, a platform cell does not express enough HLA II protein on the cell surface for an immune response to be mounted by a subject if administered to a non-HLA matched subject.

Stimulation of Innate Immune Cell Activity:

[0193] A platform cell described herein is engineered to express an exogenous protein that binds to an innate immune cell such as an NK cell, a dendritic cell, a neutrophil, a macrophage or a mast cell; and stimulates activity (*e.g.*, cytolytic activity, proinflammatory cytokine secretion) of the innate immune cell.

[0194] In some embodiments, the platform cell comprises a nucleic acid that encodes an exogenous protein that binds to an innate immune cell such as an NK cell, a dendritic cell, a neutrophil, a macrophage or a mast cell; and stimulates activity (*e.g.*, trogocytosis) of the innate immune cell.

[0195] Innate immune cell activation can be determined by analyzing at least one of: degranulation/activation markers (CD107a, CD63, CD107b, CD69,) levels of Granzyme B, IFN γ , MIP-1b, perforin, TNF α , or any combination thereof. Activation can also be determined by imaging, flow cytometry, ELISA, quantitative PCR, or any combination thereof.

NK Cells:

[0196] NK cells express multiple activating and inhibitory receptors that recognize proteins expressed on the surface of other cells. Normal healthy cells express MHC class I molecules on the surface which act as ligands for inhibitory receptors on NK cells and contribute to the self-tolerance of NK cells. Pathogen-infected cells lose surface MHC class I expression, leading to lower inhibitory signals in NK cells. Cellular stress associated with viral infection, such as DNA damage response or senescence program, up-regulates ligands for activating receptors in infected cells. As a result, the signal from activating receptors in NK cell shifts the balance toward NK cell activation and elimination of target cells, directly through NK cell-mediated cytotoxicity or indirectly through secretion of pro-inflammatory cytokines.

[0197] In some embodiments, the platform and vaccine cells described herein express one or more NK cell activation ligand. In some embodiments, the platform and vaccine cells described herein are genetically engineered to decrease or eliminate expression of an NK cell inhibition ligand.

[0198] Full NK cell activation requires recognition of NK cell activating receptors by one or more NK cell ligand expressed on the surface of a target cell. In some embodiments, platform cells described herein are engineered to enhance their recognition and lysis of the platform cell by NK cells. In some cases, platform cells are engineered to express (or over express) one or more NK cell activating ligand. For example, in one embodiment, the cells can be engineered to express cell MICA/B, Necl-2, or any other ligands listed in Table 2 on the cell surface, or one or more functional domains thereof sufficient to bind an NK cell. In order to express an NK cell activating ligand, or an NK cell binding domain therefrom, the cells can be genetically engineered to introduce an exogenous gene encoding the ligand or domain (*e.g.*, using methods described herein or otherwise known in the art). In some embodiments, a genetically engineered cell described herein expresses at least one ligand of Table 2, or a variant thereof, or domain therefrom.

Table 2. NK cell activating receptors and corresponding ligands.

Receptors	Ligands
NKG2D	MICA, MICB, ULBP1-6, Rae-1, MULT1, H60
CD94-NKG2C	HLA-E
KIR2DL4	HLA-G
KIR2DS1	HLA-C2
KIR2DS2	HLA-C1
KIR2DS3	-
KIR2DS4	HLA-A11
KIR2DS5	-
KIR3DS1	HLA-Bw4
NKp30	B7-H6, BAT3
NKp46	Heparin, viral HA and HN
NKp44	viral HA and HN, PCNA, proteoglycans
CD27	CD70
LFA-1	ICAM-1
CD16	IgG
CRTAM	Nectin-like molecules (Necl)-2
DNAM-1 (CD226)	CD155, CD112 (Nectin-2)

[0199] Additionally, NK cells express inhibitory receptors which bind to inhibitory ligands on target cells and inhibit activation of the NK cell. NK cell inhibitory receptors signal through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) present in their cytoplasmic tails. Upon ligand engagement, ITIMs undergo phosphorylation and recruit phosphatases such as Src homology-containing tyrosine phosphatase 1 (SHP-1), SHP-2, and lipid phosphatase SH2 domain-containing inositol-5-phosphatase (SHIP) which further neutralize the activating signals. During NK cell inhibitory signaling, the phosphatases SHP-1 and SHP-2 dephosphorylate the ITAM-bearing Vav-1 molecules and prevent the downstream signaling. Table 3 provides an exemplary list of inhibiting receptors and their corresponding ligands expressed on target cells. Such receptors and ligands are well known in the art.

[0200] In some embodiments, the target cells described herein are engineered to enhance their recognition and lysis by NK cells by engineering the cells to not express (or decrease expression of) one or more NK cell inhibitory ligand. For example, in one embodiment, the cells can be engineered to induce a genomic disruption in one or more HLA class I molecule or any other ligands listed in Table 3 on the cell surface. The platform and vaccine cells described herein can be engineered to knockout any one or any combination of genes encoding an NK cell inhibitory ligand, *e.g.*, those listed in Table 3.

Table 3. NK cell inhibiting receptors and corresponding ligands.

Receptors	Ligands
Ly49 (murine)	MHC-1 (murine)
KIR2DL1	HLA-C2
KIR2DL2	HLA-C1
KIR2DL3	HLA-C1
KIR3DL1	HLA-Bw4
KIR3DL2	HLA-A3, -A11
NKR-P1A	LLT1
CD94-NKG2A	HLA-E Qa-1b
ILT2 (CD85j)	HLA-A, -B, -C, HLA-G1, HCMA UL18
CD244 (2B4)	CD48
TIGIT	CD155, CD112 (Nectin-2), CD113
CD96	CD155, CD111

Increase or Mimic Antibody Opsonisation to Increase ADCC and/or Phagocytosis

[0201] In some embodiments, a platform cell is opsonized ex vivo in order to mediate increased phagocytosis and/or ADCC activity in vivo. In some embodiments, a cell is engineered to express additional exogenous proteins on the surface. In some embodiments, the cell is engineered to express a high number of exogenous proteins on the cell surface. In some embodiments, the engineered cell is contacted ex vivo with an antibody (*e.g.*, that comprises an antigen binding domain and an Fc domain) that binds to an exogenous protein such that the cell is coated with antibody. The opsonization of the cell can mediate increased phagocytosis and/or ADCC by phagocytes (*e.g.*, macrophages) and NK cells, respectively. In some embodiments, the cell is engineered ex vivo to increase opsonization in vivo. In some embodiments, said cell is engineered to express an Fc domain on the surface of the cell, such that the CH2 domain is proximal to the cell membrane and the CH3 domain is distal to the cell membrane.

Stimulation of Phagocytosis

[0202] In some embodiments, a cell described herein expresses an exogenous protein that binds to a phagocytic cell and stimulates activity (*e.g.*, phagocytosis) of the phagocytic cell. In some embodiments, the cell comprises an exogenous nucleic acid that encodes a protein that binds to a phagocytic cell and stimulates activity (*e.g.*, phagocytosis) of the phagocytic cell. In some embodiments, the phagocytic cell is a macrophage, dendritic cell, eosinophil, or neutrophil. In some embodiments, the exogenous protein is selected from the group consisting of phosphatidylserine, calreticulin, and c1q.

[0203] In some cases, cells undergoing apoptosis secrete molecules, so-called “find-me” signals (also referred to as “come-to-get-me” signals), to attract phagocytes toward them. Any and all of these signals can be incorporated into a cell provided herein. Four representative “find-me” signals released by apoptotic cells have been identified, including S1P (sphingosine-1-phosphate), LPC (lysophosphatidylcholine), nucleotides (ATP or UTP) and CX3CL1 (CX3C motif chemokine ligand 1; fractalkine). They bind to S1PR, G2A, P2Y2 and CX3CR, respectively, on the phagocyte surface, promoting phagocyte migration to apoptotic cells.

[0204] In some embodiments, a platform cell described herein comprises a genomic disruption in at least one gene that inhibits phagocytosis of the cell. In some embodiments, the disruption is of a gene selected from the group consisting of CD47 and CD31.

Immune Cell Recruitment

[0205] In some embodiments described herein, are provided platform cells that express and secrete an exogenous protein that binds to an immune cell and attracts the immune cell to the

platform cell. In some embodiments, the cell comprises an exogenous nucleic acid that encodes a secretory agent that binds to an immune cell and attracts the immune cell, for instance innate or adaptive immune cell to the platform cell.

[0206] In some embodiments, the exogenous protein is a cytokine or chemokine. In some embodiments, said protein selected from the group consisting of S1P (sphingosine-1-phosphate), LPC (lysophosphatidylcholine), nucleotides (ATP or UTP) and CX3CL1 (CX3C motif chemokine ligand 1; fractalkine), CX3CL1, and ICAM3. IL-8/CXCL8 chemokines seem to be important for Neutrophil migration to CCL2, CXCL9, CXCL10 appear to recruit CTLs and monocytes.

Methods of Genetic Modification

[0207] Genetic modification of a platform cell or vaccine cell (*e.g.*, knocking-in transgenes or knocking-out undesirable genes) can be achieved by any known genetic engineering techniques, for instance, but not restricted to endonucleases, including but are not limited to zinc-finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and RNA-guided CRISPR-Cas9 nuclease (CRISPR/Cas9; Clustered Regular Interspaced Short Palindromic Repeats Associated 9).

CRISPR System

[0208] The methods of making genetically engineered cells described herein can take advantage of a CRISPR system, including but not limited to knockout of NK cell inhibition ligands and knocking-in of NK cell activation ligands.

[0209] There are at least five types of CRISPR systems which all incorporate RNAs and Cas proteins. Types I, III, and IV assemble a multi-Cas protein complex that is capable of cleaving nucleic acids that are complementary to the crRNA. Types I and III both require pre-crRNA processing prior to assembling the processed crRNA into the multi-Cas protein complex. Types II and V CRISPR systems comprise a single Cas protein complexed with at least one guiding RNA.

[0210] The general mechanism and recent advances of CRISPR system are discussed in Cong, L. et al, "Multiplex genome engineering using CRISPR systems," *Science*, 339(6121): 819-823 (2013); Fu, Y. et al., "High-frequency off-target mutagenesis induced by CRISPR-Cas nucleases in human cells," *Nature Biotechnology*, 31, 822-826 (2013); Chu, VT et al. "Increasing the efficiency of homology-directed repair for CRISPR-Cas9-induced precise gene editing in mammalian cells," *Nature Biotechnology* 33, 543-548 (2015); Shmakov, S. et al, "Discovery and functional characterization of diverse Class 2 CRISPR-Cas systems," *Molecular Cell*, 60, 1-

13 (2015); Makarova, KS et al , "An updated evolutionary classification of CRISPR-Cas systems," , Nature Reviews Microbiology, 13, 1-15 (2015). Site-specific cleavage of a target DNA occurs at locations determined by both 1) base-pairing complementarity between the guide RNA and the target DNA (also called a protospacer) and 2) a short motif in the target DNA referred to as the protospacer adjacent motif (PAM). For example, an engineered cell can be generated using a CRISPR system, *e.g.*, a type II CRISPR system. A Cas enzyme used in the methods disclosed herein can be Cas9, which catalyzes DNA cleavage. Enzymatic action by Cas9 derived from *Streptococcus pyogenes* or any closely related Cas9 can generate double stranded breaks at target site sequences which hybridize to 20 nucleotides of a guide sequence and that have a protospacer-adjacent motif (PAM) following the 20 nucleotides of the target sequence.

[0211] A CRISPR system can be introduced to a cell or to a population of cells using any means. In some embodiments, a CRISPR system may be introduced by electroporation or nucleofection. Electroporation can be performed for example, using the Neon® Transfection System (ThermoFisher Scientific) or the AMAXA® Nucleofector (AMAXA® Biosystems). Electroporation parameters may be adjusted to optimize transfection efficiency and/or cell viability. Electroporation devices can have multiple electrical wave form pulse settings such as exponential decay, time constant and square wave. Every cell type has a unique optimal Field Strength (E) that is dependent on the pulse parameters applied (*e.g.*, voltage, capacitance and resistance). Application of optimal field strength causes electroporation through induction of transmembrane voltage, which allows nucleic acids to pass through the cell membrane. In some embodiments, the electroporation pulse voltage, the electroporation pulse width, number of pulses, cell density, and tip type may be adjusted to optimize transfection efficiency and/or cell viability.

Cas protein

[0212] A vector can be operably linked to an enzyme-coding sequence encoding a CRISPR enzyme, such as a Cas protein (CRISPR-associated protein). In some embodiments, a nuclease or a polypeptide encoding a nuclease is from a CRISPR system (*e.g.*, CRISPR enzyme). In some embodiments, the CRISPR enzyme directs cleavage of one or both strands at a target sequence. In some embodiments, the CRISPR enzyme mediates cleavage of both strands at a target DNA sequence (*e.g.*, creates a double strand break in a target DNA sequence).

[0213] Non-limiting examples of Cas proteins can include Cas1, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 or Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Cse3, Cse4, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6,

Csbl, Csb2, Csb3, Csx17, Csx14, Csx1O, Csx16, CsaX, Csx3, Csx1, Csx1S, Csf1, Csf2, CsO, Csf4, Cpf 1, c2cl, c2c3, Cas9HiFi, homologues thereof, or modified versions thereof. In some embodiments, a catalytically dead Cas protein can be used (*e.g.*, catalytically dead Cas9 (dCas9)). An unmodified CRISPR enzyme can have DNA cleavage activity, such as Cas9. In some embodiments, a nuclease is Cas9. In some embodiments, a polypeptide encodes Cas9. In some embodiments, a nuclease or a polypeptide encoding a nuclease is catalytically dead. In some embodiments, a nuclease is a catalytically dead Cas9 (dCas9). In some embodiments, a polypeptide encodes a catalytically dead Cas9 (dCas9). A Cas protein can be a high fidelity Cas protein such as Cas9HiFi.

[0214] While *S. pyogenes* Cas9 (SpCas9) is commonly used as a CRISPR endonuclease for genome engineering, it may not be the best endonuclease for every target excision site. For example, the PAM sequence for SpCas9 (5' NGG 3') is abundant throughout the human genome, but an NGG sequence may not be positioned correctly to target a desired gene for modification. In some embodiments, a different endonuclease may be used to target certain genomic targets. In some embodiments, synthetic SpCas9-derived variants with non-NGG PAM sequences may be used. Additionally, other Cas9 orthologues from various species have been identified and these "non-SpCas9s" bind a variety of PAM sequences that could also be useful for the present disclosure. For example, the relatively large size of SpCas9 (approximately 4kb coding sequence) means that plasmids carrying the SpCas9 cDNA may not be efficiently expressed in a cell. Conversely, the coding sequence for *Staphylococcus aureus* Cas9 (SaCas9) is approximately 1 kilo base shorter than SpCas9, possibly allowing it to be efficiently expressed in a cell. Similar to SpCas9, the SaCas9 endonuclease is capable of modifying target genes in mammalian cells *in vitro* and in mice *in vivo*.

[0215] Alternatives to *S. pyogenes* Cas9 may include RNA-guided endonucleases from the Cpf 1 family that display cleavage activity in mammalian cells. Unlike Cas9 nucleases, the result of Cpf1-mediated DNA cleavage is a double-strand break with a short 3' overhang. Cpf1's staggered cleavage pattern may open up the possibility of directional gene transfer, analogous to traditional restriction enzyme cloning, which may increase the efficiency of gene editing. Like the Cas9 variants and orthologues described above, Cpf1 may also expand the number of sites that can be targeted by CRISPR to AT-rich regions or AT-rich genomes that lack the NGG PAM sites favored by SpCas9.

[0216] A vector that encodes a CRISPR enzyme comprising one or more nuclear localization sequences (NLSs), such as at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, NLSs can be used. For example, a CRISPR enzyme can comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, NLSs at or near the amino-terminus, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, NLSs at or near the carboxyl-terminus, or any

combination of these (*e.g.*, one or more NLS at the amino-terminus and one or more NLS at the carboxyl terminus). When more than one NLS is present, each can be selected independently of others, such that a single NLS can be present in more than one copy and/or in combination with one or more other NLSs present in one or more copies. The NLS can be located anywhere within the polypeptide chain, *e.g.*, near the N- or C-terminus. For example, the NLS can be within or within about 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 40, 50 amino acids along a polypeptide chain from the N- or C-terminus. Sometimes the NLS can be within or within about 50 amino acids or more, *e.g.*, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 amino acids from the N- or C-terminus.

[0217] Any functional concentration of Cas protein can be introduced to a cell. For example, 15 micrograms of Cas mRNA can be introduced to a cell. In other cases, a Cas mRNA can be introduced from 0.5 micrograms to 100 micrograms. A Cas mRNA can be introduced from 0.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 micrograms.

[0218] In some embodiments, a dual nickase approach may be used to introduce a double stranded break or a genomic break. Cas proteins can be mutated at known amino acids within either nuclease domains, thereby deleting activity of one nuclease domain and generating a nickase Cas protein capable of generating a single strand break. A nickase along with two distinct guide RNAs targeting opposite strands may be utilized to generate a double strand break (DSB) within a target site (often referred to as a "double nick" or "dual nickase" CRISPR system). This approach can increase target specificity because it is unlikely that two off-target nicks will be generated within close enough proximity to cause a DSB.

Guiding polynucleic acids (gRNA or gDNA)

[0219] A guiding polynucleic acid (or a guide polynucleic acid) can be DNA (gDNA) or RNA (gRNA). A guiding polynucleic acid can be single stranded or double stranded. In some embodiments, a guiding polynucleic acid can contain regions of single stranded areas and double stranded areas. A guiding polynucleic acid can also form secondary structures.

[0220] In some embodiments, said guide nucleic acid is a gRNA. In some embodiments, said gRNA comprises a guide sequence that specifies a target site and guides an RNA/Cas complex to a specified target DNA for cleavage. Site-specific cleavage of a target DNA occurs at locations determined by both 1) base-pairing complementarity between a gRNA and a target DNA (also called a protospacer) and 2) a short motif in a target DNA referred to as a protospacer adjacent motif (PAM). Similarly, a gRNA can be specific for a target DNA and can form a complex with a nuclease to direct its nucleic acid-cleaving activity.

[0221] In some embodiments, said gRNA comprises two RNAs, *e.g.*, CRISPR RNA (crRNA) and transactivating crRNA (tracrRNA). In some embodiments, said gRNA comprises a single-guide RNA (sgRNA) formed by fusion of a portion (*e.g.*, a functional portion) of crRNA and tracrRNA. In some embodiments, said gRNA comprises a dual RNA comprising a crRNA and a tracrRNA. In some embodiments, said gRNA comprises a crRNA and lacks a tracrRNA. In some embodiments, said crRNA hybridizes with a target DNA or protospacer sequence.

[0222] In some embodiments, said gRNA targets a nucleic acid sequence of or of about 20 nucleotides. In some embodiments, said gRNA targets a nucleic acid sequence of or of about 5, 10, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30 or more nucleotides. In some embodiments, said gRNA binds a genomic region from about 1 base pair to about 20 base pairs away from a PAM. In some embodiments, said gRNA binds a genomic region from about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or up to about 20 base pairs away from a PAM. In some embodiments, said gRNA binds a genomic region within about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 base pairs away from a PAM.

[0223] A guide RNA can also comprise a dsRNA duplex region that forms a secondary structure. For example, a secondary structure formed by a guide RNA can comprise a stem (or hairpin) and a loop. The length of a loop and a stem can vary. For example, a loop can range from about 3 to about 10 nucleotides in length, and a stem can range from about 6 to about 20 base pairs in length. A stem can comprise one or more bulges of 1 to about 10 nucleotides. The overall length of a second region can range from about 16 to about 60 nucleotides in length. For example, a loop can be or can be about 4 nucleotides in length and a stem can be or can be about 12 base pairs. A dsRNA duplex region can comprise a protein-binding segment that can form a complex with an RNA-binding protein, such as a RNA-guided endonuclease, *e.g.*, Cas protein.

[0224] In some embodiments, a Cas protein, such as a Cas9 protein or any derivative thereof, is pre-complexed with a gRNA to form a ribonucleoprotein (RNP) complex. In some embodiments, the RNP complex is introduced into a cell to mediate editing.

[0225] In some embodiments, said gRNA is modified. The modifications can comprise chemical alterations, synthetic modifications, nucleotide additions, and/or nucleotide subtractions. The modifications can also enhance CRISPR genome engineering. A modification can alter chirality of a gRNA. In some embodiments, chirality may be uniform or stereopure after a modification. In some embodiments, the modification enhances stability of said gRNA.

[0226] In some embodiments, the modification is a chemical modification. A modification can be selected from 5' adenylate, 5' guanosine-triphosphate cap, 5' N7-Methylguanosine-triphosphate cap, 5' triphosphate cap, 3' phosphate, 3' thiophosphate, 5' phosphate, 5' thiophosphate, Cis-Syn thymidine dimer, trimers, C12 spacer, C3 spacer, C6 spacer, dSpacer, PC

spacer, rSpacer, Spacer 18, Spacer 9, 3'-3' modifications, 5'-5' modifications, abasic, acridine, azobenzene, biotin, biotin BB, biotin TEG, cholesteryl TEG, desthiobiotin TEG, DNP TEG, DNP-X, DOTA, dT-Biotin, dual biotin, PC biotin, psoralen C2, psoralen C6, TINA, 3' DABCYL, black hole quencher 1, black hole quencher 2, DABCYL SE, dT-DABCYL, IRDye QC-1, QSY-21, QSY-35, QSY-7, QSY-9, carboxyl linker, thiol linkers, 2' deoxyribonucleoside analog purine, 2' deoxyribonucleoside analog pyrimidine, ribonucleoside analog, 2'-O-methyl ribonucleoside analog, and sugar modified analogs, wobble/universal bases, fluorescent dye label, 2' fluoro RNA, 2' O-methyl RNA, methylphosphonate, phosphodiester DNA, phosphodiester RNA, phosphothioate DNA, phosphorothioate RNA, UNA, pseudouridine-5'-triphosphate, and 5-methylcytidine-5'-triphosphate, and any combination thereof.

[0227] In some embodiments, said modification comprise a phosphorothioate internucleotide linkage. In some embodiments, said gRNA comprises from 1 to 10, 1 to 5, or 1-3 phosphorothioate. In some embodiments, said gRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 phosphorothioates linkages. In some embodiments, said gRNA comprises phosphorothioate internucleotide linkages at the N terminus, C terminus, or both N terminus and C terminus. For example, in some embodiments, said gRNA comprises phosphorothioates internucleotide linkages between the N terminal 3-5 nucleotides, the C terminal 3-5 nucleotides, or both.

[0228] In some embodiments, the modification is a 2'-O-methyl phosphorothioate addition. In some embodiments, said gRNA comprises 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, or 1-2 2'-O-methyl phosphorothioates. In some embodiments, said gRNA comprises from 1 to 10, 1 to 5, or 1-3 2'-O-methyl phosphorothioates. In some embodiments, said gRNA comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 2'-O-methyl phosphorothioates. In some embodiments, said gRNA comprises 2'-O-methyl phosphorothioate internucleotide linkages at the N terminus, C terminus, or both N terminus and C terminus. For example, in some embodiments, said gRNA comprises 2'-O-methyl phosphorothioate internucleotide linkages between the N terminal 3-5 nucleotides, the C terminal 3-5 nucleotides, or both.

[0229] A gRNA can be introduced at any functional concentration. In some embodiments, 0.5 micrograms to 100 micrograms of said gRNA is introduced into a cell. In some embodiments, 0.5, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 micrograms of said gRNA is introduced into a cell.

Other Endonucleases

[0230] Other endonuclease based gene editing systems known in the art can be used to make an engineered cell described herein. For example, zinc finger nuclease systems and TALEN systems.

[0231] ZFNs are targeted nucleases comprising a nuclease fused to a zinc finger DNA binding domain. A "zinc finger DNA binding domain" or "ZFBD" is a polypeptide domain that binds DNA in a sequence-specific manner through one or more zinc fingers. A zinc finger is a domain of about 30 amino acids within the zinc finger binding domain whose structure is stabilized through coordination of a zinc ion. Examples of zinc fingers include, but are not limited to, C₂H₂ zinc fingers, C₃H zinc fingers, and C₄ zinc fingers. A "designed" zinc finger domain is a domain not occurring in nature whose design/composition results principally from rational criteria, *e.g.*, application of substitution rules and computerized algorithms for processing information in a database storing information of existing ZFN designs and binding data. A "selected" zinc finger domain is a domain not found in nature whose production results primarily from an empirical process such as phage display, interaction trap or hybrid selection. The most recognized example of a ZFN in the art is a fusion of the FokI nuclease with a zinc finger DNA binding domain.

[0232] A TALEN is a targeted nuclease comprising a nuclease fused to a TAL effector DNA binding domain. A "transcription activator-like effector DNA binding domain", "TAL effector DNA binding domain", or "TALE DNA binding domain" is a polypeptide domain of TAL effector proteins that is responsible for binding of the TAL effector protein to DNA. TAL effector proteins are secreted by plant pathogens of the genus *Xanthomonas* during infection. These proteins enter the nucleus of the plant cell, bind effector-specific DNA sequences via their DNA binding domain, and activate gene transcription at these sequences via their transactivation domains. TAL effector DNA binding domain specificity depends on an effector-variable number of imperfect 34 amino acid repeats, which comprise polymorphisms at select repeat positions called repeat variable-di-residues (RVD). The most recognized example of a TALEN in the art is a fusion polypeptide of the FokI nuclease to a TAL effector DNA binding domain.

[0233] Another example of a targeted nuclease that finds use in the methods described herein is a targeted Spoil nuclease, a polypeptide comprising a Spoil polypeptide having nuclease activity fused to a DNA binding domain, *e.g.*, a zinc finger DNA binding domain, a TAL effector DNA binding domain, etc. that has specificity for a DNA sequence of interest. Additional examples of targeted nucleases suitable for the present invention include, but are not limited to Bxb1, phiC31, R4, PhiBTi, and WO/SPBc/TP9O1-1, whether used individually or in combination.

[0234] Any one of the aforementioned methods comprising genomically editing via use of an endonuclease can result in a genomic disruption. The genomic disruption can be sufficient to

result in reduction or elimination of expression of the protein encoded by the gene. In some cases, a genomic disruption can also refer to the incorporation of an exogenous transgene into the cellular genome. In such cases, an exogenous transgene can also be detected. The genomic disruption can be detected in at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of cells tested. Detection can be performed by evaluating the disruption at the genomic level via sequencing, at the mRNA level, or protein level. Suitable methods include PCR, qPCR, flow cytometry, imaging, ELISA, NGS, and any combination thereof. In some cases, protein expression can be reduced by about 1 fold, 2 fold, 3 fold, 5 fold, 10 fold, 20 fold, 30 fold, 50 fold, 70 fold, 100 fold, 125 fold, 150 fold, 200 fold, 250 fold, 300 fold, 350 fold, 500 fold, or up to about 1000 fold as compared to a comparable method that lacks the use of the gene editing, such as with CRISPR.

Transgenes

[0235] A transgene polynucleic acid encoding an exogenous protein or polypeptide that is knocked into a platform or vaccine cell described herein can be DNA or RNA, single-stranded or double stranded and can be introduced into a cell in linear or circular form. A transgene sequence(s) can be contained within a DNA minicircle, which may be introduced into the cell in circular or linear form. If introduced in linear form, the ends of a transgene sequence can be protected (*e.g.*, from exonucleolytic degradation) by any method. For example, one or more dideoxynucleotide residues can be added to the 3' terminus of a linear molecule and/or self-complementary oligonucleotides can be ligated to one or both ends. Additional methods for protecting exogenous polynucleotides from degradation include, but are not limited to, addition of terminal amino group(s) and the use of modified internucleotide linkages such as, for example, phosphorothioates, phosphoramidates, and O-methyl ribose or deoxyribose residues.

[0236] A transgene can be flanked by recombination arms. In some instances, recombination arms can comprise complementary regions that target a transgene to a desired integration site. A transgene can also be integrated into a genomic region such that the insertion disrupts an endogenous gene. A transgene can be integrated by any method, *e.g.*, non-recombination end joining and/or recombination directed repair. A transgene can also be integrated during a recombination event where a double strand break is repaired. A transgene can also be integrated with the use of a homologous recombination enhancer. For example, an enhancer can block non-homologous end joining so that homology directed repair is performed to repair a double strand break.

[0237] A transgene can be flanked by recombination arms where the degree of homology between the arm and its complementary sequence is sufficient to allow homologous

recombination between the two. For example, the degree of homology between the arm and its complementary sequence can be 50% or greater. Two homologous non-identical sequences can be any length and their degree of non-homology can be as small as a single nucleotide (*e.g.*, for correction of a genomic point mutation by targeted homologous recombination) or as large as 10 or more kilobases (*e.g.*, for insertion of a gene at a predetermined ectopic site in a chromosome). Two polynucleotides comprising the homologous non-identical sequences need not be the same length.

[0238] A polynucleotide can be introduced into a cell as part of a vector molecule having additional sequences such as, for example, replication origins, promoters and genes encoding antibiotic resistance. Moreover, transgene polynucleotides can be introduced as naked nucleic acid, as nucleic acid complexed with an agent such as a liposome or poloxamer, or can be delivered by viruses (*e.g.*, adenovirus, AAV, herpesvirus, retrovirus, lentivirus and integrase defective lentivirus (IDLV)). A virus that can deliver a transgene can be an AAV virus.

[0239] A transgene is generally inserted so that its expression is driven by the endogenous promoter at the integration site, namely the promoter that drives expression of the endogenous gene into which a transgene is inserted. A transgene may comprise a promoter and/or enhancer, for example a constitutive promoter or an inducible or tissue/cell specific promoter. A minicircle vector can encode a transgene.

[0240] A transgene can be inserted into an endogenous gene such that all, some or none of the endogenous gene is expressed. For example, a transgene as described herein can be inserted into an endogenous locus such that some (N-terminal and/or C-terminal to a transgene) or none of the endogenous sequences are expressed, for example as a fusion with a transgene. In other cases, a transgene (*e.g.*, with or without additional coding sequences such as for the endogenous gene) is integrated into any endogenous locus, for example a safe-harbor locus.

[0241] When endogenous sequences (endogenous or part of a transgene) are expressed with a transgene, the endogenous sequences can be full-length sequences (wild-type or mutant) or partial sequences. The endogenous sequences can be functional. Non-limiting examples of the function of these full length or partial sequences include increasing the serum half-life of the polypeptide expressed by a transgene (*e.g.*, therapeutic gene) and/or acting as a carrier.

[0242] Furthermore, although not required for expression, exogenous sequences may also include transcriptional or translational regulatory sequences, for example, promoters, enhancers, insulators, internal ribosome entry sites, sequences encoding 2A peptides and/or polyadenylation signals.

Cell Compositions:

[0243] Platform cells described herein can be stored long term for use in vaccine cells as appropriate. Specifically, these cells can be incorporated in appropriate compositions that are stable when frozen or cryopreserved. In some cases, provided are compositions for maintaining the pluripotency of engineered induced pluripotent stem cells (iPSCs) that are used as platform cells. In some cases, the composition comprises (i) engineered iPSC platform cells, and (ii) a pluripotency maintenance composition, for instance a small molecule composition comprising a MEK inhibitor, a GSK3 inhibitor and a ROCK inhibitor. In some embodiments, the platform cells are obtained from reprogramming engineered non-pluripotent cells, wherein the obtained iPSCs comprise the same targeted integration and/or in/del at selected sites in the engineered non-pluripotent cells. In some embodiments, the engineered iPSCs are obtained from engineering a clonal iPSC or a pool of iPSCs by introducing one or more targeted integration and/or in/del at one or more selected sites. In some other embodiments, the genome-engineered iPSCs are obtained from genome engineering by introducing one or more targeted integration and/or in/del at one or more selected sites to a pool of reprogramming non-pluripotent cells in contact with one or more reprogramming factors and optionally a small molecule composition comprising a TGFP receptor/ALK inhibitor, a MEK inhibitor, a GSK3 inhibitor and/or a ROCK inhibitor.

[0244] Engineered platform cells of the composition can comprise one or more exogenous polynucleotides encoding safety switch proteins, targeting modality, receptors, signaling molecules, transcription factors, pharmaceutically active proteins and peptides, drug target candidates, or proteins promoting engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the non-pluripotent cell reprogrammed iPSCs or derivative cells thereof; and /or in/dels at one or more endogenous genes associated with targeting modality, receptors, signaling molecules, transcription factors, drug target candidates, immune response regulation and modulation, or proteins suppressing engraftment, trafficking, homing, viability, self-renewal, persistence, and/or survival of the non-pluripotent cell reprogrammed iPSCs or derivative cells thereof.

[0245] In some embodiments, one or more exogenous polynucleotides encoding one or more exogenous polypeptides or proteins are operatively linked to (1) one or more exogenous promoters comprising CMV, EF1a, PGK, CAQ UBC, or other constitutive, inducible, temporal-, tissue-, or cell type- specific promoters; or (2) one or more endogenous promoters comprised in selected sites in the platform cell comprising AAVS1, CCR5, ROSA26, collagen, HTRP, Hll, beta-2 microglobulin, GAPDH, TCR or RUNX1. In some embodiments, the composition further

comprises one or more endonuclease capable of selected site recognition for introducing double strand break at selected sites.

Vaccine Cells

[0246] Vaccine cells described herein are made by further engineering said cells to comprise a nucleic acid encoding an exogenous microbial protein, or an antigenic fragment thereof, or express said exogenous microbial protein, or an antigenic fragment thereof. Thereby, the vaccine cells, when administered to a subject will induce an immune response specifically against said exogenous microbial protein.

[0247] Therefore, in one aspect, provided herein are genetically engineered cells that comprise i) a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; and ii) expression of an exogenous protein that binds to a phagocytic or cytolytic innate immune cell and stimulates activity (*e.g.*, phagocytosis, cytolytic activity, proinflammatory cytokine secretion) of the innate immune cell; and iii) express an exogenous microbial protein or antigenic fragment thereof. In some embodiments, the vaccine cells comprise an antigen expression construct described herein.

[0248] The vaccine cells described herein can be any cell suitable for administration to a subject and delivery of a microbial protein. In some embodiments, said cells are differentiated from said platform cells. In some embodiments, said cells are differentiated from platform cells, wherein said platform cells are stem cells (*e.g.*, iPSCs). In some embodiments, said cells are epithelial cells. In some embodiments, said cells are endothelial cells.

Methods of Differentiation

[0249] In some embodiments, the vaccine cells are differentiated from the platform cells, wherein said platform cells are stem cells. In some embodiments, said stem cells are induced pluripotent stem cells. In some embodiments, the iPSCs are differentiated into epithelial cells or endothelial cells. In certain aspects, the iPSCs are differentiated into a cell type that has inherently low-immunogenicity to recipient T cells and allows the differentiated cells to be a focused target for NK cell-mediated vaccination. In certain additional aspects, the iPSCs are engineered to present kill-tags or suicide switch genes that can be activated to target the cell by administration of an antibody or small molecule.

[0250] Differentiation of pluripotent stem cells requires a change in the culture system, such as changing the stimuli agents in the culture medium or the physical state of the cells. The most conventional strategy utilizes the formation of embryoid bodies (EBs) as a common and critical intermediate to initiate the lineage-specific differentiation. "Embryoid bodies" are three-

dimensional clusters that have been shown to mimic embryo development as they give rise to numerous lineages within their three-dimensional area. Through the differentiation process, typically a few hours to days, simple EBs (for example, aggregated pluripotent stem cells elicited to differentiate) continue maturation and develop into a cystic EB at which time, typically days to a few weeks, they are further processed to continue differentiation. EB formation is initiated by bringing pluripotent stem cells into close proximity with one another in three-dimensional multilayered clusters of cells, typically this is achieved by one of several methods including allowing pluripotent cells to sediment in liquid droplets, sedimenting cells into "U" bottomed well-plates or by mechanical agitation. To promote EB development, the pluripotent stem cell aggregates require further differentiation cues, as aggregates maintained in pluripotent culture maintenance medium do not form proper EBs. As such, the pluripotent stem cell aggregates need to be transferred to differentiation medium that provides eliciting cues towards the lineage of choice. EB-based culture of pluripotent stem cells typically results in generation of differentiated cell populations (ectoderm, mesoderm and endoderm germ layers) with modest proliferation within the EB cell cluster. Although proven to facilitate cell differentiation, EBs, however, give rise to heterogeneous cells in variable differentiation state because of the inconsistent exposure of the cells in the three-dimensional structure to differentiation cues from the environment. In addition, EBs are laborious to create and maintain. Moreover, cell differentiation through EB is accompanied with modest cell expansion, which also contributes to low differentiation efficiency.

[0251] The engineered iPSCs described herein can be differentiated using biomaterial scaffolds. Biomaterial scaffolds promote the viability and differentiation of stem cells seeded inside depending on the intrinsic properties of the material as well as the incorporation of specific chemical and physical cues into the material. Both natural and synthetic biomaterials can serve as the starting point for generating bioactive scaffolds for controlling stem cell differentiation into the desired tissue type. These scaffolds can take several different forms, which in turn have unique features. These scaffolds can also be combined to yield novel hybrid materials that certain formulations enable better cell survival. Suitable biomaterial scaffolds include but not limited to hydrogels, electrospun scaffolds, nano- and micro-particles using protein-based biomaterials (*e.g.*, collagen, fibrin, silk, laminin, fibronectin and vitronectin), polysaccharide-based biomaterials (*e.g.*, agarose, alginate, hyaluronan, chitosan, cellulose and its derivatives and decellularized extracellular matrix), synthetic biomaterials (*e.g.*, poly (lactic-co-glycolic acid) (PLGA), poly (ethylene glycol) (PEG), poly caprolactone (PCL), polypyrrole (Ppy) and polydimethylsiloxane (PDMS)), and ceramic-based biomaterials.

[0252] In some cases, a method provided herein can yield reduced toxicity as compared to a comparable method. In some cases, toxicity can be reduced by about 1 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, 10 fold, 11 fold, 15 fold, 20 fold, 25 fold, 50 fold, 100 fold, 300 fold, 500 fold, 800 fold, 1000 fold.

Cell Types

[0253] In some cases, a method can comprise differentiating iPS cells. In some embodiments, a stem cell (*e.g.*, iPSC) can be differentiated into an epithelial cell. In some cases, an iPS cell can be differentiated into a skin epithelial cell, lung epithelial cell, a gastrointestinal epithelial cell, a lung alveoli epithelial cell, mouth epithelial cell, vaginal epithelial cell, renal epithelial cell, renal tube epithelial cell, respiratory tract epithelial cell, bladder epithelial cell, urinary tract epithelial cell, blood vessel epithelial cell, brain epithelial cell, heart epithelial cell, ear epithelial cell, tongue epithelial cell, a cervical epithelial cell, a prostate epithelial cell, a breast epithelial cell, a uterus epithelial cell, tracheal epithelial cell, a large intestine epithelial cell, a small intestine epithelial cell, a colon epithelial cell, or a liver epithelial cell.

APC Mimicry

[0254] In some cases, an iPS cell can be differentiated into an epithelial (dendritic) Antigen Presenting Cell (APC). By differentiating an iPS cell into a dendritic cell one can achieve APC mimicry thereby conferring upon a vaccine an MHC null and/or NK/Mo Innate Immunity+ APC being presented to a subject's native MHC specific APC/innate immune system. This can result in a superior and/or safer immune antigenic response and/or naturalizing Ab production thereby conferring a lasting immunity post administration. In other cases, an iPS cell can be differentiated into a skin, lung, or GI/gut epithelial cell thus allowing for natural physiologic presentation of the immunogen to the host immune system. This may facilitate and/or enhance vaccine application for pulmonary application of the vaccine directly delivery to the lungs and/or p.o. per oral route of administration in addition to standard sub-cutaneous and intradermal and other skin and dermal based vaccine delivery methods.

Antigen Expression Construct

[0255] Provided herein are antigen expression constructs and engineered cells comprising said constructs. In some embodiments, the antigen expression construct comprises a nucleic acid encoding an exogenous protein, or antigenic fragment thereof. In some embodiments, said exogenous protein comprises an exogenous antigenic protein. In some embodiments, said construct comprises a two or more exogenous proteins, or antigenic fragments thereof. In some

embodiments, said nucleic acid is DNA or RNA. In some embodiments, said nucleic acid is cDNA. In some embodiments, said nucleic acid is mRNA.

In some embodiments, the exogenous protein is a microbial protein. In some embodiments, the microbial protein is a viral, bacterial, parasitic, or protozoa protein.

COVID-19

[0256] In some embodiments, the microbial protein is a viral protein. In some embodiments, the viral protein is of a virus of order Nidovirales. In some embodiments, the viral protein is of a virus of family Coronaviridae. In some embodiments, the viral protein is of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, the viral protein is of a virus of genus Betacoronavirus. In some embodiments, the viral protein is of a virus of subgenus Sarbecovirus. In some embodiments, the viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, the viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, the viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2.

[0257] In some embodiments, a cellular vaccine described herein is used to treat coronavirus disease 2019 (COVID-19). As a severe respiratory disease firstly reported in Wuhan, Hubei province, China, COVID-19 is also known as COVID-2019, 2019 novel coronavirus, or 2019-nCoV.

[0258] The COVID-19 disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2's genome has been sequenced and the order of genes (from 5' to 3') are as follows: replicase ORF1ab, spike (S), envelope (E), membrane (M) and nucleocapsid (N). Wu, F., Zhao, S., Yu, B., Chen, Y., Wang, W., Song, Z., Hu, Y., Tao, Z., Tian, J. Pei, Y., Yuan, M., Zhang, Y., Dai, F., Liu, Y., Wang, Q., Zheng, J., Xu, L., Holmes, E., & Zhang, Y., *A new coronavirus associated with human respiratory disease in China*. Nature 579, 265–269 (2020) (the entire contents of which is incorporated by reference herein for all purposes).

[0259] SARS-CoV-2 makes use of a densely glycosylated spike protein (S protein) to gain entry into host cells. The coronavirus spike protein is a trimeric class I fusion protein that exists in a metastable prefusion conformation that undergoes a substantial structural rearrangement to fuse the viral membrane with the host cell membrane. This process is triggered when the S1 subunit binds to a host cell receptor. Receptor binding destabilizes the prefusion trimer, resulting in shedding of the S1 subunit and transition of the S2 subunit to a stable post-fusion conformation. To engage a host cell receptor, the receptor-binding domain (RBD) of S1 undergoes hinge-like conformational movements that transiently hide or expose the determinants of receptor binding.

These two states are referred to as the "down" conformation and the "up" conformation, where down corresponds to the receptor-inaccessible state and up corresponds to the receptor-accessible state, which is thought to be less stable. Because of the indispensable function of the S protein, it represents a target for antibody-mediated neutralization, and characterization of the prefusion S structure would provide atomic-level information to guide vaccine design and development. Wrapp, D., Wang, N., Corbett, K., Goldsmith, J., Hsieh, C., Abiona, O., Graham, B. & McLellan, J., *Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation*, SCIENCE, 13 MAR 2020: 1260-1263 (the entire contents of which is incorporated by reference herein for all purposes).

[0260] The amino acid sequence of the SARS-CoV-2 S protein is (obtained from NCBI):

>YP_009724390.1 surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]
 MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFS
 NVTWFHAIHVSGTNGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIV
 NNATNVVIKVC EFQFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMD
 LEGKQGNFKNLREFVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQT
 LLALHRSYLT PGDSSSGWTAGAAAYVGYLQPRTFLLKYNENGTITDAVDCALDPLSET
 KCTLKSFTVEKGIYQTSNFRVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNKRKRISN
 CVADYSVLYNSASFSTFKCYGVSPTKLNDLCFTNVYADSFVIRGDEV RQIAPGQTGKIA
 DYNKLPDDFTGCVIAWNSNNLDSKVGGNYNLYRLFRKSNLKPFERDISTEIIYQAGST
 PCNGVEGFNCYFPLQSYGFQPTNGVGYQPYRVVLSFELLHAPATVCGPKKSTNLVKN
 KCVNFNFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVS
 VITPGTNTSNQVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAEH
 VNNSYECDIPIGAGICASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNNSIAIPT
 NFTISVTTEILPVSMTKTSVDCTMYICGDSTECSNLLLQYGSFCTQLNRALTGIAVEQDK
 NTQEVFAQVKQIYKTPPIKDFGGFNFSQILPDPSKPSKRSFIEDLLFNKVTLADAGFIKQY
 GDCLGDIAARDLICAQKFNGLTVLPPLLTDEMIAQYTSALLAGTITSGWTFGAGAALQIP
 FAMQMAYRFNGIGVTQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQN
 AQALNTLVKQLSSNFGAIVSLNDILSRLDKVEAEVQIDRLITGRLQSLQTYVTQQLIRAA
 EIRASANLAATKMSECVLGQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTYVPAQEK
 FTTAPAICHGDKAHFPREGVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVN
 NTVYDPLQPELDSFKEELDKYFKNHTSPDVDLGDISGINASVVNIQKEIDRLNEVAKNLN
 ESLIDLQELGKYEQYIKWPWYIWLGFIAGLIAIVMTIMLCCMTSCCSCCLKGCCSCGSCC
 KFEDEDDSEPV LKGVKLHYT (SEQ ID NO: 1).

[0261] The SARS-CoV-2's spike protein is composed of about 1,273 amino acids and contain several domains. Wu et al., Wrapp et al., and Xia, S., Zhu, Y., Liu, M., Lan, Q., Xu, W., Wu, Y.,

Ying, T., Liu, S., Shi, Z., Jiang, S. & Lu, L., *Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein*. Cell Mol Immunol (2020).

<https://doi.org/10.1038/s41423-020-0374-2>. The spike protein contains: signal sequence (SS); N-terminal domain (NTD, 14-305 aa); receptor-binding domain (RBD, 319-541 aa); S1/S2 protease cleavage site (S1/S2, R685/S686); fusion peptide (FP, 788-806 aa from Zhu et al. or 816-833 aa from Wrapp et al.); heptad repeat 1 (HR1, 912-984 aa); central helix (CH, 986-1035 aa from Wrapp et al.); connector domain (CD, 1076-1141 aa from Wrapp et al.); heptad repeat 2 (HR2, 1163-1213 aa); transmembrane domain (TM, 1214-1237 aa); and cytoplasmic tail (CT, 1238-1273 aa).

[0262] The NTD sequence is:

QCVNLTTTRTQLPPAYTNSFTRGVYYPDKVFRSSVLHSTQDLFLPFFSNVTWFHAIHVSGT
NGTKRFDNPVLPFNDGVYFASTEKSNIIRGWIFGTTLDSKTQSLIVNNATNVVIKVCEF
QFCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLMDLEGKQGNFKNLRE
FVFKNIDGYFKIYSKHTPINLVRDLPQGFSALEPLVDLPIGINITRFQTLLALHRSYLT
PGDSSSGWTAGAAAYYVGYLQPRFTLLKYNENGTITDAVDCALDPLSETKCTLKS (SEQ ID NO: 2).

[0263] The RBD sequence is:

RVQPTESIVRFPNITNLCPFGEVFNATRFASVYAWNRRKRISNCVADYSVLVNSASFSTFK
CYGVSPTKLNDLCFTNVYADSFVIRGDEVQRQIAPGQTGKIADYNYKLPPDDFTGCVIAWN
SNNLDSKVGGNYNYLYRLFRKSNLKPFERDISTEYQAGSTPCNGVEGFNCYFPLQSYGF
QPTNGVGYQPVRVVLSFELLHAPATVCGPKKSTNLVKNKCVNF (SEQ ID NO: 3).

[0264] The FB sequence is: IYKTPPIKDFGGFNFSQIL (SEQ ID NO: 4) (Zhu et al.) or
SFIEDLLFNKVTADAGF (SEQ ID NO: 5) (Wrapp et al.).

[0265] The HR1 sequence is:

TQNVLYENQKLIANQFNSAIGKIQDSLSTASALGKLQDVVNQNAQALNTLVKQLSSNF
GAISSVLNDILSRL (SEQ ID NO: 6).

[0266] The CH sequence is:

KVEAEVQIDRLITGRLQSLQTYVTQQLIRAAEIRASANLAATKMSECVLG (SEQ ID NO: 7)

[0267] The CD sequence is:

TTAPAICHGDKAHFPREGVVFVSNGTHWFVTQRNFYEPQIITDNTFVSGNCDVVIGIVNN
TVYDPL (SEQ ID NO: 8).

[0268] The HR2 sequence is:

DVDLGDISGINASVVNIQKEIDRLNEVAKNLNESLIDLQELGKYEQYIKWP (SEQ ID NO: 9).

[0269] The TM sequence is: WYIWLGFIAGLIAIVMVTIMLCCM (SEQ ID NO: 10).

[0270] The CT sequence is: TSCCCLKGCCSCGSCCKFDEDDSEPVLKGVKLHYT (SEQ ID NO: 11).

[0271] In some embodiments, said antigen expression construct comprises a nucleic acid sequence encoding a protein with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11. In some embodiments, said antigen expression construct comprises a nucleic acid sequence encoding a protein with at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11, or an antigen fragment thereof that is at least 10, 15, 20, 25, 30, 40, 50, or 100 amino acid acids in length.

[0272] A phylogenetic and genetic comparison analysis of the S gene and its regions showed minor variations among strains. The RBD sequences of WHCV (WH-Human 1 coronavirus: the SARS-CoV-2 strain identified in Wu et al.) were more closely related to those of SARS-CoVs (73.8–74.9% amino acid identity) and SARS-like CoVs, including strains Rs4874, Rs7327 and Rs4231 (75.9–76.9% amino acid identity), that are able to use the human ACE2 receptor for cell entry. In addition, the RBD of the spike protein from WHCV was only one amino acid longer than the RBD of the spike protein from SARS-CoV. The previously determined crystal structure of the RBD of the spike protein of SARS-CoV complexed with human ACE2 (Protein Data Bank (PDB) 2AJF) revealed that regions 433–437 and 460–472 directly interact with human ACE2 and hence may be important in determining species specificity. Thus, the S protein is a primary target for the development of effective vaccines against SARS-CoV-2. In some embodiments, the inventors of the present application develop a cellular vaccine against SARS-CoV-2 using a living cell transfected with a construct containing SARS-CoV-2 S protein.

[0273] The nucleic acid sequence encoding the SARS-CoV-2 S protein is:

>NC_045512.2:21563-25384 Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome (Gene ID: 43740568)

ATGTTTGTTCCTTCTGTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAAC
CAGAACTCAATTACCCCTGCATACACTAATTCTTTCACACGTGGTGTTCCTTACCCT
GACAAAGTTTTCAGATCCTCAGTTTACATTCAACTCAGGACTTGTTCTTACCTTTCT
TTTCCAATGTTACTTGGTTCATGCTATACATGTCTCTGGGACCAATGGTACTAAGA
GGTTTGATAACCCTGTCCTACCATTTAATGATGGTGTTCCTTCCACTGAGAA
GTCTAACATAATAAGAGGCTGGATTTTGGTACTACTTTAGATTTCGAAGACCCAGTC
CCTACTTATTGTTAATAACGCTACTAATGTTGTTATTAAAGTCTGTGAATTTCAATTT
TGTAATGATCCATTTTGGGTGTTTATTACCACAAAAACAACAAAAGTTGGATGGAA
AGTGAGTTCAGAGTTTATTCTAGTGCGAATAATTGCACTTTTGAATATGTCTCTCAGC

CTTTTCTTATGGACCTTGAAGGAAAACAGGGTAATTTCAAAAATCTTAGGGAATTTG
TGTTTAAGAATATTGATGGTTATTTTAAAATATATTCTAAGCACACGCCTATTAATTT
AGTGC GTGATCTCCCTCAGGGTTTTTCGGCTTTAGAACCATTTGGTAGATTTGCCAAT
AGGTATTAACATCACTAGGTTTCAAACCTTACTTGCTTTACATAGAAGTTATTTGACT
CCTGGTGATTCTTCTTCAGGTTGGACAGCTGGTGCTGCAGCTTATTATGTGGGTTATC
TTCAACCTAGGACTTTTCTATTAAAATATAATGAAAATGGAACCATACAGATGCTG
TAGACTGTGCACTTGACCCTCTCTCAGAAACAAAGTGTACGTTGAAATCCTTCACTG
TAGAAAAAGGAATCTATCAAACCTTCTAACTTTAGAGTCCAACCAACAGAATCTATTG
TTAGATTTCTTAATATTACAAACCTTGTGCCCTTTTGGTGAAGTTTTTAACGCCACCAG
ATTTGCATCTGTTTATGCTTGGAACAGGAAGAGAATCAGCAACTGTGTTGCTGATTA
TTCTGTCCTATATAATTCCGCATCATTTTCCACTTTTAAGTGTTATGGAGTGTCTCCT
ACTAAATTAAATGATCTCTGCTTTACTAATGTCTATGCAGATTCATTTGTAATTAGAG
GTGATGAAGTCAGACAAATCGCTCCAGGGCAAACCTGGAAAGATTGCTGATTATAAT
TATAAATTACCAGATGATTTTACAGGCTGCGTTATAGCTTGGAATTCTAACAATCTT
GATTCTAAGGTTGGTGGTAATTATAATTACCTGTATAGATTGTTTAGGAAGTCTAAT
CTCAAACCTTTTGAGAGAGATATTTCAAACCTGAAATCTATCAGGCCGGTAGCACACCT
TGTAATGGTGTGGAAGGTTTTAATTGTTACTTTTCTTTACAATCATATGGTTTCCAAC
CCACTAATGGTGTGTTGGTTACCAACCATACAGAGTAGTAGTACTTTCTTTTGAACCTCT
ACATGCACCAGCAACTGTTTGTGGACCTAAAAAGTCTACTAATTTGGTTAAAAACAA
ATGTGTCAATTTCAAACCTTCAATGGTTTAAACAGGCACAGGTGTTCTTACTGAGTCTAA
CAAAAAGTTTCTGCCTTTCCAACAATTTGGCAGAGACATTGCTGACACTACTGATGC
TGTC CGTGATCCACAGACACTTGAGATTCTTGACATTACACCATGTTCTTTTGGTGGT
GTCAGTGTTATAACACCAGGAACAAATACTTCTAACCAGGTTGCTGTTCTTTATCAG
GATGTTAACTGCACAGAAGTCCCTGTTGCTATTCATGCAGATCAACTTACTCCTACTT
GGCGTGTTTATTCTACAGGTTCTAATGTTTTTCAAACACGTGCAGGCTGTTTAATAGG
GGCTGAACATGTCAACAACCTCATATGAGTGTGACATACCCATTGGTGCAGGTATATG
CGCTAGTTATCAGACTCAGACTAATTCTCCTCGGCGGGCACGTAGTG TAGCTAGTCA
ATCCATCATTGCCTACACTATGTCACCTGGTGCAGAAAATTCAGTTGCTTACTCTAAT
AACTCTATTGCCATACCCACAAATTTTACTATTAGTGTTACCACAGAAATTCTACCA
GTGTCTATGACCAAGACATCAGTAGATTGTACAATGTACATTTGTGGTGATTCAACT
GAATGCAGCAATCTTTTGTGCAATATGGCAGTTTTTGTACACAATTAAACCGTGCT
TTAACTGGAATAGCTGTTGAACAAGACAAAAACACCCAAGAAGTTTTTGCACAAGT
CAAACAAATTTACAAAACACCACCAATTAAAGATTTTGGTGGTTTTAATTTTTCACA
AATATTACCAGATCCATCAAAACCAAGCAAGAGGTCATTTATTGAAGATCTACTTTT
CAACAAAGTGACACTTGCAGATGCTGGCTTCATCAACAATATGGTGATTGCCTTGG

TGATATTGCTGCTAGAGACCTCATTTGTGCACAAAAGTTTAACGGCCTTACTGTTTTG
CCACCTTTGCTCACAGATGAAATGATTGCTCAATACACTTCTGCACTGTTAGCGGGT
ACAATCACTTCTGGTTGGACCTTTGGTGCAGGTGCTGCATTACAAATACCATTTGCT
ATGCAAATGGCTTATAGGTTTAATGGTATTGGAGTTACACAGAATGTTCTCTATGAG
AACCAAAAATTGATTGCCAACCAATTTAATAGTGCTATTGGCAAAAATTCAAGACTCA
CTTTCTTCCACAGCAAGTGCACCTTGGAAAACCTCAAGATGTGGTCAACCAAAAATGCA
CAAGCTTTAAACACGCTTGTTAAACAACCTTAGCTCCAATTTTGGTGCAATTTCAAGT
GTTTTAAATGATATCCTTTCACGTCTTGACAAAGTTGAGGCTGAAGTGCAAATTGAT
AGGTTGATCACAGGCAGACTTCAAAGTTTGCAGACATATGTGACTCAACAATTAATT
AGAGCTGCAGAAATCAGAGCTTCTGCTAATCTTGCTGCTACTAAAATGTCAGAGTGT
GTACTTGGACAATCAAAAAGAGTTGATTTTTGTGGAAAGGGCTATCATCTTATGTCC
TTCCCTCAGTCAGCACCTCATGGTGTAGTCTTCTTGCATGTGACTTATGTCCCTGCAC
AAGAAAAGAACTTCACAACCTGCTCCTGCCATTTGTCATGATGGAAAAGCACACTTTC
CTCGTGAAGGTGTCTTTGTTTCAAATGGCACACACTGGTTTGTAACACAAAGGAATT
TTTATGAACCACAAATCATTACTACAGACAACACATTTGTGTCTGGTAACTGTGATG
TTGTAATAGGAATTGTCAACAACACAGTTTATGATCCTTTGCAACCTGAATTAGACT
CATTCAAGGAGGAGTTAGATAAATATTTTAAGAATCATACATCACCAGATGTTGATT
TAGGTGACATCTCTGGCATTAAATGCTTCAGTTGTAAACATTCAAAAAGAAATTGACC
GCCTCAATGAGGTTGCCAAGAATTTAAATGAATCTCTCATCGATCTCCAAGAACTTG
GAAAGTATGAGCAGTATATAAAATGGCCATGGTACATTTGGCTAGGTTTTATAGCTG
GCTTGATTGCCATAGTAATGGTGACAATTATGCTTTGCTGTATGACCAGTTGCTGTA
GTTGTCTCAAGGGCTGTTGTTCTTGTGGATCCTGCTGCAAATTTGATGAAGACGACT
CTGAGCCAGTGCTCAAAGGAGTCAAATTACATTACACATAA (SEQ ID NO: 12). When
incorporating the nucleic acid sequence into the construct, the above sequence can be codon-
optimized.

[0274] In some cases, a nucleic acid sequence to be incorporated into a construct provided herein may be modified. Modifications can comprise truncations of a sequence. For example, modifications can comprise deletion of the cytoplasmic tail, deletion of the transmembrane domain, deletion of a furin cleavage site, and any combination thereof. Modifications can also include additions of a sequence. Additions can comprise a trimerization tag, transgene sequences, and both. In some cases, modifications can also comprise mutations, for example a proline mutation. Any number of modifications can be introduced such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or up to about 10 mutations.

Other Pathogens

[0275] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) of a rabies virus, Ebola virus, HIV, influenza virus, avian influenza virus, SARS coronavirus, herpes virus, Caliciviruses, hepatitis viruses, zika virus, West Nile virus, LaCrosse encephalitis, California encephalitis, Venezuelan equine encephalitis, Eastern equine encephalitis, Western equine encephalitis, Japanese encephalitis virus, St. Louis encephalitis virus, Yellow fever virus, Chikungunya virus or norovirus.

[0276] In some cases, an influenza antigen peptide may be utilized. In influenza viral antigen may be human or non-human. In some cases, an influenza viral antigen that is used originates from type A, B, C, and/or D. In some cases, an influenza viral antigen is A(H1N1), A(H3N2), B(Victoria), or B(Yamagata). In some cases, an influenza viral antigen can be from a non-human species, such as swine, bird, bat, bovine, canine, horse, poultry, feline, and the like.

[0277] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) from a virus from any of the following viral families: Arenaviridae, Arterivirus, Astroviridae, Baculoviridae, Badnavirus, Barnaviridae, Birnaviridae, Bromoviridae, Bunyaviridae, Caliciviridae, Capillovirus, Carlavirus, Caulimovirus, Circoviridae, Closterovirus, Comoviridae, Coronaviridae (*e.g.*, Coronavirus, such as severe acute respiratory syndrome (SARS) virus), Corticoviridae, Cystoviridae, Deltavirus, Dianthovirus, Enamovirus, Filoviridae (*e.g.*, Marburg virus and Ebola virus (EBOV) (*e.g.*, Zaire, Reston, Ivory Coast, or Sudan strain)), Flaviviridae, (*e.g.*, Hepatitis C virus, Dengue virus 1, Dengue virus 2, Dengue virus 3, and Dengue virus 4), Hepadnaviridae, Herpesviridae (*e.g.*, Human herpesvirus 1, 3, 4, 5, and 6, and Cytomegalovirus (CMV)), chikungunya, Hantavirus, Hypoviridae, Iridoviridae, Leviviridae, Lipothrixviridae, Microviridae, Orthomyxoviridae (*e.g.*, Influenza virus A, such as H1N1 strain, and B and C), Papovaviridae, Paramyxoviridae (*e.g.*, measles, mumps, and human respiratory syncytial virus), Parvoviridae, Picornaviridae (*e.g.*, poliovirus, rhinovirus, hepatovirus, and aphthovirus), Poxviridae (*e.g.*, vaccinia and smallpox virus), Reoviridae (*e.g.*, rotavirus), Retroviridae (*e.g.*, lentivirus, such as human immunodeficiency virus (HIV) 1 and HIV 2), Rhabdoviridae (for example, rabies virus, measles virus, respiratory syncytial virus, etc.), Togaviridae (for example, rubella virus, dengue virus, etc.), and Totiviridae. Suitable viral antigens also include all or part of Dengue protein M, Dengue protein E, Dengue D1NS1, Dengue D1NS2, and Dengue D1NS3. Viral antigens may be derived from a particular strain such as a papilloma virus, a herpes virus, *i.e.*, herpes simplex 1 and 2; a hepatitis virus, for example, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), the delta hepatitis D virus (HDV), hepatitis E virus (HEV) and hepatitis G virus

(HGV), the tick-borne encephalitis viruses; parainfluenza, varicella-zoster, JC virus, west nile virus, cytomegalavirus, Epstein- Barr, rotavirus, rhinovirus, adenovirus, coxsackieviruses, equine encephalitis, BK virus, malaria, MuLV, VSV, HTLV, Japanese encephalitis, yellow fever, Rift Valley fever, and lymphocytic choriomeningitis.

[0278] In some cases, an antigen can be from a coronavirus and is SARS-CoV-2, SARS-CoV, and/or MERS-CoV. In some cases, a viral peptide can be from a variant of SARS-CoV-2. In some cases, a variant of SARS-CoV-2 can comprise B.1.1.7 (or the U.K. variant), B.1.1.207, Cluster 5, B.1.351 (or RSA variant), P.1 (or Brazil variant), B.1.617 (or India variant), B.1525, NS3, WIV04/2019, or CAL.20C. In some embodiments, the B.1.617 variant includes a mutation in a spike protein comprising at least one of E154K, E484Q, L452R, P681R, Q1071H, or any combination thereof. In some embodiments, a variant of SARS-CoV-2 comprises lineage A.1, A.2, A.3, A.4, A.5, A.6, B.1, B.2, B.3, B.4, B.5, B.6, B.7, B.8, B.9, B.10, B.11, B.12, B.13, B.14, B.15, or B.16.

[0279] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) from a virus of one or more of Influenza virus A, Influenza virus B, Influenza virus C, Isavirus, Thogotovirus and Quarantavirus. Exemplary influenza A virus subtypes include H1N1, H1N2, H3N2, H3N1, H5N1, H2N2, and H7N7. Exemplary influenza virus antigens include one or more proteins or glycoproteins such as hemagglutinin, such as HA1 and HA2 subunits, neuraminidase, viral RNA polymerase, such as one or more of PB1, PB2 PA and PB1-F2, reverse transcriptase, capsid protein, non-structured proteins, such as NS 1 and NEP, nucleoprotein, matrix proteins, such as M1 and M2 and pore proteins. In some embodiments, Influenza A virus antigens include one or more of the Hemagglutinin (HA) or Neuraminidase (NA) glycoproteins or fragments of the HA or NA, including the antigenic sites of the Hemagglutinin HA1 glycoprotein. In an exemplary embodiment, MDNPs include RNA encoding the influenza A/WSN/33 HA protein.

[0280] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) from a virus of one or more of Ebolavirus, for example, the Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Tai Forest ebolavirus (TAFV), Reston ebolavirus (RESTV), and Bundibugyo ebolavirus (BDBV). In an exemplary embodiment, MDNPs include RNA, such as repRNA, encoding the Zaire ebolavirus glycoprotein (GP), or one or more fragments of the Zaire ebolavirus glycoprotein (GP).

[0281] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) from a virus of one or more of the genus Flavivirus, for example, the Zika virus (ZIKV).

[0282] In some cases, more than one nucleic acids are expressed in a cell vaccine. For example, at least 2, at least 3, or at least 4 can be comprised in a cellular vaccine. In some cases, at least 2 are expressed by a cellular vaccine and are from SARS-CoV-2 and influenza (H1N1).

[0283] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) of *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Variola major*, *Francisella tularensis*, poxviridae, *Burkholderia pseudomallei*, *Coxiella burnetii*, *Brucella* species, *Burkholderia mallei*, *Chlamydia psittaci*, *Staphylococcus enterotoxin B*, *Diarrheagenic E.coli*, *Pathogenic Vibrios*, *Shigella* species, *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter* spp., *Salmonellae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Shigella* spp.

[0284] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a microbial protein (or antigenic fragment thereof) of *Cryptosporidium parvum*, *Cyclospora cayatanensis*, *Giardia lamblia*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Naegleria fowleri* or *Balamuthia mandrillaris*.

[0285] In some embodiments, a peptide or fragment thereof from another pathogen to be utilized in a vaccine can have from about 50%, 60%, 70%, 75%, 80%, 85%, 88%, 90%, 93%, 95%, 96%, 97%, 98%, 99%, or 100% identity to any sequence from **Table 4**.

Table 4: Exemplary viral peptides and corresponding MHC alleles

SEQ ID No		Protein	IEDB ID	Peptide	MHC Allele	MHC Allele Class
55	HIV 1	Gag	59613	SLYNTVATL	HLA-A*02:01/HLA-A*02:02/HLA-A*02:03/HLA-A*02:06/HLA-A*02:11/HLA-A*02:19/HLA-A2/HLA-B*15:01/HLA-A*02:05/HLA-A*02:14/HLA-A*68:02/HLA-A*69:01/HLA-B*07:02	I
13	HIV 1	Gag	33250	KRWIILGLNK	HLA-B*27:05/HLA-B*27:03/HLA-A*03:01/HLA-B*27:02/HLA-DRB1*01:01/Mamu-B*017:04/HLA-DRB1*01:03	I

14	HIV 1	Gag	21635	GPGHKARVL	HLA-B*07:02/H2-Dd/HLA-A*01:01/HLA-A*02:01/HLA-A*03:01/HLA-A*11:01/HLA-A*31:01/HLA-A*69:01/HLA-B*15:01/HLA-B*27:05/HLA-B*40:01/HLA-B*58:01	I
15	HIV 1	Gag	29804	KAFSPEVIPMF	HLA-B*57:01/HLA-B*57:03	I
16	HIV 1	Gag	69360	VLAEAMSQV	HLA-A*02:01/HLA-A*02:02/HLA-A*02:03/HLA-A*02:06/HLA-A*68:02/HLA-A*02:11/HLA-A*02:19/HLA-A*69:01	I
17	HIV 1	Gag	131070	SLFNTVATL	HLA-A*02:01	I
18	HIV 1	Nef	56620	RYPLTFGWCF	HLA-A*24:02	I
19	HIV 1	Nef	5295	AVDLSHFLK	HLA-A*11:01/HLA-A*03:01/HLA-A*01:01/HLA-A*02:01/HLA-A*24:02	I
20	HIV 1	Nef	52760	QVPLRPMTYK	HLA-A*03:01/HLA-A*11:01/HLA-A*02:01/HLA-A*31:01/HLA-A*33:01/HLA-A*68:01/HLA-A*01:01/HLA-A*02:02/HLA-A*02:05/HLA-A*24:02	I
21	HIV 1	Nef	102046	RYPLTFGW	HLA-A*24:02	I
22	HIV 1	Nef	193060	RFPLTFGWCF	HLA-A*24:02	I
23	HIV 1	Envelope glycoprotein gp160	53935	RGPGRAFVTI	H2-Dd/H2-Db/H2-Ld/H2-Kb/H2-Kd/HLA-A*02:01/HLA-A*02:01/HLA-A2/HLA-A*02:02/HLA-A*02:03/HLA-A*02:06/HLA-A*68:02	I
24	HIV 1	Env gp160	32201	KLTPLCVTL	HLA-A*02:01/HLA-A*02:02/HLA-A*02:03/HLA-A*02:06/HLA-	I

					A*02:11/HLA-A*02:19/HLA-A*68:02/HLA-A*69:01	
25	HIV 1	Env gp160	54226	RIQRGPGRFV TIGK	HLA-DQA1*03:01/DQB1*03:02	II
26	HIV 1	Env gp160	53114	RAIEAQQHL	HLA-C*12:02/HLA-B*40:01/HLA-A*31:01/HLA-A*69:01/HLA-B*07:02/HLA-B*15:01/HLA-B*58:01/HLA-C*03:01/HLA-A*01:01/HLA-A*02:01/HLA-A*03:01/HLA-A*11:01/HLA-B*27:05	I
27	HIV 1	Env gp160	54226	RIQRGPGRFV TIGK	HLA-DQA1*03:01/DQB1*03:02	II
28	HIV 1	Env gp160	67245	TVYYGVPVWK	HLA-A*11:01/HLA-A*03:01/HLA-A*31:01/HLA-A*68:01/HLA-A*33:01	I
29	HIV 1	Env gp160	54730	RLRDLLIVTR	HLA-A*11:01/HLA-A*03:01/HLA-A*01:01/HLA-A*02:01/HLA-A*24:02/HLA-A*33:03/HLA-A*07:02	I
30	Zaire ebola virus	Nucleoprotein	16888	FLSFASLFL	HLA-A*02:01/HLA-A*24:02/HLA-B*15:01/HLA-C*03:03/HLA-A*03:01/HLA-A*25:01/HLA-A*26:01/HLA-A*80:01/HLA-A*18:01/HLA-B*18:01/HLA-B*27:03/HLA-B*46:01/HLA-B*57:01	I
31	Zaire ebola virus	Nucleoprotein	17527	FQQTNAMVT	HLA-A*02:01/HLA-B*15:01	I
32	Zaire ebola virus	Nucleoprotein	32188	KLTEAITAA	HLA-A*02:02/HLA-B*15:01	I

33	Zaire ebola virus	Nucleoprotein	54673	RLMRTNFLI	HLA-A*02:01/HLA-A*24:02/HLA-A*03:01/HLA-A*11:01/HLA-A*08:01/HLA-B*15:01/HLA-B*07:02/HLA-A*25:01/HLA-A*26:01/HLA-B*18:01/HLA-B*46:01	I
34	Zaire ebola virus	Nucleoprotein	75566	YQNNLEEI	HLA-A*24:02/HLA-A*02:01/HLA-B*15:01	I
35	Zaire ebola virus	Envelope glycoprotein	91144	ATDVPSATK	HLA-A*11:01/HLA-A*01:01/HLA-A*03:01/HLA-A*24:02	I
36	Zaire ebola virus	Envelope glycoprotein	54480	RLASTVIYR	HLA-A*03:01/HLA-A*11:01/HLA-A*02:01/HLA-A*31:01/HLA-A*02:03/HLA-A*02:12/HLA-A*02:19/HLA-A*23:01/HLA-A*24:03/HLA-A*25:01/HLA-A*26:01/HLA-A*68:02/HLA-A*69:01/HLA-A*80:01/HLA-B*15:01/HLA-B*15:17/HLA-B*18:01/HLA-B*27:03/HLA-B*39:01/HLA-B*46:01/HLA-B*51:01/HLA-B*57:01	I
37	Zaire ebola virus	Envelope glycoprotein	66646	TTIGEWAFW	HLA-A*24:02/HLA-A*32:07/HLA-A*32:15/HLA-A*68:23/HLA-B*15:42/HLA-B*45:06/HLA-B*58:01/HLA-B*83:01/HLA-C*04:01/HLA-A*02:01/HLA-A*02:03/HLA-A*02:11/HLA-A*02:12/HLA-A*02:16/HLA-	I

					A*02:19/HLA-A*03:01/HLA-A*26:01/HLA-A*68:02/HLA-A*69:01/HLA-A*80:01/HLA-B*15:01/HLA-B*18:01/HLA-B*27:03/HLA-B*39:01/HLA-B*46:01	
38	Zaire ebola virus	Envelope glycoprotein	91362	GFRSGVPPK	HLA-A*03:01/HLA-A*11:01/HLA-B*15:01	I
39	Zaire ebola virus	Envelope glycoprotein	91766	NQDGLICGL	HLA-A*02:01	I
40	Zika Virus	Genome polyprotein	569587	IGVSNRDFV	H2-Db/H2-Kb	I
41	Zika Virus	Genome polyprotein	741567	IRCIGVSNRDF VEGMSGGTW	HLA-DRB1*01:01/HLA-DTB1*03:01/HLA-DTB1*04:01/HLA-DTB1*07:01/HLA-DTB1*15:01/HLA-DTB5*01:01/HLA-DTB1*11:01	II
42	Zika Virus	Genome polyprotein	741871	QPENLEYRIML SVHGSQHSG	HLA-DRB5*01:01/HLA-DRB1*03:01/HLA-DRB1*04:01/HLA-DRB1*07:01/HLA-DRB1*11:01/HLA-DRB1*15:01/HLA-DRB5*01:01	II
43	Zika Virus	Genome polyprotein	741599	KGVSYSLCTA AFTFTKIPAE	HLA-DRB1*01:01/HLA-DRB1*04:01/HLA-DRB1*07:01/HLA-DRB1*11:01/HLA-DRB1*15:01/HLA-DRB5*01:01/HLA-DRB1*03:01	II
44	Zika Virus	Genome polyprotein	741402	FEATVRGAKR MAVLGDTAW D	HLA-DRB1*01:01/HLA-DRB1*03:01/HLA-DRB1*07:01/HLA-DRB1*11:01/HLA-DRB1*15:01/HLA-DRB5*01:01/HLA-DRB1*04:01	II
45	Zika Virus	Genome polyprotein	741533	HRSGSTIGKAF EATVRGAKR	HLA-DRB1*01:01/HLA-DRB1*04:01/HLA-DRB1*11:01/HLA-DRB1*15:01/HLA-DRB5*01:01/HLA-	II

					DRB1*03:01/HLA-DRB1*07:01	
46	Influenza A (H1N1, 2009)	Hemagglutinin	125913	ELLVLENER LDYHDS	HLA-DRB1*04:01	II
47	Influenza A	Hemagglutinin	125913	ELLVLENER LDYHDS	HLA-DRB1*04:01	II
48	Influenza A	Matrix protein 1	67496	TYVLSIIPSGPL KAEIAQRL	HLA-DRB1*04:01	II
49	Influenza A	Matrix protein 1	124495	LYKKLKREITF	HLA-A*24:02	I
50	Influenza A	Neuraminidase	126100	GFEMIWDPNG WTGTDN	HLA-DRB1*04:01	II
51	Influenza A	Neuraminidase	126167	GQASYKIFRIE KGKIVK	HLA-DRB1*04:01	II
52	Influenza A	Neuraminidase	126199	GWAIYSKDNS VRIGSKG	HLA-DRB1*04:01	II

Cancer Peptides

[0286] In some embodiments, the antigen expression construct comprises a nucleic acid encoding a peptide (or a fragment thereof) associated with a cancer or a tumor. In some embodiments, the nucleic acid encodes a full-length protein or a fragment or derivative thereof. Exemplary peptides can be neoantigens or oncoproteins. In some embodiments, the peptide or fragment thereof comprises at least one of 707-AP, a biotinylated molecule, a-Actinin-4, abl-bcr alb-b3 (b2a2), abl-bcr alb-b4 (b3a2), adipophilin, AFP, AIM-2, Annexin II, ART-4, BAGE, b-Catenin, bcr-abl, bcr-abl p190 (e1a2), bcr-abl p210 (b2a2), bcr-abl p210 (b3a2), BING-4, CAG-3, CAIX, CAMEL, CISH, Caspase-8, CD171, CD19, CD20, CD22, CD23, CD24, CD30, CD33, CD38, CD44v7/8, CDC27, CDK-4, CEA, CLCA2, Cyp-B, DAM-10, DAM-6, DEK-CAN, EGFRvIII, EGP-2, EGP-40, ELF2, Ep-CAM, EphA2, EphA3, erb-B2, erb-B3, erb-B4, ES-ESO-1a, ETV6/AML, FBP, fetal acetylcholine receptor, FGF-5, FN, G250, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8, GD2, GD3, GnT-V, Gp100, gp75, Her-2, HLA-A*0201-R170I, HMW-MAA, HSP70-2 M, HST-2 (FGF6), HST-2/neu, hTERT, iCE, IL-11R α , IL-13R α 2, KDR, KIAA0205, K-RAS, L1-cell adhesion molecule, LAGE-1, LDLR/FUT, Lewis Y, MAGE-1, MAGE-10, MAGE-12, MAGE-2, MAGE-3, MAGE-4, MAGE-6, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A6, MAGE-B1, MAGE-B2, Malic enzyme, Mammaglobin-A, MART-1/Melan-A, MART-2, MC1R, M-CSF, mesothelin, MUC1, MUC16, MUC2, MUM-1, MUM-2, MUM-3, Myosin, NA88-A, Neo-PAP, NKG2D, NPM/ALK, N-RAS, NY-ESO-1, OA1, OGT, oncofetal antigen (h5T4), OS-9, P polypeptide, P15, P53, PRAME, PSA, PSCA, PSMA, PTPRK, RAGE, ROR1, RU1, RU2, SART-1, SART-2,

SART-3, SOX10, SSX-2, Survivin, Survivin-2B, SYT/SSX, TAG-72, TEL/AML1, TGF α RII, TGF β RII, TP1, TRAG-3, TRG, TRP-1, TRP-2, TRP-2/INT2, TRP-2-6b, Tyrosinase, VEGF-R2, WT1, α -folate receptor, κ -light chain, or any combination thereof.

[0287] In some embodiments, a peptide comprises a neoantigen peptide. For example, a neoantigen can be a peptide that arises from polypeptide generated from genomic sequence that comprises an E805G mutation in ERBB2IP. Neoantigen and neoepitopes can be identified by whole-exome sequencing. In some cases, a gene that can comprise a mutation that gives rise to a neoantigen or neoepitope peptide can be ABL1, ACO1 1997, ACVR2A, AFP, AKT1, ALK, ALPPL2, ANAPC1, APC, ARID1A, AR, AR-v7, ASCL2, β 2M, BRAF, BTK, C15ORF40, CDH1, CLDN6, CNOT1, CT45A5, CTAG1B, DCT, DKK4,EEF1B2, EEF1DP3, EGFR, EIF2B3, env, EPHB2, ERBB3, ESR1, ESRP1, FAM11 IB, FGFR3, FRG1B,GAGE1, GAGE 10, GATA3, GBP3, HER2, IDH1, JAK1, KIT, KRAS, LMAN1, MABEB 16, MAGEA1,MAGEA10, MAGEA4, MAGEA8, MAGEB 17, MAGEB4, MAGEC1, MEK, MLANA, MLL2, MMP13, MSH3, MSH6, MYC, NDUFC2, NRAS, NY-ESO, PAGE2, PAGE5, PDGFR α , PIK3CA, PMEL, pol protein, POLE, PTEN, RAC1, RBM27, RNF43, RPL22, RUNX1, SEC31A, SEC63, SF3B 1, SLC35F5, SLC45A2, SMAP1, SMAP1, SPOP, TFAM, TGFR2, THAP5, TP53, TTK, TYR, UBR5, VHL, XPOT.

[0288] In some embodiments, the peptide(s) or fragment(s) thereof are derived from a polypeptide, a polypeptide generated from a nucleic acid sequence, or a neoantigen derived from at least one of A1CF, ABI1, ABL1, ABL2, ACKR3, ACSL3, ACSL6, ACVR1, ACVR1B, ACVR2A, AFDN, AFF1, AFF3, AFF4, AKAP9, AKT1, AKT2, AKT3, ALDH2, ALK, AMER1, ANK1, APC, APOBEC3B, AR, ARAF, ARHGAP26, ARHGAP5, ARHGEF10, ARHGEF10L, ARHGEF12, ARID1A, ARID1B, ARID2, ARNT, ASPSCR1, ASXL1, ASXL2, ATF1, ATIC, ATM, ATP1A1, ATP2B3, ATR, ATRX, AXIN1, AXIN2, B2M, BAP1, BARD1, BAX, BAZ1A, BCL10, BCL11A, BCL11B, BCL2, BCL2L12, BCL3, BCL6, BCL7A, BCL9, BCL9L, BCLAF1, BCOR, BCORL1, BCR, BIRC3, BIRC6, BLM, BMP5, BMPR1A, BRAF, BRCA1, BRCA2, BRD3, BRD4, BRIP1, BTG1, BTK, BUB1B, C15orf65, CACNA1D, CALR, CAMTA1, CANT1, CARD11, CARS, CASP3, CASP8, CASP9, CBFA2T3, CBFB, CBL, CBLB, CBLC, CCDC6, CCNB1IP1, CCNC, CCND1, CCND2, CCND3, CCNE1, CCR4, CCR7, CD209, CD274, CD28, CD74, CD79A, CD79B, CDC73, CDH1, CDH10, CDH11, CDH17, CDK12, CDK4, CDK6, CDKN1A, CDKN1B, CDKN2A, CDKN2C, CDX2, CEBPA, CEP89, HCHD7, CHD2, CHD4, CHEK2, CHIC2, CHST11, CIC, CIITA, CLIP1, CLP1, CLTC, CLTCL1, CNBD1, CNBP, CNOT3, CNTNAP2, CNTRL, COL1A1, COL2A1, COL3A1, COX6C, CPEB3, CREB1, CREB3L1, CREB3L2, CREBBP, CRLF2, CRNKL1, CRTCL1, CRTCL3, CSF1R, CSF3R, , CSMD3, CTCF, CTNNA2, CTNNB1, CTNND1, CTNND2, CUL3,

CUX1, CXCR4, CYLD, CYP2C8, CYSLTR2, DAXX, DCAF12L2, DCC, DCTN1, DDB2, DDIT3, DDR2, DDX10, DDX3X, DDX5, DDX6, DEK, DGCR8, DICER1, DNAJB1, DNM2, DNMT1, DNMT3A, DROSHA, EBF1, ECT2L, EED, EGFR, EIF1AX, EIF3E, EIF4A2, ELF3, ELF4, ELK4, ELL, ELN, EML4, EP300, EPAS1, EPHA3, EPHA7, EPS15, ERBB2, ERBB3, ERBB4, ERC1, ERCC2, ERCC3, ERCC4, ERG, ESR1, ETNK1, ETV1, ETV4, ETV5, ETV6, EWSR1, EXT1, EXT2, EZH2, EZR, FAM131B, FAM135B, FAM46C, FAM47C, FANCA, FANCC, FANCD2, FANCE, FANCF, FANCG, FAS, FAT1, FAT3, FAT4, FBLN2, FBXO11, FBXW7, FCGR2B, FCRL4, FEN1, FES, FEV, FGFR1, FGFR1OP, FGFR2, FGFR3, FGFR4, FH, FHIT, FIP1L1, FKBP9, FLCN, FLI1, FLNA, FLT3, FLT4, FNBP1, FOXA1, FOXL2, FOXO1, FOXO3, FOXO4, FOXP1, FOXR1, FSTL3, FUBP1, FUS, GAS7, GATA1, GATA2, GATA3, GLI1, GMPS, GNA11, GNAQ, GNAS, , GOLGA5, GOPC, GPC3, GPC5, GPHN, GRIN2A, GRM3, H3F3A, H3F3B, HERPUD1, HEY1, HIF1A, HIP1, HIST1H3B, HIST1H4I, HLA-A, HLF, HMGA1, HMGA2, HNF1A, HNRNPA2B1, HOOK3, HOXA11, HOXA13, HOXA9, HOXC11, HOXC13, HOXD11, HOXD13, HRAS, HSP90AA1, HSP90AB1, ID3, IDH1, IDH2, IGF2BP2, IKBKB, IKZF1, IL2, IL21R, IL6ST, IL7R, IRF4, IRS4, ISX, ITGAV, ITK, JAK1, JAK2, JAK3, JAZF1, JUN, KAT6A, KAT6B, KAT7, KCNJ5, KDM5A, KDM5C, KDM6A, KDR, KDSR, KEAP1, KIAA1549, KIF5B, KIT, KLF4, KLF6, KLK2, KMT2A, KMT2C, KMT2D, KNL1, KNSTRN, KRAS, KTN1, LARP4B, LASP1, LCK, LCP1, LEF1, LEPROTL1, LHFPL6, LIFR, LMNA, LMO1, LMO2, LPP, LRIG3, LRP1B, LSM14A, LYL1, LZTR1, MAF, MAFB, MALT1, MAML2, MAP2K1, MAP2K2, MAP2K4, MAP3K1, MAP3K13, MAPK1, MAX, MB21D2, MDM2, MDM4, MDS2, MECOM, MED12, MEN1, MET, MGMT, MITF, MKL1, MLF1, MLH1, MLLT1, MLLT10, MLLT11, MLLT3, MLLT6, MN1, MNX1, MPL, MSH2, MSH6, MSI2, MSN, MTCP1, MTOR, MUC1, MUC16, MUC4, MUTYH, MYB, MYC, MYCL, MYCN, MYD88, MYH11, MYH9, MYO5A, MYOD1, N4BP2, NAB2, NACA, NBEA, NBN, NCKIPSD, NCOA1, NCOA2, NCOA4, NCOR1, NCOR2, NDRG1, NF1, NF2, NFATC2, NFE2L2, NFIB, NFKB2, NFKBIE, NIN, NKX2-1, NONO, NOTCH1, NOTCH2, NPM1, NR4A3, NRAS, NRG1, NSD1, NSD2, NSD3, NT5C2, NTHL1, NTRK1, NTRK3, NUMA1, NUP214, NUP98, NUTM1, NUTM2A, NUTM2B, OLIG2, OMD, P2RY8, PABPC1, PAFAH1B2, PALB2, PATZ1, PAX3, PAX5, PAX7, PAX8, PBRM1, PBX1, PCBP1, PCM1, PD-1, PDCD1LG2, PDGFB, PDGFRA, PDGFRB, PDL1, PER1, PHF6, PHOX2B, PICALM, PIK3CA, PIK3CB, PIK3R1, PIM1, PLAG1, PLCG1, PML, PMS1, PMS2, POLD1, POLE, POLG, POT1, POU2AF1, POU5F1, PPARG, PPFIBP1, PPM1D, PPP2R1A, PPP6C, PRCC, PRDM1, PRDM16, PRDM2, PREX2, PRF1, PRKACA, PRKAR1A, PRKCB, PRPF40B, PRRX1, PSIP1, PTCH1, PTEN, PTK6, PTPN11, PTPN13, PTPN6, PTPRB, PTPRC, PTPRD, PTPRK, PTPRT, PWWP2A, QKI, RABEP1, RAC1, RAD17, RAD21, RAD51B,

RAF1, RALGDS, RANBP2, RAP1GDS1, RARA, RB1, RBM10, RBM15, RECQL4, REL, RET, RFWD3, RGPD3, RGS7, RHOA, RHOH, RMI2, RNF213, RNF43, ROBO2, ROS1, RPL10, RPL22, RPL5, RPN1, RSPO2, RSPO3, RUNX1, RUNX1T1, S100A7, SALL4, SBDS, SDC4, SDHA, SDHAF2, SDHB, SDHC, SDHD, SEPT5, SEPT6, SEPT9, SET, SETBP1, SETD1B, SETD2, SF3B1, SFPQ, SFRP4, SGK1, SH2B3, SH3GL1, SHTN1, SIRPA, SIX1, SIX2, SKI, SLC34A2, SLC45A3, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMARCD1, SMARCE1, SMC1A, SMO, SND1, SNX29, SOCS1, SOX2, SOX21, SOX9, SPECC1, SPEN, SPOP, SRC, SRGAP3, SRSF2, SRSF3, SS18, SS18L1, SSX1, SSX2, SSX4, STAG1, STAG2, STAT3, STAT5B, STAT6, STIL, STK11, STRN, SUFU, SUZ12 SYK, TAF15, TAL1, TAL2, TBL1XR1, TBX3, TCEA1, TCF12, TCF3, TCF7L2, TCL1A, TEC, TERT, TET1, TET2, TFE3, TFEB, TFG, TFPT, TFRC, TGFB2, THRAP3, TLX1, TLX3, TMEM127, TMPRSS2, TNC, TNFAIP3, TNFRSF14, TNFRSF17, TOP1, TP53, TP63, TPM3, TPM4, TPR, TRAF7, TRIM24, TRIM27, TRIM33, TRIP11, TRRAP, TSC1, TSC2, TSHR, U2AF1, UBR5, USP44, USP6, USP8, VAV1, VHL, VTI1A, WAS, WDCP, WIF1, WNK2, WRN, WT1, WWTR1, XPA, XPC, XPO1, YWHAE, ZBTB16, ZCCHC8, ZEB1, ZFH3, ZMYM2, ZMYM3, ZNF331, ZNF384, ZNF429, ZNF479, ZNF521, ZNRF3, ZRSR2, or any combination thereof.

Methods of Delivery

[0289] The antigen expression constructs described herein can be delivered to a target cell by any suitable means, *e.g.*, any potentially be stably integrated into the cell genome at designated sites via genome engineering, such as safe harbor sites, for constitutive and predictable expression.. An antigen expression construct can be targeted into a preferred genomic location. In some cases, an antigen expression construct can be stably integrated into a cellular genome. In some cases, an antigen expression construct is integrated into a safe harbor site, MHC locus, TCR locus, HLA locus, inhibitory receptor locus, and any combination thereof. Non-limiting examples of safe harbors can include HPRT, AAVS SITE (*e.g.*, AAVS1, AAVS2, ETC.), CCR5, or Rosa26. In some cases, an antigen expression construct is transiently expressed. Conventional viral and non-viral based gene transfer methods can be used to introduce nucleic acids into cells. Methods of non-viral delivery of nucleic acids include electroporation, lipofection, nucleofection, gold nanoparticle delivery, microinjection, biolistics, virosomes, liposomes, immunoliposomes, polycation or lipid-nucleic acid conjugates, naked DNA, mRNA, artificial virions, and agent-enhanced uptake of DNA. Sonoporation using, *e.g.*, the Sonitron 2000 system (Rich-Mar), can also be used for delivery of nucleic acids.

[0290] Non-viral vector delivery systems can include DNA plasmids, naked nucleic acids, nucleic acids complexed with a delivery vehicle such as a liposome or poloxamer, and delivery of an mRNA.

[0291] In one embodiment, the antigen expression construct is electroporated into the cell. In some embodiments, the antigen expression construct comprises mRNA and said mRNA is electroporated into said cell. Additional exemplary nucleic acid delivery systems include those provided by AMAXA Biosystems (Cologne, Germany), Life Technologies (Frederick, Md.), MAXCYTE, Inc. (Rockville, Md.), BTX Molecular Delivery Systems (Holliston, Mass.) and Copernicus Therapeutics Inc. (see for example U.S. Pat. No. 6,008,336). Lipofection reagents are sold commercially (e.g., TRANSFECTAM® and LIPOFECTIN®). Delivery can be to cells (*ex vivo* administration) or target tissues (*in vivo* administration).

[0292] The pathogen protein encoding polynucleotides and compositions comprising the polynucleotides described herein can be delivered using vectors containing sequences encoding one or more of the proteins. Any vector systems can be used including but not limited to plasmid vectors, retroviral vectors, lentiviral vectors, adenovirus vectors, poxvirus vectors, herpesvirus vectors and adeno-associated virus vectors, etc. Viral vector delivery systems can include DNA and RNA viruses, which have either episomal or integrated genomes after delivery to the cell.

Suicide Gene

[0293] In some cases, a cell provided herein can comprise a genomic integration of a “kill-switch” suicide gene. A suicide gene can allow for removal of the cell by treatment with a drug that selectively kills those cells comprising the suicide gene. Inclusion of a suicide gene in a cell can also allow for increased safety when utilizing cells provided herein for treatment.

[0294] In some cases, a suicide gene may be incorporated into a cellular product. A suicide gene allows for the elimination of gene modified cells in the case of an adverse event, self-reactivity of infused cells, eradication of infection, and the like. In some embodiments, the suicide gene is introduced to a random genomic position, or a targeted locus (e.g., a metabolic gene locus, DNA/RNA replication gene locus, safe harbor, MHC locus, HLA locus, TCR locus, exhaustion locus, inhibitory receptor locus (PD-1, CTLA-4, Tim 3, CISH, and the like). Non-limiting examples of safe harbors can include HPRT, AAVS SITE (e.g., AAVS1, AAVS2, ETC.), CCR5, or Rosa26. In some cases, a suicide gene may be driven by an exogenous promoter or take advantage of an endogenous promoter of an integrated locus.

[0295] Various suicide genes are known in the art and can be utilized in the cellular compositions provided herein. Exemplary suicide genes can be: thymidine kinase/Ganciclovir,

cytosine deaminase/5-fluorocytosine, nitroreductase/CB1954, carboxypeptidase G2/nitrogen mustard, cytochrome P450/oxazaphosphorine, purine nucleoside phosphorylase/6-methylpurine deoxyriboside (PNP/MEP), (HRP/IAA), and combinations thereof. In a specific embodiment, a suicide gene is an inducible caspase-9 gene (see US Pre-Grant Patent Publication No. US 2013/0071414, which suicide genes are incorporated by reference herein). Other suicide genes include a gene that encodes any one or more of: a conformationally intact binding epitope for pharmaceutical-grade anti-EGFR monoclonal antibody, cetuximab (Erbiximab); EGFRt, a caspase polypeptide (*e.g.*, iCasp9; Straathof et al., *Blood* 105:4247-4254, 2005; Di Stasi et al., *N. Engl. J. Med.* 365: 1673-1683, 2011; Zhou and Brenner, *Exp. Hematol.* pii: S0301 -472X (16)30513-6. doi: 10.1016/j.exphem.2016.07.011), RQR8 (Philip et al., *Blood* 124: 1277-1287, 2014), a 10-amino acid tag of the human c-myc protein (Myc) (Kieback et al., *Proc. Natl. Acad. Sci. USA* 105:623-628, 2008), as discussed herein, and a marker/safety switch polypeptide, such as RQR (CD20 + CD34; Philip et al., 2014). In some embodiments, the suicide gene is sr39TK, which allows elimination of cells by the introduction of ganciclovir. This gene may also be used to image gene modified cells using positron emission tomography to localized cells in the recipient / host. A suicide gene may also be a chemically induced caspase, dimerization induced by a small molecule/chemically induced dimerizer (CID). The suicide gene may also be a selectable surface marker (CD 19 or CD20 or CD34 or EGFR or LNGFR, etc.) allowing the cells to be eliminated by introduction of an antibody through antibody dependent cellular cytotoxicity, complement cascade, etc.

[0296] In some cases, a suicide gene can be included within a vector comprising a viral antigen peptide provided herein. In other cases, a suicide gene is separately introduced into a cell, using for example a CRISPR system, a viral system, electroporation, transfection, transduction, and any combination thereof. In some cases, a suicide gene is knocked into a targeted locus.

Methods of Vaccinating A Subject

[0297] In one aspect, provided herein are methods of immunizing a subject against a pathogen by administering a population of vaccine cells described herein tailored to induce an adaptive immune response against said pathogen in said subject (*e.g.*, said vaccine cells comprise a protein or antigen fragment of said pathogen).

[0298] In some embodiments, the pathogen is a virus, bacteria, or parasite.

[0299] In some embodiments, the pathogen is a virus. In some embodiments, said virus is a rabies virus, Ebola virus, HIV, influenza virus, avian influenza virus, SARS coronavirus, herpes virus, Caliciviruses, hepatitis viruses, zika virus, West Nile virus, LaCrosse encephalitis, California encephalitis, Venezuelan equine encephalitis, Eastern equine encephalitis, Western

equine encephalitis, Japanese encephalitis virus, St. Louis encephalitis virus, Yellow fever virus, Chikungunya virus, or norovirus.

[0300] In some embodiments, said virus is of order Nidovirales. In some embodiments, said virus is of family Coronaviridae. In some embodiments, said virus is of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. In some embodiments, said virus is of genus Betacoronavirus. In some embodiments, said virus is of subgenus Sarbecovirus. In some embodiments, said virus is of species severe acute respiratory syndrome-related coronavirus 2. In some embodiments, said virus is of strain severe acute respiratory syndrome coronavirus 2. In some embodiments, said virus is of severe acute respiratory syndrome coronavirus 2.

[0301] In some embodiments, said pathogen is a bacteria. In some embodiments, said bacteria is *Bacillus anthracis*, *Clostridium botulinum*, *Yersinia pestis*, *Variola major*, *Francisella tularensis*, poxviridae, *Burkholderia pseudomallei*, *Coxiella burnetii*, *Brucella* species, *Burkholderia mallei*, *Chlamydia psittaci*, *Staphylococcus enterotoxin B*, *Diarrheagenic E.coli*, *Pathogenic Vibrios*, *Shigella* species, *Salmonella*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacteriaceae*, *Enterococcus faecium*, *Staphylococcus aureus*, *Helicobacter pylori*, *Campylobacter* spp., *Salmonellae*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae*, *Haemophilus influenzae* or *Shigella* spp.

[0302] In some embodiments, said pathogen is a parasite. In some embodiments, said parasite is *Cryptosporidium parvum*, *Cyclospora cayatanensis*, *Giardia lamblia*, *Entamoeba histolytica*, *Toxoplasma gondii*, *Naegleria fowleri* or *Balamuthia mandrillaris*.

[0303] The cellular vaccine described herein may be administered by any suitable delivery route well known in the art, include but not limited to intramuscular injection, intradermal injection, intravenous injection or subcutaneous injection. In some embodiments, said vaccine is administered locally. In some embodiments, said vaccine is administered systemically. In some embodiments, said vaccine is administered using a pen-injector device, such as is used for at-home delivery of epinephrine, could be used to allow self-administration of the vaccine. In some cases, a vaccine is administered via post intradermal/SQ injection.

[0304] In some embodiments, a vaccine is administered locally. In some embodiments, the vaccine is administered subcutaneously. In some embodiments, the vaccine is self-administered by a patient.

[0305] In some cases, a vaccine is administered via a pulmonary system. In some cases, a vaccine is inhaled. In some cases, the vaccine is administered via inhalation. In some cases, the vaccine is inhaled and is able to access the lungs. In some cases, the vaccine is inhaled and able

to access the airways. In some cases, the vaccine is administered orally. In some cases, the vaccine is administered orally and is able to access the gastrointestinal tract. In some cases a vaccine may be ingested orally via the GI system. In some cases, a vaccine is applied to the skin. In some cases the vaccine is administered through the skin. In some embodiments, the vaccine is administered via subcutaneous injection. In some embodiments, the vaccine is administered via dermal injection. In some embodiments, the vaccine is administered via intradermal injection. The use of such delivery devices may be particularly amenable to large scale immunization campaigns such as would be required during a pandemic.

Kits

[0306] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a vaccine may be in a kit, any type of cells may be provided in the kit, and/or reagents for manipulation of vaccines and/or cells may be provided in the kit. The components are provided in suitable container means.

[0307] The kits may comprise a suitably aliquoted composition. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there is more than one component in the kit, the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits also will typically include a means for containing the components in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[0308] However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

[0309] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein may be employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

EXAMPLES

Example 1. CRISPR genetic engineering of iPSCs to knockout both MHC class I and II genes.

[0310] The parental iPS cell line is transfected using the Lonza nucleofection system with Cas9 protein precomplexed with gRNAs targeting genes essential for the expression of MHC class I and II (such as B2M and CIITA). Cells are allowed to recover from transfection and grown in complete growth media for 72 hours before analysis of the targeted loci by PCR and sequencing across the modified region. Loss of MHC I and MHC II is confirmed by surface expression by flow cytometry in iPSC cells stimulated with IFN γ (and in differentiated cells generated from these iPS Cells). **FIG. 7.**

[0311] In a further experiment, control cells or B2M knock out platform cells from two different donors are cultured with T cells. MHCI deficient iPSC cells fail to activate the proliferation of MHC-mismatched T cells as compared to control iPS cells, **FIG. 8A.** Further experiments will measure NK cell killing of modified cells to demonstrate elevated cytolysis in the absence of MHCI.

Example 2. CRISPR genetic engineering of MHC-null iPSCs from Example 1 to genomically integrate critical NK cell activation ligand genes or lytic-associated genes

Activation ligand genes

[0312] MHC-null iPS cells are transfected with Cas9 and gRNA complexes targeting regions of the genome for targeted integration of either plasmid based DNA donors or rAAV templates carrying transgenes for the stimulation, activation or recruitment of innate immune cells. Target sites include genomic safe harbor sites, or genes that repress or inhibit the stimulation, activation or recruitment of innate immune cells to be inactivated via genomic cleavage and insertion of donor templates. Donor templates are designed to express the cDNAs of the ligands with constitutive promoter and terminator sequences.

Lysis genes

[0313] MHC-null iPS platform cells are transfected with Cas9 and gRNA complexes targeting regions of the genome for targeted integration of either plasmid based DNA donors or rAAV templates carrying transgenes for the lytic signals recognized by innate immune cells, exemplary signals in **Table 5.** In addition to a lack of MHC-I, innate immune cells such as NK cells, can be effectively activated to kill target iPS cells by a secondary activating signal in the form of a cell surface ligand that interacts with the NKG2D receptor on the surface of NK cells, such as those in **Table 5.** Target sites include genomic safe harbor sites, or genes that repress or inhibit the stimulation, activation or recruitment of innate immune cells to be inactivated via genomic cleavage and insertion of donor templates. Donor templates are designed to express the cDNAs of the ligands with constitutive promoter and terminator sequences.

Generation of Endothelial Cells from Platform Cells

[0314] To differentiate towards the endothelial lineage iPS cells were grown on Vitronectin coated plates and fed with base media of RPMI of B27(-insulin), Glutamax, Penicillin/Streptomycin containing on day 0-2 6 μ M CHIR99021, 10ng/ml BMP4, and 100 μ g/ml AA2P (stage1), followed by 50 ng/mL VEGF165 + 20 ng/mL FGF + 10 μ M SB431542 from days 2-7 (stage 2). Results of Day 7 Flow Cytometry for CD31+CD144+ endothelial cells is shown in **FIG. 8B**.

[0315] 48 hours post transfection flow cytometry is obtained on iPSC-derived endothelial cells overexpressing NK-activating ligands, data shown in **FIG. 9**.

Table 5: Genes that encode NK cell activating ligands

Target	Gene	
1	MICA	MICA and MICB molecules act as key ligands for activating receptor natural killer cell (NK) group 2, member D (NKG2D) and promote NK cell-mediated recognition and cytotoxicity.
2	MICB	
3	PVR	PVR overexpressed on tumor cells increases the activation of NK cells and elimination of tumor cells via its interaction with DNAM-1
4	PVRL2	
5	ULBP1	ULBP1-6 are recognized by a single NK activating receptor, NKG2D
6	ULBP2	
7	ULBP3	
8	ULBP4	
9	ULBP5	
10	ULBP6	
11	CMV pp65	Viral antigens to be used as a positive control as they have been shown to activate NK cells
12	B7-H6	
13	InfA-HA	
14	infA-NA	
15	IgG-FC	“reverse FC” expressed on cell surface to mimic an opsonised cell having been coated with antibody and thus a target for phagocytosis

Example 3. Transfection of the engineered cellular vaccine cells, such as platform cells, with SARS-Cov-2 spike protein or S1 subunit expression constructs with desired modification.

[0316] DNA donors (plasmids, linear DNA or rAAV donors) expressing the cDNA for the spike protein or the S1 subunit were transfected into cellular vaccine cells (iPSC or differentiated cells) using Lonza nucleofector, Thermo Neon or any other lipid-based transfection. Endothelial cells expressing the SARS-CoV-2 Spike protein variants were lysed, and lysates analyzed for spike protein antigen by ELISA. Both protein antigen variants could be detected abundantly and showed a dose-dependent increase with vaccine cell number, **FIG. 10**.

[0317] In an exemplary strategy, DNA sequences encoding the Spike antigen variant expression construct are inserted into the AAVS1 safe-harbor site using CRISPR gene engineering.

Example 4. Coculture of the transfected cells in Example 3 with donor-derived NK cells and analysis of NK-mediated cytotoxicity of the antigen loaded vaccine cells by standard *ex vivo* NK-killing assays and/or analysis of killing by confocal imaging.

[0318] Cellular Vaccine cells are cocultured with PBMC derived NK cells at varying effector: target (E: T) ratios for several days. NK-mediated cell lysis is measured using CyQUANT LDH Cytotoxicity Assay to measure live and dead cells using a plate reader. NK cell degranulation is also measured by analysis of NK cell CD107a expression by flow cytometry.

[0319] In another assay, 24 hours before performing an NK cell killing assay, iPS derived Endothelial Cells (differentiated from platform cells) were harvested with TRYPLE and seeded into geltrex coated 96 well plate wells (2×10^4 /well) and incubated overnight in stage 2 endothelial differentiation medium. On the day of the assay K562 cells were seeded out into 96 well plates at (2×10^4 /well) and both endothelial cells and K562 cells were stained with Cell Tracker Blue dye. Primary NK cells were added to the wells at 0; 0.25:1; 1.25:1; 2.5:1; or 5:1 and incubated for 4 hours containing RPMI, 10% FCS with 200IU/ml IL2 and 10ng/ml IL15. Samples were then run on a flow cytometer using 7AAD staining to identify dead cells within the Cell Tracker Blue target population, **FIG. 12**.

[0320] The data demonstrates effective NK Cell killing in the absence of MHC-I. NK Cell assays measuring the cytolytic killing of MHC-I deficient, iPSC derived Endothelial cells (differentiated from platform cells), show robust, dose dependent lysis that is equivalent to the gold-standard K562 cell line for NK killing. Platform cells or cells differentiated or derived therefrom can be engineered to express NK activating ligands to potentiate this targeted cell lysis further and ensure robust and rapid cytotoxicity when administered *in vivo*.

Example 5. Comparing the engineered vaccine cells with non-engineered cells

[0321] NK-mediated killing and degranulation assays are performed as outlined above comparing cellular vaccine cells (iPSC and differentiated cells) to the same cell type without engineering of either ligands to activate the innate immune system or MHC I & II knockout.

Example 6. Detection of SARS-Cov-2 spike protein in the culture media upon NK cell-mediated lysis

[0322] The release of the SARS-Cov-2 spike protein, exemplary schematic at **FIG. 11**, expressed by the cellular vaccine cells is detected in the supernatant using a Spike protein specific ELISA kit, such as those provided in [sinobiological.com/elisa-kits/cov-spike-kit40591](https://www.sinobiological.com/elisa-kits/cov-spike-kit40591).

Non-human Primate Study

[0323] 10 adult rhesus macaques (6-12 years old) are inoculated with with a total of 1.1×10^6 PFU (Group 1; N = 3), 1.1×10^5 PFU (Group 2; N = 3), or 1.1×10^4 PFU (Group 3; N = 3) SARS-CoV-2, administered as 1 ml by the intranasal (IN) route and 1 ml by the intratracheal (IT) route. 10 comparable macaques receive control inoculations. Following viral challenge, viral RNA level are assessed by RT-PCR in multiple anatomic compartments, such as bronchoalveolar lavage and nasal swabs.

[0324] SARS-CoV-2-specific humoral and cellular immune responses are detected in the animals by evaluating binding antibody responses to the SARS-CoV-2 Spike (S) protein by ELISA and neutralizing antibody (NAb) responses using both a pseudovirus neutralization assay and a live virus neutralization assay. Antibody responses are evaluated against the receptor binding domain (RBD), the prefusion S ectodomain (S), and the nucleocapsid (N). Additionally, the presence of various immune responses are evaluated: antibody-dependent complement deposition (ADCD), antibody-dependent cellular phagocytosis (ADCP), antibody-dependent neutrophil phagocytosis (ADNP), and antibody-dependent NK cell degranulation (NK CD107a) and cytokine secretion (NK MIP1 β , NK IFN γ).

Example 7. A Universal Vaccine Cell (UVC) for SAR-CoV-2

[0325] As shown in **FIG. 13**, the UVC is MHC-I deficient (B2M KO) and does not express MHC-II. The lack of expression of MHC-I potentiates the lysis of the UVC by NK cells. Expression of the NK ligand MICA also further potentiates the NK cell engagement and the UVC cytolysis. The UVC expresses a high level of intracellular SARS-CoV-2 spike protein and nucleocapsid proteins. Upon cell lysis by the NK cell, these proteins are released into the immune microenvironment.

[0326] The UVC does not express MHC-II, preventing it from presenting any peptide (*e.g.*, a SARS-CoV-2 spike protein peptide) to any recipient immune cells and unable to be stimulated by IFN γ .

[0327] At the site of vaccination, the UVC will activate the innate immune cell (*e.g.*, NK cells) to trigger its own lysis. The spike and nucleocapsid protein will then be released following the apoptosis of the UVC. Phagocytosis and pinocytosis of the UVC apoptotic bodies will enable APCs to present the spike protein and nucleocapsid peptides to the adaptive immune system through MHC presentation. The UVC expresses both a full-length SARS-CoV-2 spike protein and a full-length nucleoplasmid protein. To ensure a robust response by the adaptive immune system, the UVC is engineered to express a full-length SARS-CoV-2 spike protein with disrupted furin cleavage sites and two proline residues substitutions. The nucleotide sequence encoding this spike protein is shown in **SEQ ID NO: 53**.

[0328] cDNA of the RSA Spike protein without the furin cleavage site – **SEQ ID: NO 53**

ATGGCATTCGTGTTTCTGGTGCTGCTGCCTCTGGTGTCCAGCCAGTGCGTGAAC TTCA
CCACCAGAACACAGCTGCCTCCAGCCTACACCAACAGCTTTACCAGAGGCGTGTACT
ACCCCGACAAGGTGTT CAGATCCAGCGTGCTGCACTCTACCCAGGACCTGTT CCTGC
CTTTCTTCAGCAACGTGACCTGGTTCCACGCCATCCACGTGTCCGGCACCAATGGCA
CCAAGAGATTGCGCAATCCTGTGCTGCCCTTCAACGACGGGGTGTACTTTGCCAGCA
CCGAGAAGTCCAACATCATCAGAGGCTGGATCTTCGGCACCACACTGGACAGCAAG
ACCCAGAGCCTGCTGATCGTGAACAACGCCACCAACGTGGTCATCAAAGTGTGCGA
GTTCCAGTTCTGCAACGACCCCTTCCTGGGCGTCTACTACCACAAGAACAACAAGAG
CTGGATGGAAAGCGAGTTCGGGTGTACAGCAGCGCCAACA ACTGCACCTTCGAGT
ACGTGTCCCAGCCTTTCCTGATGGACCTGGAAGGCAAGCAGGGCAACTTCAAGAAC
CTGCGCGAGTTCGTGTTCAAGAACATCGACGGCTACTTCAAGATCTACAGCAAGCAC
ACCCCTATCAACCTCGTGCGGGGACTGCCTCAGGGCTTTTCTGCTCTGGAACCCCTG
GTGGATCTGCCCATCGGCATCAACATCACCCGGTTTCAGACCCTGCACCGGTCCTAT
CTGACACCCGGCGATTCTTCTAGCGGATGGACAGCTGGCGCCGCTGCCTACTATGTG
GGATACCTGCAGCCTCGGACCTTCCTGCTGAAGTACAACGAGAACGGCACCATCAC
CGACGCCGTGGATTGTGCTCTGGATCCTCTGAGCGAGACAAAGTGCACCTGAAGTC
CTTCACCGTGGA AAAAGGGCATCTACCAGACCAGCAACTTCCGGGTGCAGCCCACCG
AATCCATCGTGCGGTTCCCAATATCACCAATCTGTGCCCTTCGGCGAGGTGTTCA
ATGCCACCAGATTCGCCTCTGTGTACGCCTGGAACCGGAAGCGGATCAGCAATTGC
GTGGCCGACTACTCCGTGCTGTACAACTCCGCCAGCTTCAGCACCTTCAAGTGCTAC
GGCGTGTCCTTACCAAGCTGAACGACCTGTGCTTCACAAACGTGTACGCCGACAGC
TTCGTGATCCGGGGAGATGAAGTGCGGCAGATTGCCCTGGACAGACCGGCAATAT
CGCCGACTACA ACTACAAGCTGCCCCGACGACTTACC GGCTGTGTGATTGCCTGGAA
CAGCAACAACCTGGACTCCAAAGTCGGCGGCAACTACAATTACCTGTACCGGCTGTT
CCGGAAGTCCAATCTGAAGCCCTTCGAGCGGGACATCTCCACCGAGATCTATCAGG
CCGGCAGCACCCCTTGCAATGGCGTGAAGGGCTTTAACTGCTACTTCCC ACTGCAGT
CCTACGGCTTCAGCCAACATACGGCGTGGGCTACCAGCCTTACAGAGTGGTGGTGC
TGAGCTTCGAGCTGCTGCATGCTCCTGCCACAGTGTGCGGCCCTAAGAAAAGCACCA
ATCTCGTGAAGAACAAATGCGTCAACTTCAATTTCAACGGCCTGACCGGCACCGGC
GTGCTGACAGAGAGCAACAAGAAGTTCCTGCCATTCCAGCAGTTCGGCCGGGACAT
TGCCGATACCACAGATGCTGTCAGAGATCCCCAGACACTGGAAATCCTGGACATCA
CCCCATGCAGCTTCGGCGGAGTGTCTGTGATCACCCCTGGCACCAACACCAGCAATC
AGGTGGCAGTGCTGTACCAGGGCGTCAACTGTACAGAGGTGCCAGTGGCCATTAC
GCCGATCAGCTGACCCCTACTTGGCGGGTGTACTCCACAGGCAGCAATGTGTTCCAG

ACCAGAGCCGGCTGTCTGATCGGAGCCGAGCACGTGAACAATAGCTACGAGTGCGA
CATCCCCATCGGCGCTGGCATCTGTGCCAGCTACCAGACACAGACAAACAGCCCCG
GCGGCAGCGGATCTGTGGCCAGCCAGAGCATCATTGCCTACACAATGTCTCTGGGC
GTCGAGAACAGCGTGGCCTACTCCAACAACCTCTATCGCTATCCCCACCAATTTACC
ATCAGCGTGACCACAGAGATCCTGCCTGTGTCCATGACCAAGACCAGCGTGGACTG
CACCATGTACATCTGCGGCGATAGCACCGAGTGCTCCAACCTGCTGCTGCAGTACGG
CAGCTTCTGCACCCAGCTGAATAGAGCCCTGACCGGAATCGCCGTGGAACAGGACA
AGAACACCCAAGAGGTGTTGCCCCAAGTGAAGCAGATCTACAAGACCCCTCCTATC
AAGGACTTCGGCGGCTTCAACTTCAGCCAGATTCTGCCCCGATCCTAGCAAGCCCAGC
AAGCGGAGCTTCATCGAGGACCTGCTGTTCAACAAAGTGACACTGGCCGACGCCGG
CTTCATCAAGCAGTATGGCGATTGTCTGGGCGACATTGCAGCCCCGGGATCTGATTTG
CGCCCAGAAGTTTAACGGACTGACCGTGCTGCCTCCTCTGCTGACCGATGAGATGAT
CGCCCAGTACACATCTGCCCTGCTGGCCGGCACAATCACAAGCGGCTGGACATTTGG
AGCTGGCGCTGCCCTGCAGATCCCCTTTGCTATGCAGATGGCCTACCGGTTCAACGG
CATCGGAGTGACCCAGAATGTGCTGTACGAGAACCAGAAGCTGATCGCCAACCAGT
TCAACAGCGCCATCGGCAAGATCCAGGACAGCCTGAGCAGCACAGCCAGCGCTCTG
GGAAAACCTGCAGGACGTGGTCAACCAGAACGCCAGGCTCTGAATACCCTGGTCAA
GCAGCTGTCCTCCAACCTTCGGCGCCATCAGCTCTGTGCTGAACGATATCCTGAGCAG
ACTGGACCCTCCTGAAGCCGAGGTGCAGATCGACAGACTGATCACCGGAAGGCTGC
AGTCCCTGCAGACCTACGTTACCCAGCAGCTGATCAGAGCCGCCGAGATTAGAGCC
TCTGCCAATCTGGCCGCCACCAAGATGTCTGAGTGTGTGCTGGGCCAGAGCAAGAG
AGTGGACTTTTGCGGCAAGGGCTACCACCTGATGAGCTTCCCTCAGTCTGCACCACA
CGGCGTGGTGTCTTCTGCACGTGACATACGTGCCCGCTCAAGAGAAGAACTTCACAAC
AGCCCCTGCCATCTGCCACGACGGCAAAGCCCACTTTCCTAGAGAAGGCGTGTTCTGT
GTCCAACGGCACCCATTGGTTCGTGACCCAGCGGAACCTTCTACGAGCCCCAGATCAT
CACCACCGACAACACCTTCGTGTCTGGCAACTGCGACGTCGTGATCGGCATTGTGAA
CAATACCGTGTACGACCCTCTGCAGCCCGAGCTGGACAGCTTCAAAGAGGAACTGG
ATAAGTACTTTAAGAACCACACAAGCCCCGACGTGGACCTGGGCGATATCAGCGGA
ATCAATGCCAGCGTCGTGAACATCCAGAAAGAGATCGACCGGCTGAACGAGGTGGC
CAAGAATCTGAACGAGAGCCTGATCGACCTGCAAGAACTGGGGAAGTACGAGCAGT
ACATCAAGTGGCCTTGGTACATCTGGCTGGGCTTTATCGCCGGACTGATTGCCATCG
TGATGGTCACAATCATGCTGTGCTGTATGACCAGCTGCTGTAGCTGCCTGAAGGGCT
GTTGCAGCTGTGGCTCCTGCTGCAAGTTCGACGAGGACGACAGTGAGCCGGTGCTTA
AGGGCGTAAAACTTCATTACACTTCCGGATGA

[0329] These modifications allow the spike protein to remain intact and a natural cell surface active conformation, because its multiple subunits will dissociate inside the host. The amino acid sequence of the nucleocapsid protein is shown in **FIG. 14A** or **SEQ ID NO: 54**.

[0330] GenBank: QHD43423.2 **SEQ ID NO: 54**

MSDNGPQNQRNAPRITFGGSPDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQ
 HGKEDLKFPRGQGVPIINTNSSPDDQIGYYRRATTRIRGGDGKMKDLSPRWYFYLLGTG
 PEAGLPYGANKDGIWVATEGALNTPKDHIGTRNPANNAIIVLQLPQGTTLPGFYAEG
 SRGGSQASSRSSSRNSSRNSTPGSSRGTSARMAGNGGDAALALLLDRLNQLESKM
 SGKGGQQQGGQTVTKKSAAEASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQEL
 IRQGTDYKHWPQIAQFAPSASAFFGMSRIGMEVTPSGTWLTYTGAIKLDDKDPNFKDQV
 ILLNKHIDAYKTFPPTEPKDKKKKADETQALPQRQKKQQTVTLLPAADLDDFSKQLQQ
 SMSSADSTQA

[0331] As shown in **FIG. 14B**, an EF1a promoter are used to drive and ensure the maximum expression of the SARS-CoV-2 spike protein and the nucleocapsid protein. To maintain a 1:1 expression ratio, the two proteins are expressed from the same transcript with a T2A peptide cleave sequence connecting them.

Example 8. Polyvalent SARS-CoV-2 UVC design

[0332] A genome-wide screening technology called T-Scan, described in Kula et al., “T-Scan: A Genome-wide Method for the Systematic Discovery of T Cell Epitopes.” 2019, Cell, 178:1016–1028.e3, which is herein incorporated by reference in its entirety for all purposes, was used to determine the global landscape of CD8⁺ T cell recognition of SARS-CoV-2 in an unbiased fashion: CD8⁺ T cells were co-cultured with a genome-wide library of target cells (modified HEK293 cells), engineered to express a single HLA allele. Each target cell in the library also expressed a unique coronavirus-derived 61-amino acid (aa) protein fragment. These fragments were processed naturally by the target cell, and the appropriate peptide epitopes were displayed on major histocompatibility complex (MHC) class I molecules on the cell surface. When a CD8⁺ T cell encountered its target in the co-culture, it secreted cytotoxic granules into the target cell, inducing the apoptosis of its target. Early apoptotic cells were then isolated from the co-culture, and the expression cassettes were sequenced, revealing the identity of the protein fragment. To optimize sorting and isolating rare recognized target cells, the target cells were engineered to express a Granzyme B (GzB)-activated fluorescent reporter as described previously as well as a GzB-activated version of the scramblase enzyme XKR8, which drives rapid and efficient transfer of phosphatidylserine to the outer membrane of early apoptotic cells. Early apoptotic cells were then enriched by magnetic-activated cell sorting with Annexin V, followed by fluorescence-activated sorting with the fluorescent reporter.

[0333] A library of 61-aa protein fragments that tiled across all 11 open reading frames (ORFs) of SARS-CoV-2 in 20-aa steps. To capture the known genetic diversity of SARS-CoV-2, all protein-coding variants from the 104 isolates that had been reported as of March 15, 2020 and the complete set of ORFs (ORFeome) of SARS-CoV and the four endemic coronaviruses that cause the common cold (betacoronaviruses HKU1 and OC43 and alphacoronaviruses NL63 and 229E) were included. Known immunodominant antigens from CMV, EBV, and influenza virus were included as positive controls. Each protein fragment with a unique nucleotide barcode to provide internal replicates in our screens was represented 10 times for a final library size of 43,420 clones.

[0334] As shown in **FIG. 14C**, broad reactivity CD8⁺ T cells to many SARS-CoV-2 proteins, including ORF1ab, S, N, M, and ORF3a, were observed. As shown in **FIG. 14D**, 3 of the 29 epitopes were located in the spike protein. Most epitopes (15 of 29) were located in ORF1ab, and the highest density of epitopes were located in the N protein. Shared epitopes in the S protein for HLA-A*02:01, HLA-A*03:01, and HLA-A*24:02 but not for HLA-A*01:01, HLA-A*11:01, or HLA-B*07:02 were observed. Only one recurrent response in the RBD of the S protein (KCY on HLA-A*03:01).

Example 9: Gene expression in a CRISPR engineered UVC

[0335] The expression levels of various proteins in the UVC was examined.

[0336] Using CRISPR, a NK ligand MICA was knocked in the UVC genome, and the B2M locus was knocked out to eliminate the expression of MHC-I. Flow cytometry analysis was used to examine the expression level of MICA and MHC-I in the UVC and the parental iPSC. As shown in **FIG. 15**, most cells in the UVC population showed a high level expression of MICA and minimal expression of MHC-I, compared to the control parent iPSC.

Example 10: Effective lysis of UVCs by NK cells

[0337] The UVC can induce effective lysis by the NK cell.

[0338] To measure cell lysis of the UVC, a flow cytometry-based NK cytotoxicity assay using calcein AM (CAM) staining of the NK cells, described in Jang et al., “An Improved Flow Cytometry-Based Natural Killer Cytotoxicity Assay Involving Calcein AM Staining of Effector Cells.” 2012, Ann. Clin. Lab. Sci. Winter;42(1):42-9, which is herein incorporated by reference in its entirety for all purposes, was used. Macaque NK cells (effector) were stained with CAM and seeded with a fixed number of MHC-I deficient (B2M KO) endothelial cells (EC) as target cells derived from the UVC iPSc. The cells were mixed with an E:T ratio of 1:1 or 5:1. Wildtype ECs were used as a control. Forward scatter profiles with CAM staining were used to distinguish the NK cells and the EC cells. Propidium iodide was used to detect the amount of dead EC cells. The percentage of cytotoxicity was scored as the percentage of dead cells in the total amount of

EC cells. As shown in **FIG. 16**, the NK cell induced an increased amount of lysis in B2M KO-EC than that of WT-EC in either E:T ratio.

[0339] Therefore, the UVC can induce effective lysis *in vitro* by the monkey NK cell.

Example 11: Additional responses from NK cells by NK ligands

[0340] The NK ligand can induce additional responses from the NK cell.

[0341] To show that the NK ligand can increase the NK cell, intracellular cytokine staining (ICS) was used to determine the expression of CD107a, MIP1- β , IFN- γ , or TNF- α in an MHC-I deficient UVC (KO), UVC transfected with a MICA expression construct (KO-MICA), UVC transfected with a MICB expression construct (KO-MICB), or UVC transfected with a ULBP1 expression construct (KO-ULBP1). Nucleofection was used to transfect the UVC. For the MICA and MICB construct, the transfection efficiency was about 40 to 70%. The expression construct drove a high level expression of the respective NK ligand in the transfected UVC. As shown in **FIG. 17A**, KO-MICA increased the total amounts of NK cells with CD107a or MIP1- β expression, while KO-MICA increased the total amounts of NK cells with CD107a expression, compared to that of KO. As illustrated in **FIG. 17B**, SPICE analysis shows that when responding to the UVC, MICA or MICB also increased the amount of NK cells expressing multiple cytokines. Addition of the NK ligand increases the NK cell response to the MHC-I deficient UVC.

Example 12: Cell surface expression of SARS-CoV-2 spike antigens on UVC cells

[0342] The UVC has a robust expression of the SARS-CoV-2 spike protein.

[0343] The SARS-CoV-2 spike protein knock-in construct and a MICA knock-in constructed were integrated into the genome of the UVC iPSC with B2M knocked out (B2m $-/-$). As shown in **FIG. 18A**, almost half of the engineered UVC iPSC population expressed a high amount of the spike protein. The level of the spike protein expression in the UVC iPSC was similar to HEK293T cells with transient transfection of a spike protein expression construct, as shown in **FIG. 18B**.

[0344] Multivalent antigens (*e.g.*, other SARS-CoV-2 variant spike proteins such as the RSA variant listed in **SEQ ID NO: 53**; or other proteins such as the nucleotplasmid proteins listed in **SEQ ID NO: 54**) can also be engineered in the UVC.

Example 13: UVC Non-Human Primate (NHP) Pilot -1 study

[0345] 6 monkeys negative for SARS-CoV-2 were administered B2M knock-out, MICA knock-in UVC expressing SARS-CoV-2 spike protein, variant or domain thereof. **FIG. 19A** and **FIG. 19B** show the results of antibody ELISA performed at 0, 2, 6, and 8 weeks post vaccination for both the receptor binding domain (RBD) (**FIG. 19A**) and the full-length SARS-CoV-2 spike protein (**FIG. 19B**).

[0346] RBD-specific and full-length SARS-CoV-2 spike protein-specific binding antibodies were assessed by ELISA as previously described in Chandrashekar, A. et al., Science 369, 812–817 (2020) and Yu, J. et al., Science 369, 806–811 (2020). In brief, 96-well plates were coated with $1\ \mu\text{g ml}^{-1}$ SARS-CoV-2 RBD or full-length protein (A. Schmidt, MassCPR) in $1\times$ DPBS and incubated at $4\ ^\circ\text{C}$ overnight. After incubation, plates were washed once with wash buffer (0.05% Tween 20 in $1\times$ DPBS) and blocked with $350\ \mu\text{l}$ casein block per well for 2–3 h at room temperature. After incubation, block solution was discarded and plates were blotted dry. Serial dilutions of heat-inactivated serum diluted in casein block were added to wells and plates were incubated for 1 h at room temperature, before three further washes and a 1-h incubation with a 1:1,000 dilution of anti-macaque IgG HRP (NIH NHP Reagent Program) at room temperature in the dark. Plates were then washed three times, and $100\ \mu\text{l}$ of SeraCare KPL TMB SureBlue Start solution was added to each well; plate development was halted by the addition of $100\ \mu\text{l}$ SeraCare KPL TMB Stop solution per well. The absorbance at 450 nm was recorded using a VersaMax or Omega microplate reader.

CLAIMS

What Is Claimed Is:

1. A genetically engineered human cell comprising:
 - a. a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene; and
 - b. an exogenous nucleic acid encoding a cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell.
2. The genetically engineered human cell of claim 1, wherein said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell.
3. The genetically engineered human cell of claim 2, wherein said genomic disruption completely inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said genetically engineered human cell.
4. The genetically engineered human cell of any one of claims 1-3, wherein said genomic disruption is in an HLA class I gene.
5. The genetically engineered human cell of claim 4, wherein said HLA class I gene is an HLA-A gene, HLA-B gene, HLA-C gene, or β -microglobulin gene.
6. The genetically engineered human cell of claim 5, wherein said HLA class I gene is a β -microglobulin gene.
7. The genetically engineered human cell of any one of claims 1-3, wherein said genomic disruption is in an HLA class II gene.
8. The genetically engineered human cell of claim 7, wherein said HLA class II gene is an HLA-DP gene, HLA-DM gene, HLA-DOA gene, HLA-DOB gene, HLA-DQ gene, HLA-DR gene.
9. The genetically engineered human cell of any preceding claim, wherein said at least one transcriptional regulator of said HLA gene is a CIITA gene, RFX5 gene, RFXAP gene, or RFXANK gene.
10. The genetically engineered human cell of claim 9, wherein said HLA gene is a CIITA gene.
11. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell comprises a genomic disruption in at least one HLA class I gene or said at least one transcriptional regulator of said HLA class I gene and a genomic disruption in at least one HLA class II gene or said at least one transcriptional regulator of said HLA class II gene.

12. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell comprises a genomic disruption in at least one HLA class I transcriptional regulator gene and a genomic disruption in at least one HLA class II transcriptional regulator.
13. The genetically engineered human cell of any preceding claim, wherein said immune cell is an innate immune cell.
14. The genetically engineered human cell of claim 13, wherein said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil.
15. The genetically engineered human cell of claim 14, wherein said innate immune cell is an NK cell.
16. The genetically engineered human cell of claim 15, wherein said binding results in the activation of cytolytic activity of said NK cell.
17. The genetically engineered human cell of claim 14 or 15, wherein said cell surface protein is a ligand that specifically binds to a natural killer (NK) cell activating receptor expressed on the surface of an NK cell.
18. The genetically engineered human cell of claim 14 or 15, wherein said cell surface protein is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, Necl-2, and immunoglobulin Fc.
19. The genetically engineered human cell of claim 14 or 15, wherein said cell surface protein is a natural killer (NK) cell activating ligand.
20. The genetically engineered human cell of claim 14 or 15, wherein said natural killer cell activating ligand is selected from the group consisting of MICA, MICB, ULBP1, ULBP2, ULBP3, ULBP4, ULBP5, ULBP6, CD155, CD112 (Nectin-2), B7-H6, and Necl-2.
21. The genetically engineered human cell of any preceding claim, wherein said cell comprises an exogenous nucleic acid encoding a secretory protein that binds to a receptor expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said secretory protein, wherein said protein attracts said immune cell towards said genetically engineered human cell.
22. The genetically engineered human cell of any preceding claim, further comprising a nucleic acid encoding an exogenous protein, an antigenic fragment thereof, or a suicide gene.
23. The genetically engineered human cell of claim 22, wherein said exogenous protein comprises a microbial protein.
24. The genetically engineered human cell of claim 23, wherein said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

25. The genetically engineered human cell of claim 23, wherein said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.
26. The genetically engineered human cell of claim 23 or 25, wherein said microbial protein is a viral, bacterial, parasitic, or protozoa protein.
27. The genetically engineered human cell of claim 26, wherein said microbial protein is a viral protein.
28. The genetically engineered human cell of claim 27, wherein said viral protein is of a virus of order Nidovirales.
29. The genetically engineered human cell of claim 27 or 28, wherein said viral protein is of a virus of family Coronaviridae.
30. The genetically engineered human cell of any one of claims 27-29, wherein said viral protein is of a virus of subfamily Orthocoronavirinae.
31. The genetically engineered human cell of any one of claims 27-30, wherein said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus.
32. The genetically engineered human cell of claim 31, wherein said viral protein is of a virus of genus Betacoronavirus.
33. The genetically engineered human cell of any one of claims 27-32, wherein said viral protein is of a virus of subgenus Sarbecovirus.
34. The genetically engineered human cell of any one of claims 27-33, wherein said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2.
35. The genetically engineered human cell of any one of claims 27-34, wherein said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2.
36. The genetically engineered human cell of any one of claims 27-35, wherein said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2.
37. The genetically engineered human cell of any one of claims 27-36, wherein said viral protein is a spike protein of SEQ ID NO: 1.
38. The genetically engineered human cell of any one of claims 27-36, wherein said viral protein is a spike protein encoded by SEQ ID NO: 53.
39. The genetically engineered human cell of claim 27, wherein said viral protein is of a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster

virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.

40. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell is differentiated from a stem cell.

41. The genetically engineered human cell of claim 40, wherein said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC).

42. The genetically engineered human cell of claim 40, wherein said stem cell is an induced pluripotent stem cell (iPSC).

43. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell is an epithelial cell or endothelial cell.

44. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell is not a cancer cell.

45. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell has been irradiated.

46. The genetically engineered human cell of any one of claims 1-44, wherein said genetically engineered human cell is a stem cell.

47. The genetically engineered human cell of claim 46, wherein said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC).

48. The genetically engineered human cell of claim 47, wherein said stem cell is an induced pluripotent stem cell (iPSC).

49. The genetically engineered human cell of any preceding claim, wherein said genetically engineered human cell is incapable of proliferation in vitro, in vivo, or both.

50. The genetically engineered human cell of any preceding claim, for use in a vaccine.

51. The genetically engineered human cell of any preceding claim, wherein said at least one genomic disruption is mediated by an endonuclease.

52. The genetically engineered human cell of claim 51, wherein said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN).

53. The genetically engineered human cell of any preceding claim, wherein said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA

sequence of said at least one HLA gene or at least one transcriptional regulator of said HLA gene.

54. A genetically engineered human cell comprising:

- a. a genomic disruption in at least one human leukocyte antigen (HLA) gene or at least one transcriptional regulator of an HLA gene;
- b. a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and
- c. a nucleic acid encoding an exogenous antigenic protein, or an antigenic fragment thereof.

55. The genetically engineered human cell of claim 54, wherein said exogenous antigenic protein, or antigenic fragment thereof, is a microbial protein, or an antigenic fragment thereof.

56. The genetically engineered human cell of claim 55, wherein said exogenous antigenic protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

57. The genetically engineered human cell of claim 55, wherein said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

58. The genetically engineered human cell of claim 55 or 57, wherein said microbial protein is a viral, bacterial, parasitic, or protozoa protein.

59. The genetically engineered human cell of claim 58, wherein said microbial protein is a viral protein.

60. The genetically engineered human cell of claim 59, wherein said viral protein is of a virus of order Nidovirales.

61. The genetically engineered human cell of claim 59 or 60, wherein said viral protein is of a virus of family Coronaviridae.

62. The genetically engineered human cell of any one of claims 59-61, wherein said viral protein is of a virus of subfamily Orthocoronavirinae.

63. The genetically engineered human cell of any one of claims 59-62, wherein said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus.

64. The genetically engineered human cell of claim 63, wherein said viral protein is of a virus of genus Betacoronavirus.

65. The genetically engineered human cell of any one of claims 59-64, wherein said viral protein is of a virus of subgenus Sarbecovirus.
66. The genetically engineered human cell of any one of claims 59-65, wherein said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2.
67. The genetically engineered human cell of any one of claims 59-66, wherein said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2.
68. The genetically engineered human cell of any one of claims 59-67, wherein said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2.
69. The genetically engineered human cell of any one of claims 59-68, wherein said viral protein is a spike protein of SEQ ID NO: 1.
70. The genetically engineered human cell of any one of claims 59-68, wherein said viral protein is a spike protein encoded by SEQ ID NO: 53.
71. The genetically engineered human cell of claim 59, wherein said viral protein is from a virus selected from a group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.
72. The genetically engineered human cell of any one of claims 54-71, wherein said genetically engineered human cell is differentiated from a stem cell.
73. The genetically engineered human cell of claim 72, wherein said stem cell is an induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC).
74. The genetically engineered human cell of claim 73, wherein said stem cell is an induced pluripotent stem cell (iPSC).
75. The genetically engineered human cell of any one of claims 54-74, wherein said genetically engineered human cell is an epithelial cell or endothelial cell.
76. The genetically engineered human cell of any one of claims 54-75, wherein said genetically engineered human cell is not a cancer cell.
77. The genetically engineered human cell of any one of claims 54-76, wherein said genetically engineered human cell has been irradiated.
78. The genetically engineered human cell of any one of claims 54-77, for use in a vaccine.
79. The genetically engineered human cell of any one of claims 54-78, wherein said immune cell is an innate immune cell.

80. The genetically engineered human cell of claim 79, wherein said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil.
81. The genetically engineered human cell of claim 80, wherein said innate immune cell is an NK cell.
82. A composition comprising a population of genetically engineered human cells of any one of claims 1-81.
83. A pharmaceutical composition comprising said genetically engineered human cell of any one of claims 1-81 or said composition of claim 82, and an excipient.
84. A unit dosage form comprising said composition of claim 82 or 83.
85. A method of making a population of genetically engineered human stem cells, said method comprising:
obtaining a population of human stem cells;
inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene; and
introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell;
to thereby produce a population of genetically engineered stem cells.
86. The method of claim 85, wherein said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell.
87. The method of claim 85 or 86, wherein said genomic disruption inhibits expression of an HLA protein encoded by said at least one HLA gene on the surface of said cell for a period of time sufficient to interact with a protein expressed on the surface of an immune cell.
88. The method of any one of claims 85-87, wherein said at least one genomic disruption is mediated by an endonuclease.
89. The method of claim 88, wherein said endonuclease is a CRISPR endonuclease, a Zinc finger nuclease (ZFN), or a transcription activator-Like Effector Nuclease (TALEN).
90. The method of any one of claims 85-89, wherein said at least one genomic disruption is mediated by a CRISPR system that comprises an endonuclease and a guide RNA (gRNA), wherein said gRNA comprises an RNA sequence complementary to a DNA sequence of said at least one HLA gene or at least one transcriptional regulator of an HLA gene.
91. The method of any one of claims 85-90, wherein said genomic disruption is a single strand DNA break or a double strand DNA break.

92. The method of any one of claims 85-91, further comprising introducing a nucleic acid encoding a microbial protein, or an antigenic fragment thereof.
93. The method of claim 92, wherein said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.
94. The method of claim 92, wherein said microbial protein is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.
95. The method of claim 92 or 94, wherein said microbial protein is a viral, bacterial, or parasitic protein.
96. The method of claim 95, wherein said microbial protein is a viral protein.
97. The method of any one of claims 85-96, wherein said stem cells are induced pluripotent stem cell (iPSC), an embryonic stem cell (ESC), an adult stem cell (ASC), a pluripotent stem cell (PSC), or a hematopoietic stem and progenitor cell (HSPC).
98. The method of claim 97, wherein said stem cells are induced pluripotent stem cell (iPSC).
99. The method of any one of claims 85-98, further comprising differentiating said population of genetically engineered human stem cells.
100. The method of claim 99, wherein said cells are differentiated into epithelial cells or endothelial cells.
101. The genetically engineered human cell of any one of claims 85-100, wherein said immune cell is an innate immune cell.
102. The method of claim 101, wherein said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil.
103. The method of claim 102, wherein said innate immune cell is an NK cell.
104. A method of making a population of terminally differentiated genetically engineered human cells, said method comprising:
obtaining a population of human stem cells;
inducing a genomic disruption in at least one HLA gene or at least one transcriptional regulator of said HLA gene;
introducing a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell, thereby producing a population of genetically engineered human stem cells; and

differentiating said population of genetically engineered human stem cells into a population of terminally differentiated genetically engineered human cells.

105. The method of claim 104, wherein said population of genetically engineered human stem cells are differentiated into epithelial cells or endothelial cells.

106. A method of immunizing a human subject against a microbe, said method comprising administering to said subject said genetically engineered human cell of any one of claims 1-81, said composition of claim 82, or said pharmaceutical composition of claim 83.

107. A method of immunizing a human subject against a microbe, said method comprising administering to said subject a population of genetically engineered human cells comprising:

- a. a genomic disruption in at least one HLA gene or at least one transcriptional regulator of an HLA gene;
- b. a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell; and
- c. a nucleic acid encoding a microbial protein, or an antigenic fragment thereof.

108. The method of claim 107, wherein said binding results in immune cell mediated lysis or phagocytosis of at least a portion of said population of genetically engineered human cells.

109. The method of claim 107 or 108, wherein said administering results in said subject mounting an adaptive immune response against said microbe.

110. The method of any one of claims 107-109, wherein said administering results in an increase in activation and/or proliferation of T cells that express a T cell receptor that specifically binds said microbial protein or an antigenic fragment thereof.

111. The method of any one of claims 107-110, wherein said administering results in an increase in activation and/or proliferation of B cells that express a B cell receptor that specifically binds said microbial protein or an antigenic fragment thereof.

112. The method of any one of claims 107-111, wherein said administering results in an increase in circulating antibodies that specifically bind said microbial protein or antigenic fragment thereof.

113. The method of any one of claims 107-112, wherein said microbial protein or antigenic fragment thereof, is secreted by said genetically engineered human cell, expressed on the surface of said genetically engineered human cell, or expressed within the cytoplasm of said genetically engineered human cell.

114. The method of any one of claims 107-113, wherein said microbial protein is a viral, bacterial, or parasitic protein.

115. The method of claim 114, wherein said microbial protein is a viral protein.
116. The method of claim 115, wherein said viral protein is of a virus of family Coronaviridae.
117. The method of any one of claims 115-116, wherein said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus.
118. The method of any one of claims 115-117, wherein said viral protein is of a virus of genus Betacoronavirus.
119. The method of any one of claims 115-118, wherein said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2.
120. The method of any one of claims 115-119, wherein said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2.
121. The method of any one of claims 115-120, wherein said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2.
122. The method of any one of claims 115-121, wherein said viral protein is a spike protein of SEQ ID NO: 1.
123. The method of any one of claims 115-121, wherein said viral protein is a spike protein encoded by SEQ ID NO: 53.
124. The method of claim 115, wherein said viral protein is from a virus selected from the group that comprises: influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, and any combination thereof.
125. The method of any one of claims 107-124, wherein said population of genetically engineered human cells are administered intramuscularly or subcutaneously.
126. The genetically engineered human cell of any one of claims 107-125, wherein said immune cell is an innate immune cell.
127. The method of claim 126, wherein said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil.
128. The method of claim 127, wherein said innate immune cell is an NK cell.
129. The method of any one of claims 107-128, wherein said genetically engineered human cells further comprise a suicide gene.
130. The method of claim 107, wherein said microbial protein comprises a nucleocapsid phosphoprotein comprising at least about 85% sequence identity to SEQ ID NO: 54.

131. A method of immunizing a subject, said method comprising administering to said subject a population of genetically engineered mammalian cells comprising:

- a. a genomic disruption in at least one MHC gene or at least one transcriptional regulator of an MHC gene, wherein said disruption results in a reduction of activation of T cell proliferation compared to said genetically engineered human cell without said disruption; and
- b. a nucleic acid encoding an exogenous cell surface protein that binds to a protein expressed on the surface of a phagocytic or cytolytic immune cell, or a functional fragment or functional variant of said exogenous cell surface protein, wherein said binding results in the activation of phagocytic or cytolytic activity of said immune cell.

132. The method of claim 131, wherein said immunizing is specific for an antigen, and wherein said genetically engineered mammalian cells further comprise a nucleic acid encoding the antigen or a fragment thereof.

133. The method of claim 131, wherein said immunizing is specific for an antigen, and wherein said genetically engineered mammalian cells further comprise the antigen or a fragment thereof.

134. The method of any one of claims 131-133, wherein said activation results in immune cell mediated lysis or phagocytosis of at least a portion of said population of genetically engineered mammalian cells.

135. The method of any one of claims 132-134, wherein said administration results in said subject mounting an adaptive immune response against said antigen.

136. The method of any one of claims 132-135, wherein said administration results in an increase in activation and/or proliferation of T cells that express a T cell receptor that specifically binds a peptide of said antigen.

137. The method of any one of claims 132-134, wherein said administration results in an increase in activation and/or proliferation of B cells that express a B cell receptor that specifically binds a peptide of said antigen.

138. The method of any one of claims 132-137, wherein said administration results in an increase in circulating antibodies that specifically bind said antigen.

139. The method of any one of claims 132-138, wherein said antigen is secreted by said genetically engineered mammalian cell, expressed on the surface of said genetically engineered mammalian cell, or expressed within the cytoplasm of said genetically engineered mammalian cell.

140. The method of any one of claims 132-139, wherein said antigen is a viral, bacterial, fungal, or parasitic protein.

141. The method of claim 140, wherein said viral protein is of a virus of family Coronaviridae.

142. The method of any one of claims 140-141, wherein said viral protein is of a virus of genus Alphacoronavirus, Betacoronavirus, Gammacoronavirus, or Deltacoronavirus.
143. The method of any one of claims 140-141, wherein said viral protein is of a virus of genus Betacoronavirus.
144. The method of any one of claims 141-143, wherein said viral protein is of a virus of species severe acute respiratory syndrome-related coronavirus 2.
145. The method of any one of claims 141-144, wherein said viral protein is of a virus of strain severe acute respiratory syndrome coronavirus 2.
146. The method of any one of claims 141-145, wherein said viral protein is a spike protein of severe acute respiratory syndrome coronavirus 2.
147. The method of any one of claims 141-146, wherein said viral protein is a spike protein of SEQ ID NO: 1.
148. The method of any one of claims 141-146, wherein said viral protein is a spike protein encoded by SEQ ID NO: 53.
149. The method of claim 140, wherein said viral protein is from a virus selected from the group that comprises at least one of influenza, Epstein-Barr virus (EBV), mega virus, Norwalk virus, coxsackie virus, middle east respiratory syndrome-related coronavirus, severe acute respiratory syndrome-related coronavirus, SARS-Cov-2 virus, hepatitis B, varicella zoster virus, parvovirus, adenovirus, Marburg virus, Ebola virus, Rabies, Smallpox, HIV, Hantavirus, Dengue, Rotavirus, MERS-CoV, mumps virus, cytomegalovirus (CMV), Herpes virus, papillomavirus, chikungunya virus, or any combination thereof.
150. The method of any one of claims 132-139, wherein said antigen comprises a protein or peptide associated with a cancer or a tumor.
151. The method of claim 150, wherein said antigen comprises a neoantigen.
152. The method of any one of claims 131-151, wherein said population of genetically engineered cells are administered intramuscularly or subcutaneously.
153. The genetically engineered cell of any one of claims 131-152, wherein said immune cell is an innate immune cell.
154. The method of claim 153, wherein said innate immune cell is an NK cell, a macrophage, a dendritic cell, a neutrophil, or an eosinophil.
155. The method of claim 153, wherein said innate immune cell is an NK cell.
156. The method of any one of claims 131-155, wherein said genetically engineered mammalian cells further comprise a suicide gene.

157. The method of any one of claims 131-156, wherein said genetically engineered mammalian cells comprise genetically engineered human cells, and wherein said MHC gene comprises an HLA gene.

>YP_009724390.1 surface glycoprotein [Severe acute respiratory syndrome coronavirus 2]

MFVFLVLLPLVSSQCVNLTTRTQLPPAYTNSFTRGVYYPDKVERSSVLHSTQDLFLPFFSNVTWEHA
 IHVSGTNGTKBFNDNPVLPFNDGVYFASTEKSNTIRGWIFGTTLDSKTQSLIIVMNATNVVTKVCEFO
 FCNDPFLGVYYHKNNKSWMESEFRVYSSANNCTFEYVSQPFLLMDLEGKQGNFKNLREFVFKNIDCYF
 KIYSKHTPINLVRDLPOCFSALEPLVDLPICGINITRFQTLALHRSYLTIPGDSSSSGWTAGAAAYYVG
 YLOPRTFELLKYNENGTITDAVDCALDPLSETKCTLKSFTVEKGIYQTSNFRVQPTESIVKFPNITNL
 CPPGEVFNATRFASVYAWNRKRISKCVADYSVLNSASFTEKCYGVSPTKLNLICTFNKYADSFTV
 RGDEVRLIAPGQITCKIADYNYKLPDDFTGCVIAWNSNNLDSKVGGNYNYLYRLERKSNLKPFERDIS
 TEIYQAGSTPCNGVEGFNCYFPLOS YGFQPI NGVGYQFYRVVVLSPFELLHAPATVCGPKASTNLVKN
 KCVNENFNGLTGTGVLTESNKKFLPFQQFGRDIADTTDAVRDPQTLEILDITPCSFGGVSVITPGTN
 TSNOVAVLYQDVNCTEVPVAIHADQLTPTWRVYSTGSNVFQTRAGCLIGAHEVNNSEYCDIPIGAGI
 CASYQTQTNSPRRARSVASQSIIAYTMSLGAENSVAYSNNISIAIPTNFTISVTTEILPVSMTKTSVD
 CTMYICGDSTEC SNLLQYGSFCTQLNRALTGIAVEQDKNTQEVFAQVKOLYKTPPIKDEGGFNFSQ
 ILPDPSKPSKPSFIEDLLFNKVTIADAGFIKQYGDCLGDIAARDLICAQKFNGLTVLPPLLTDEMA
 QYTSALLAGTITSGWTFGAGAALQIPFAMQMAYRFNGIGVTQNVLYENOKLIANQFNSAIGKIQDSL
 SSTASALGKLQDVVNQNAQALNTLVKOLSSNFGAISSVLNDILSRLD
 VVPCQLIRAAELIRAPFLAATKKEVLRQSKRVDFCGKGYHLMSFPQSAPHGVVFLHVTVYVPAQE
 KNF
 IAPALCHDSNAREPREGVVSNGIHWFTQRMFYEFILITIDMTFVSNGCDVVICIVHNTVYE
 PLOPELDSFKEELDKYFKNHTSPQVDELGDISGNASVNTCKETDRLNEVAKNLNESITLQELGKY
 EOTIFKWPYIWLCPASLIATVMVTIMLCMTSSSNIPAEPCSSC
 KPFEDTQEPHIGSKH

NTD RBD S1/S2 FP HR1 HR2 HR3

FIG. 1

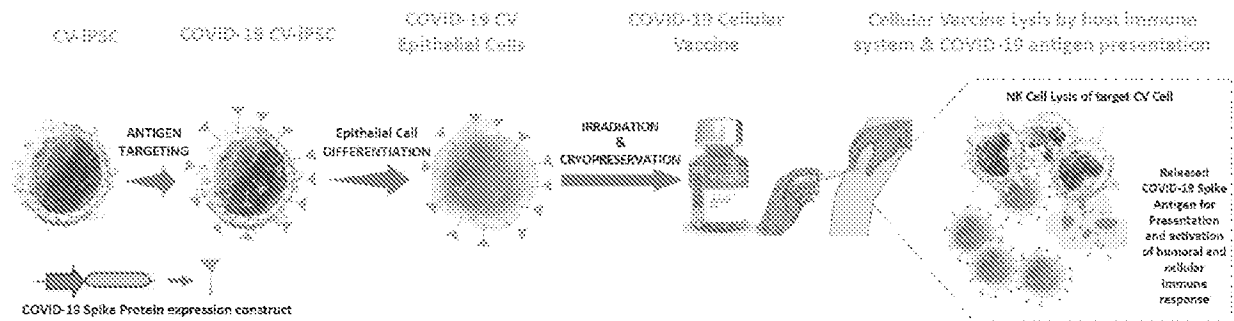
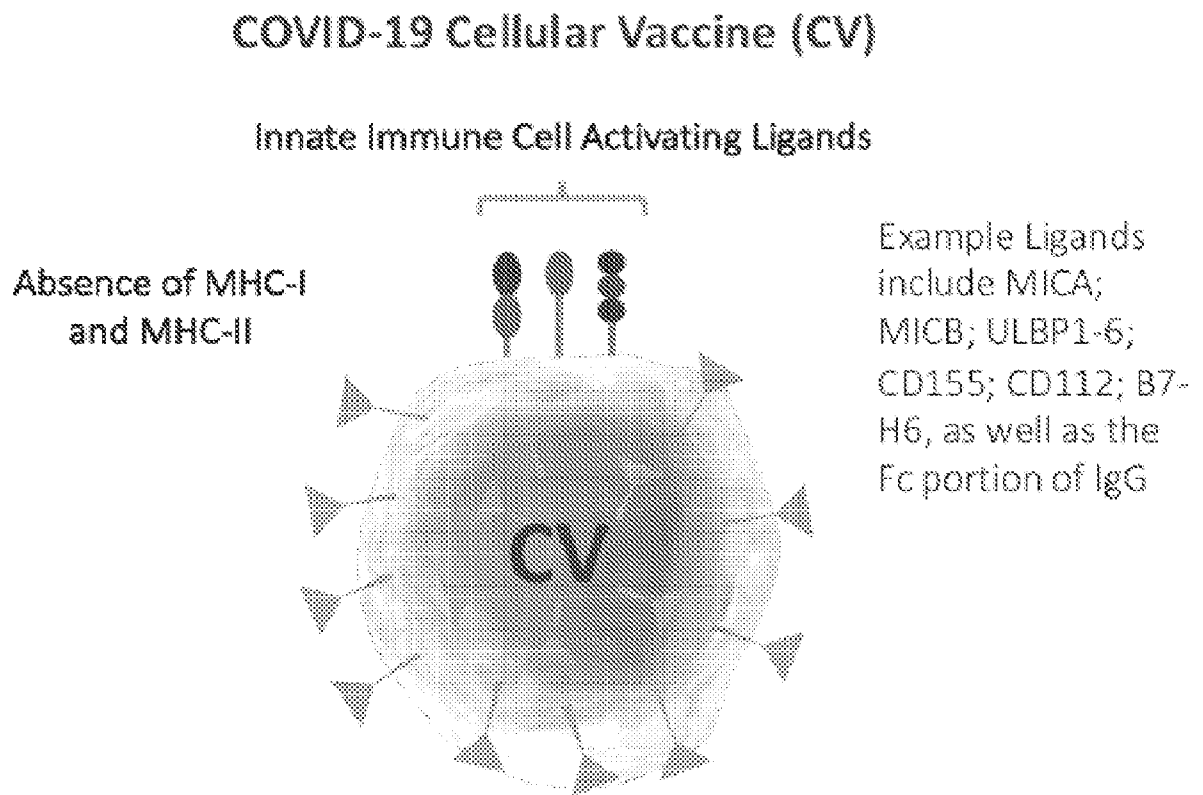


FIG. 2

**FIG. 3**

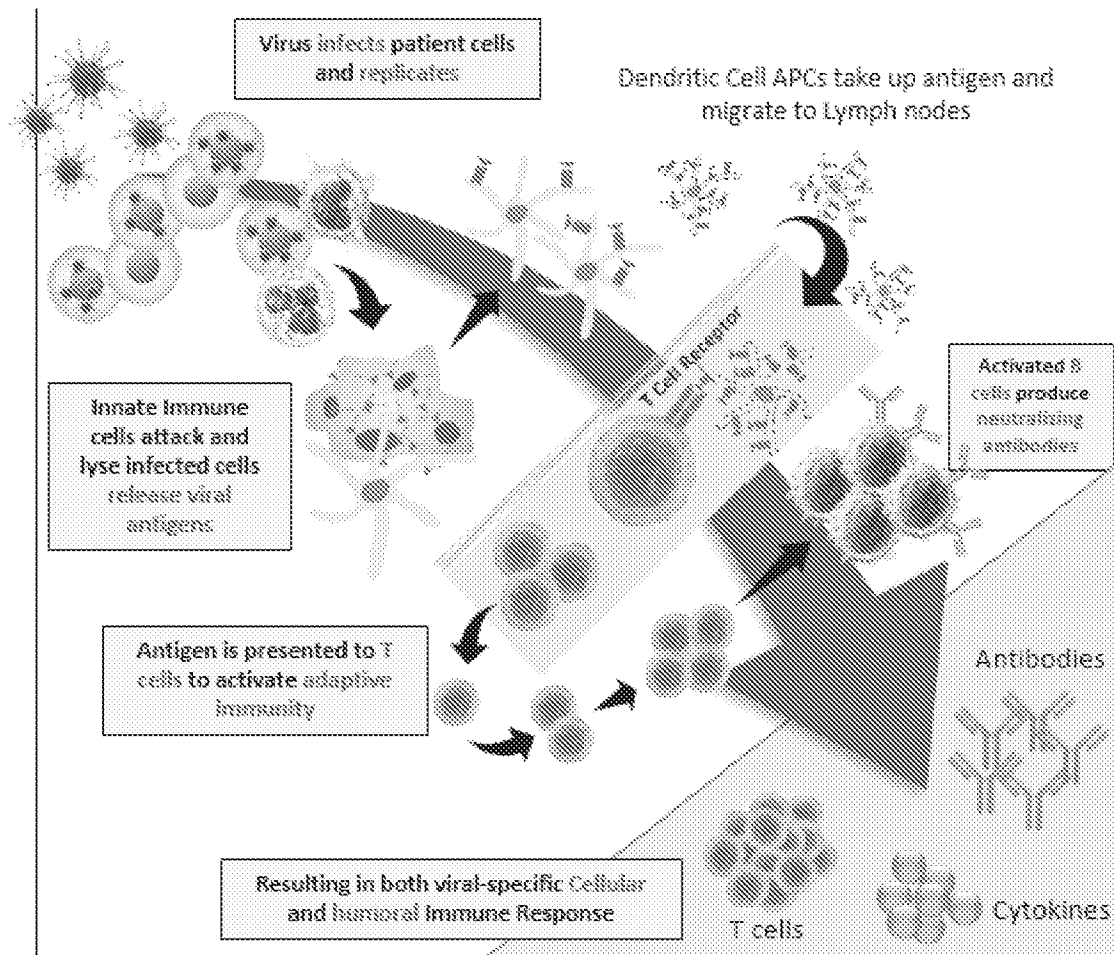
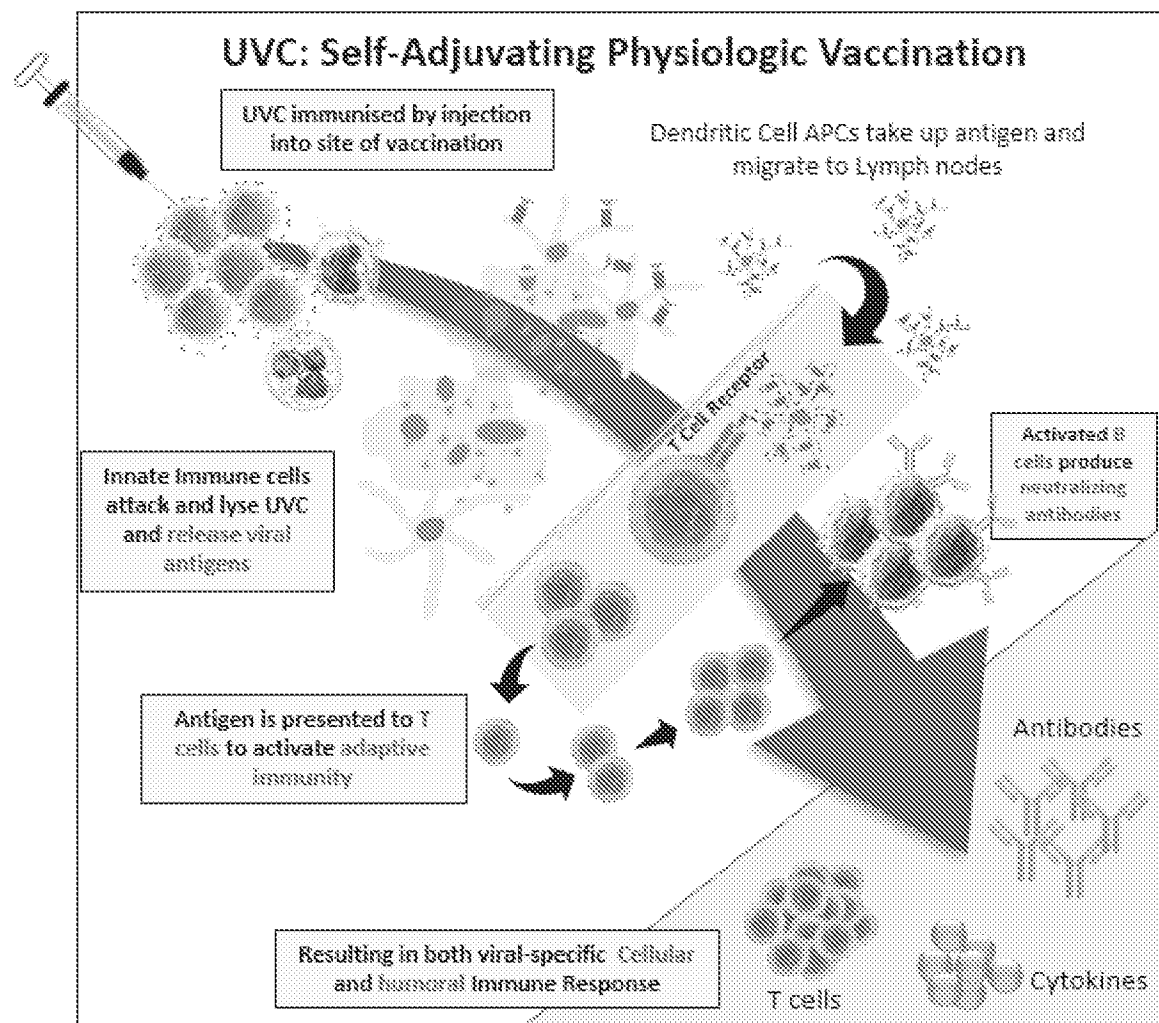


FIG. 4A

**FIG. 4B**

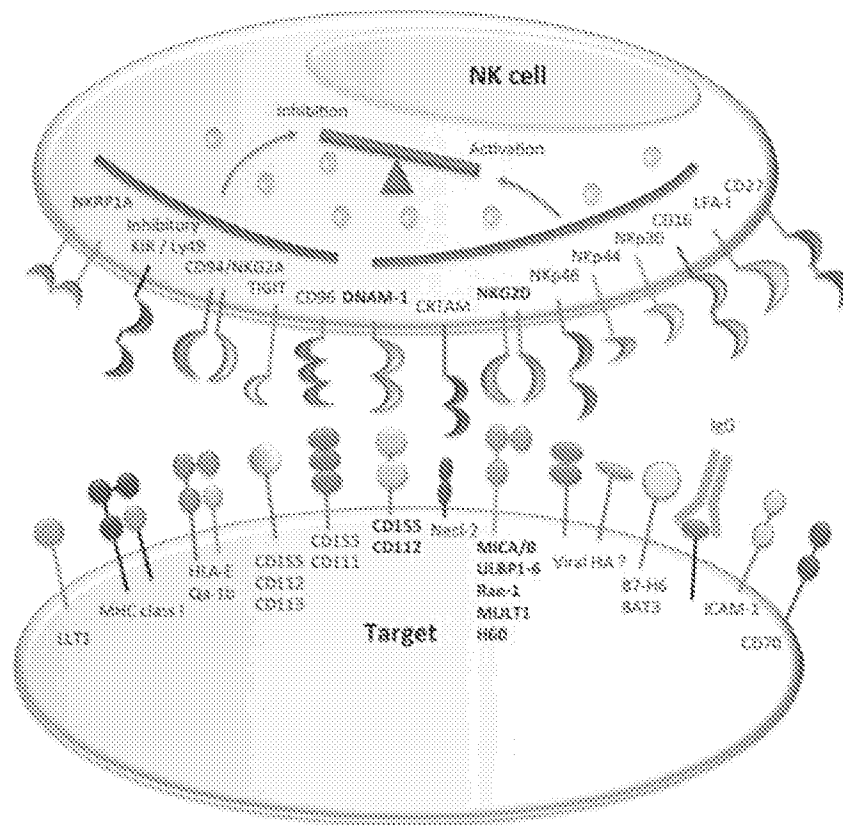


FIG. 5

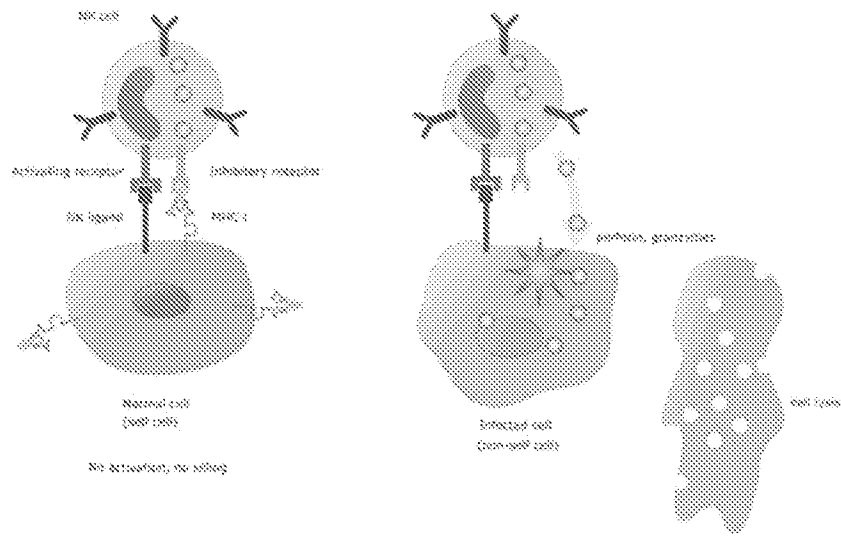


FIG. 6

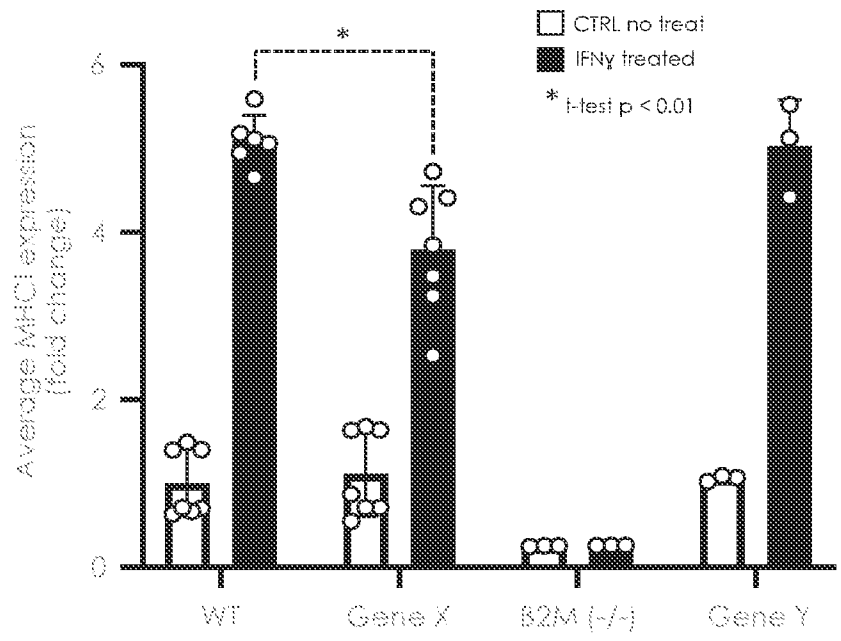
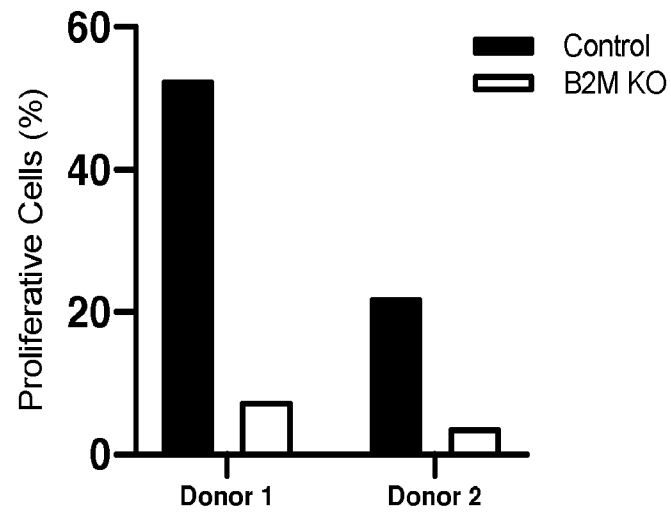
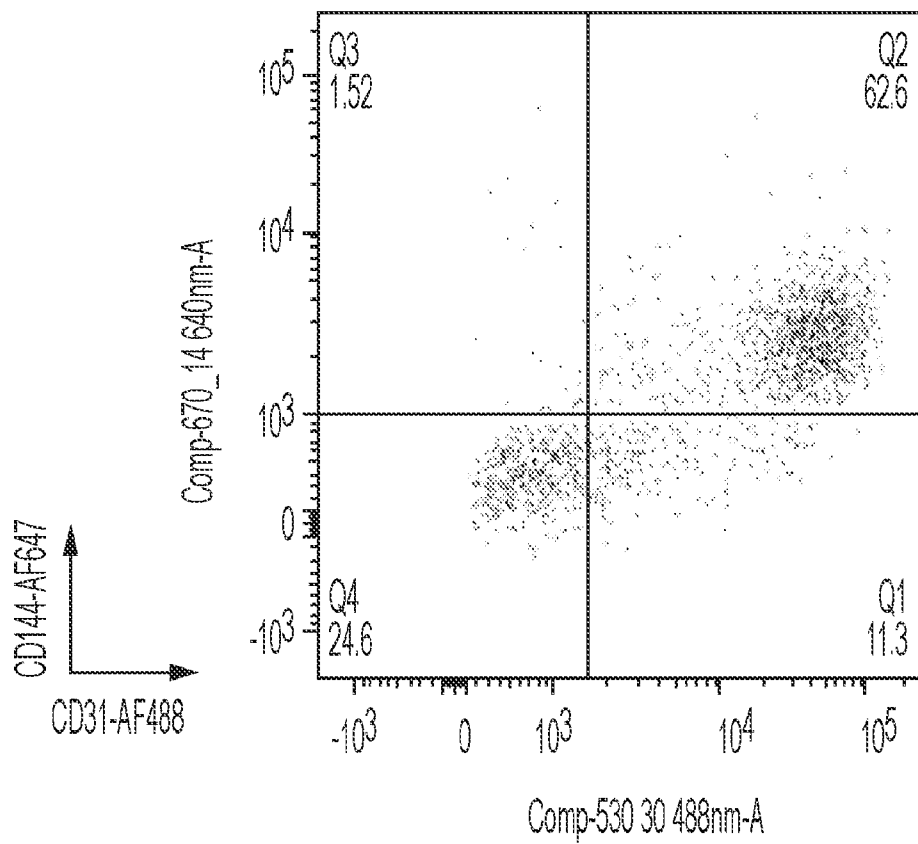


FIG. 7

**FIG. 8A****FIG. 8B**

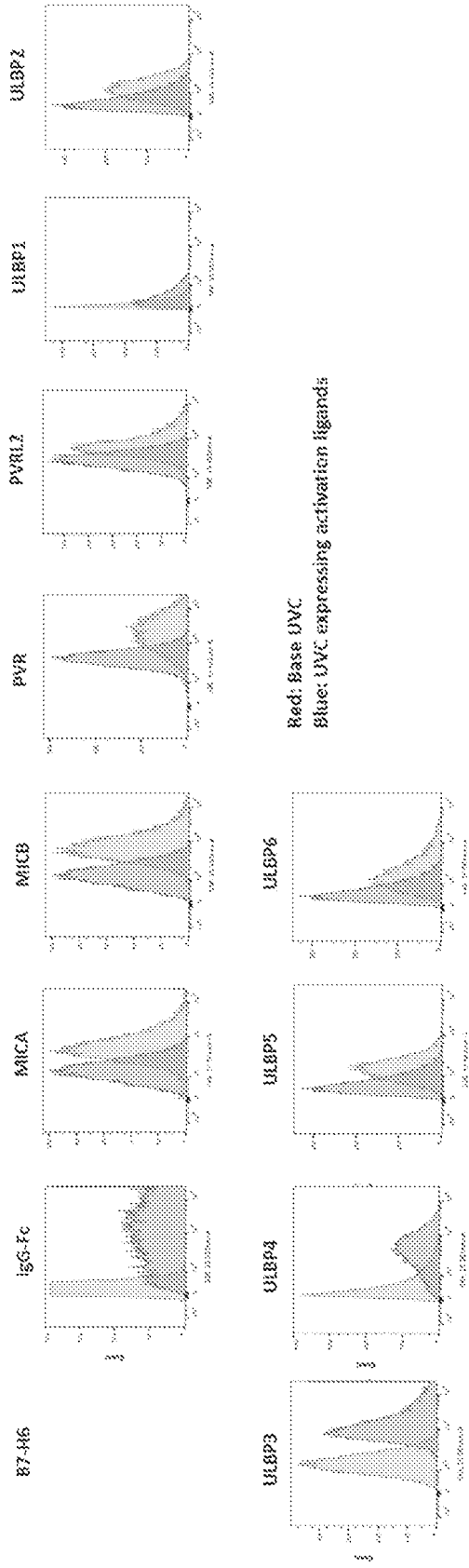


FIG. 9

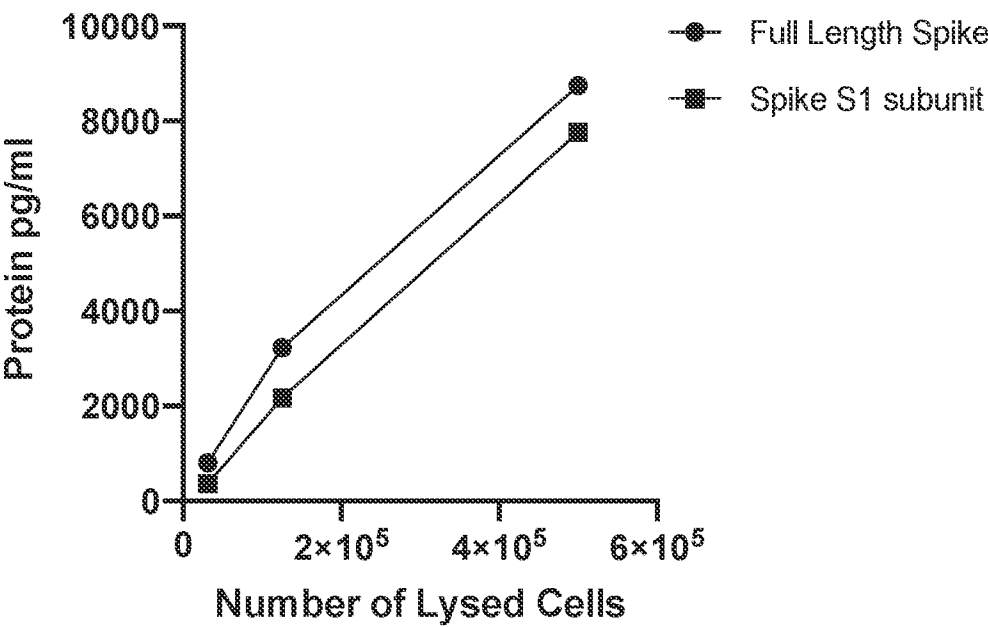


FIG. 10

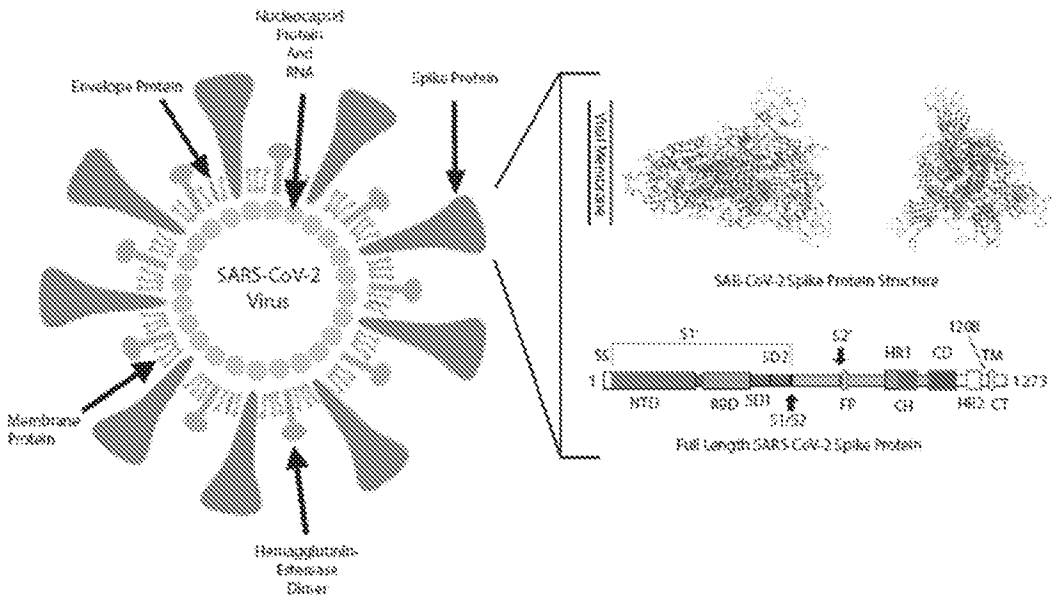
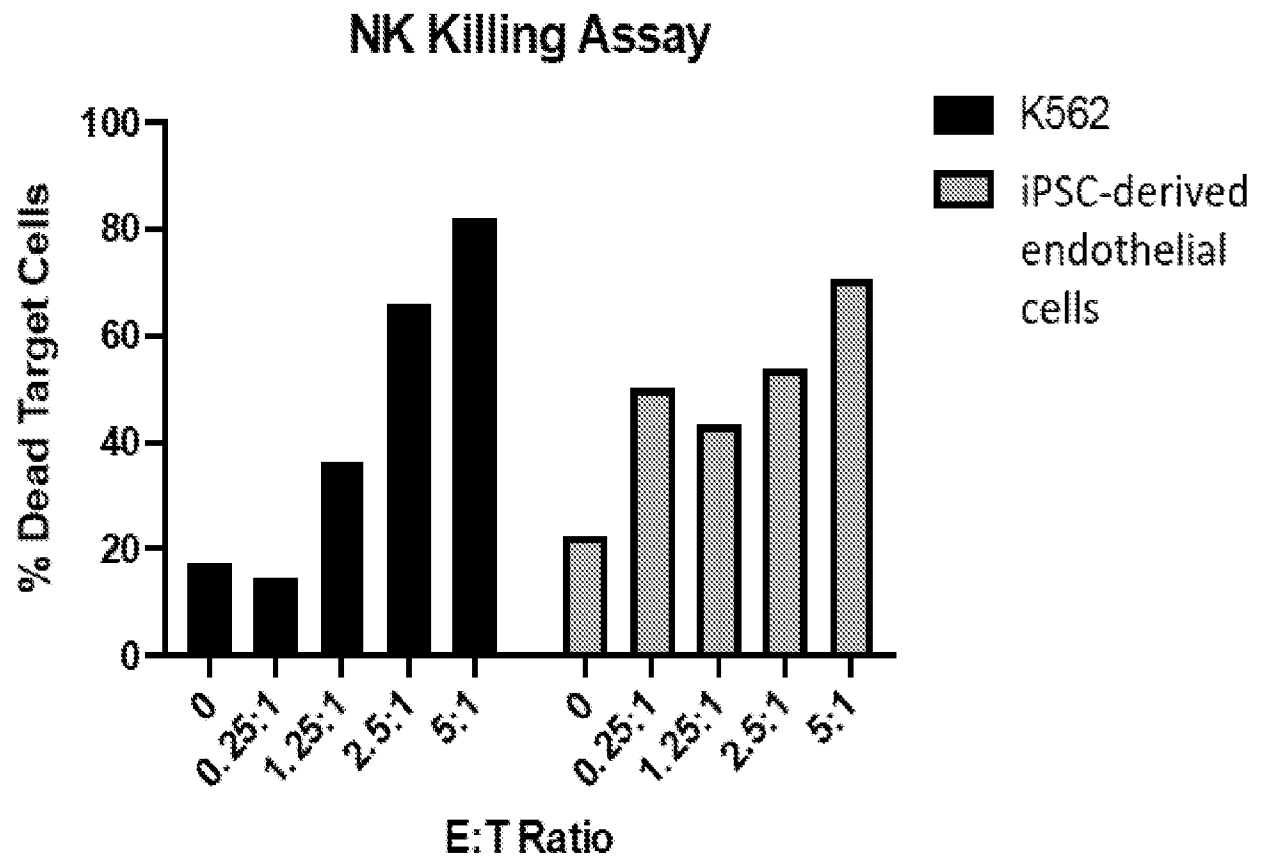


FIG. 11

**FIG. 12**

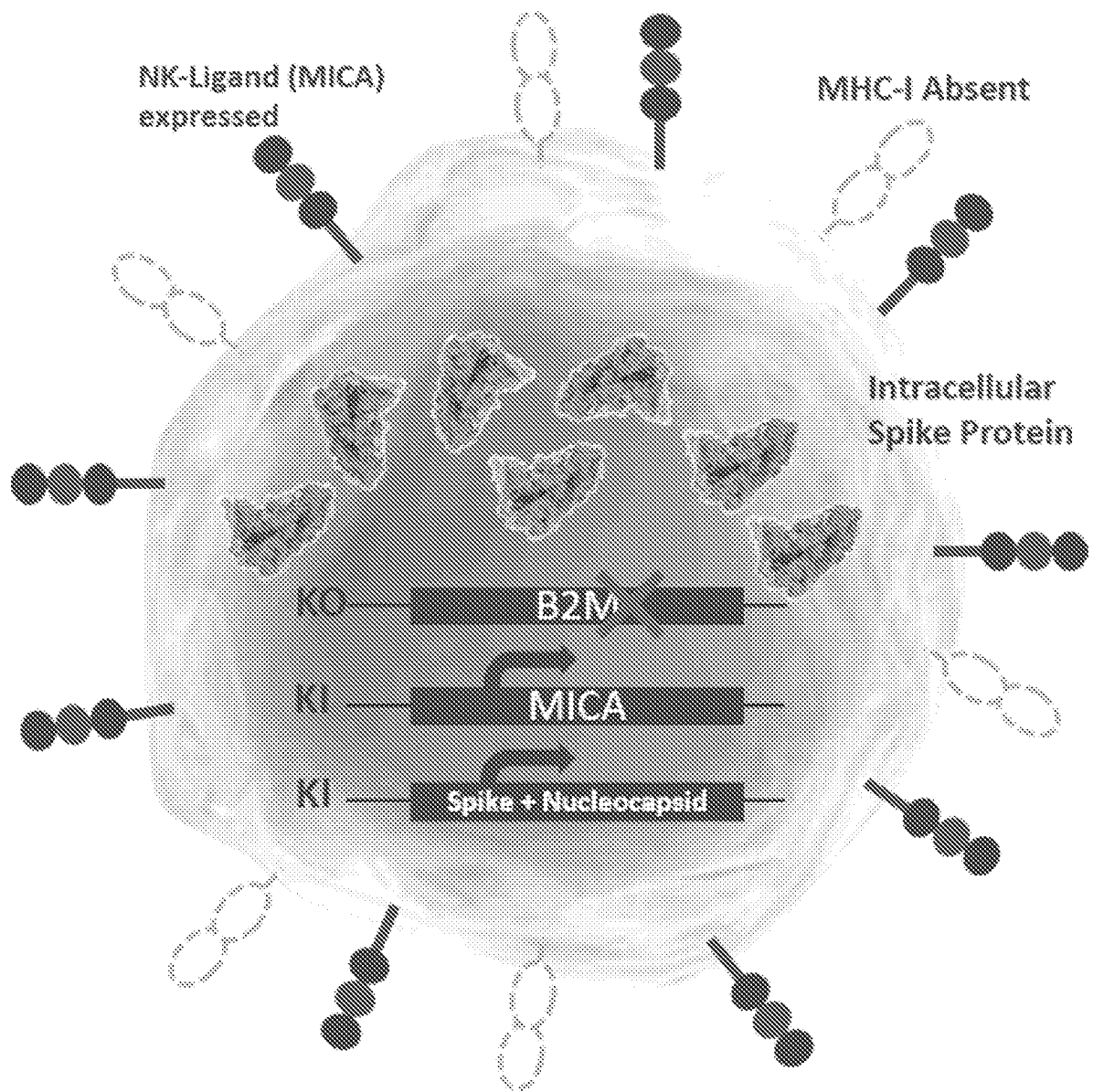


FIG. 13

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FIG. 14A

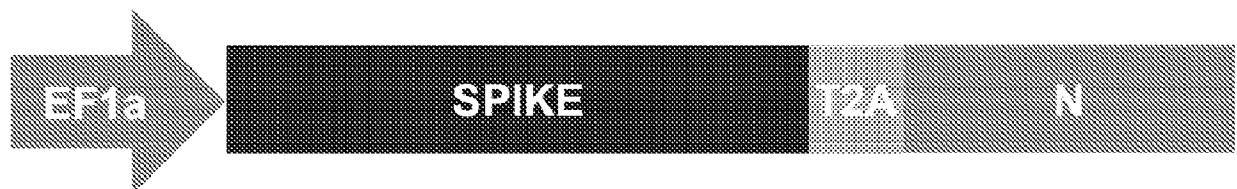


FIG. 14B

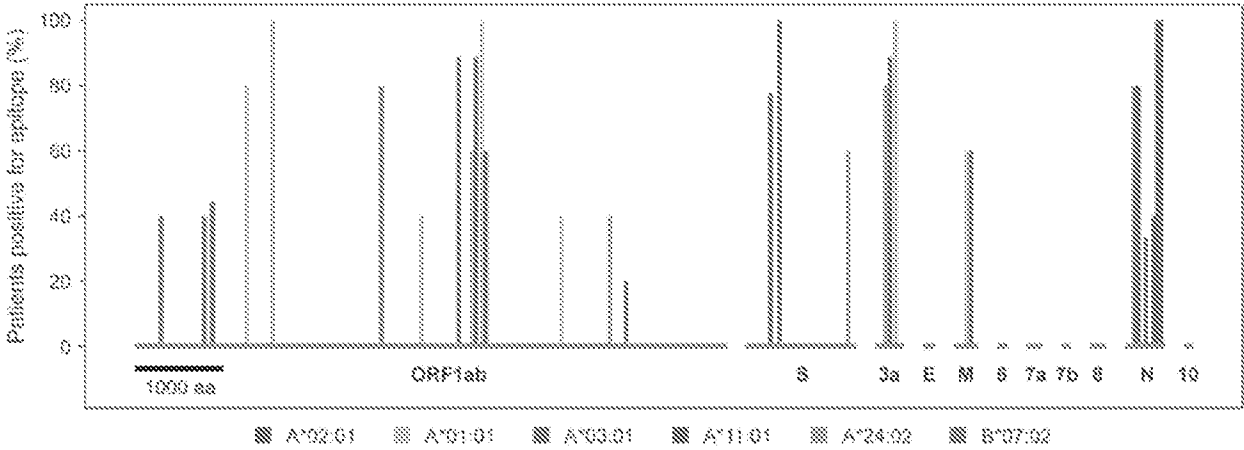


FIG. 14C

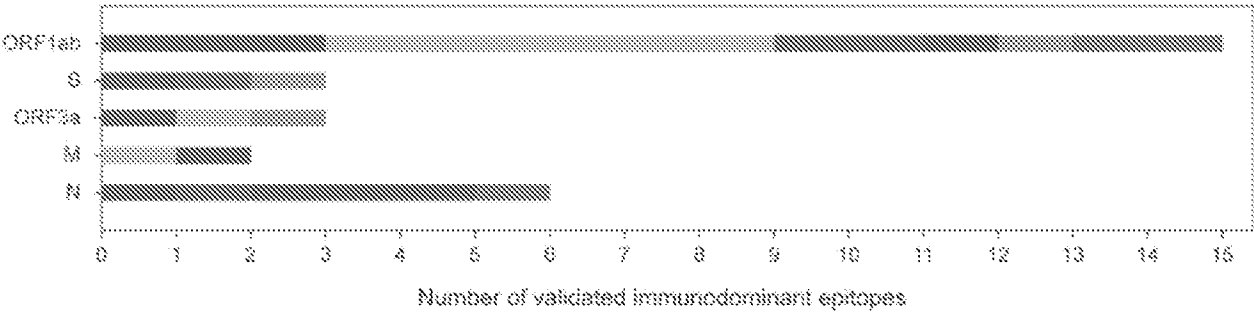


FIG. 14D

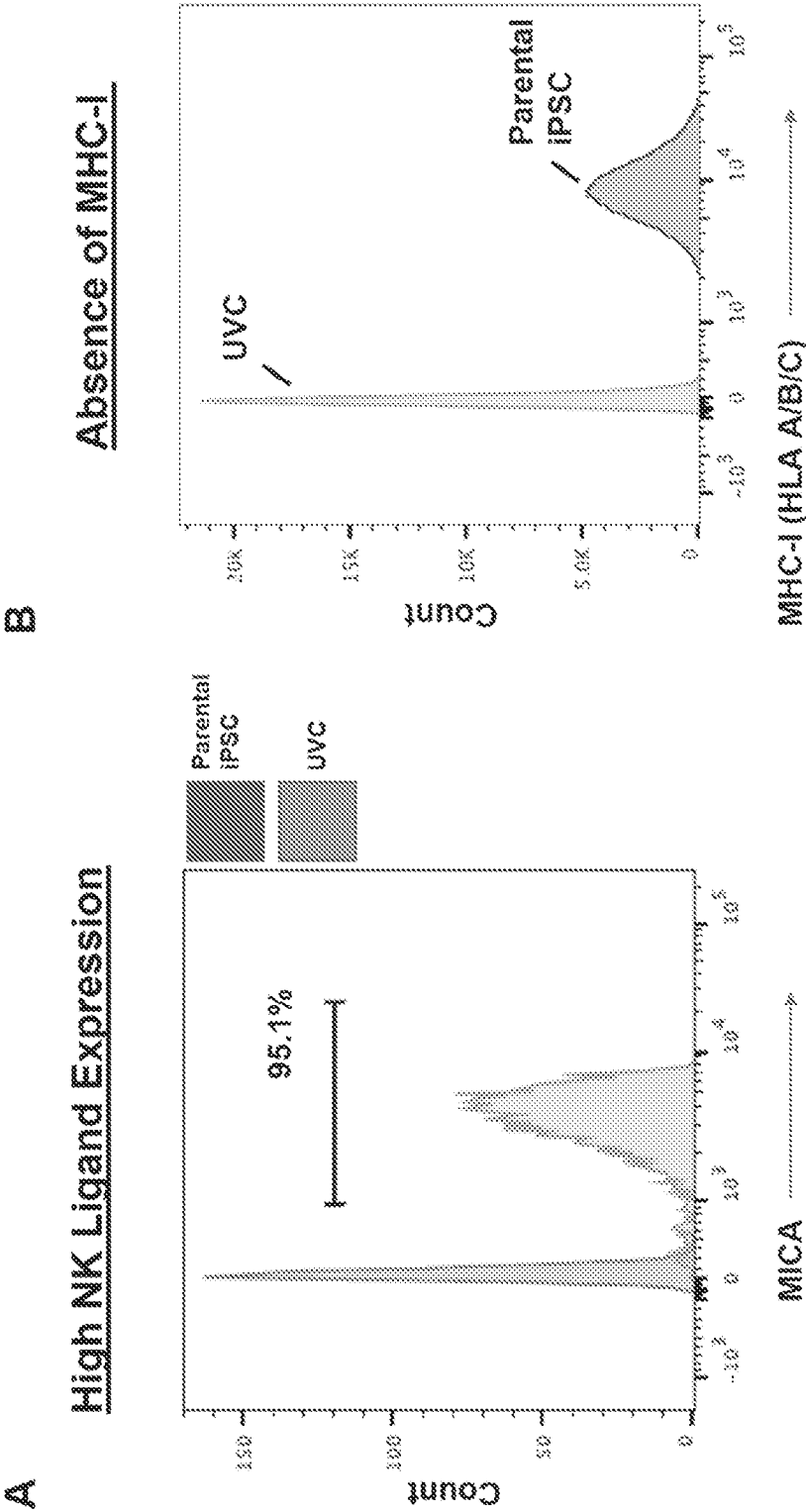
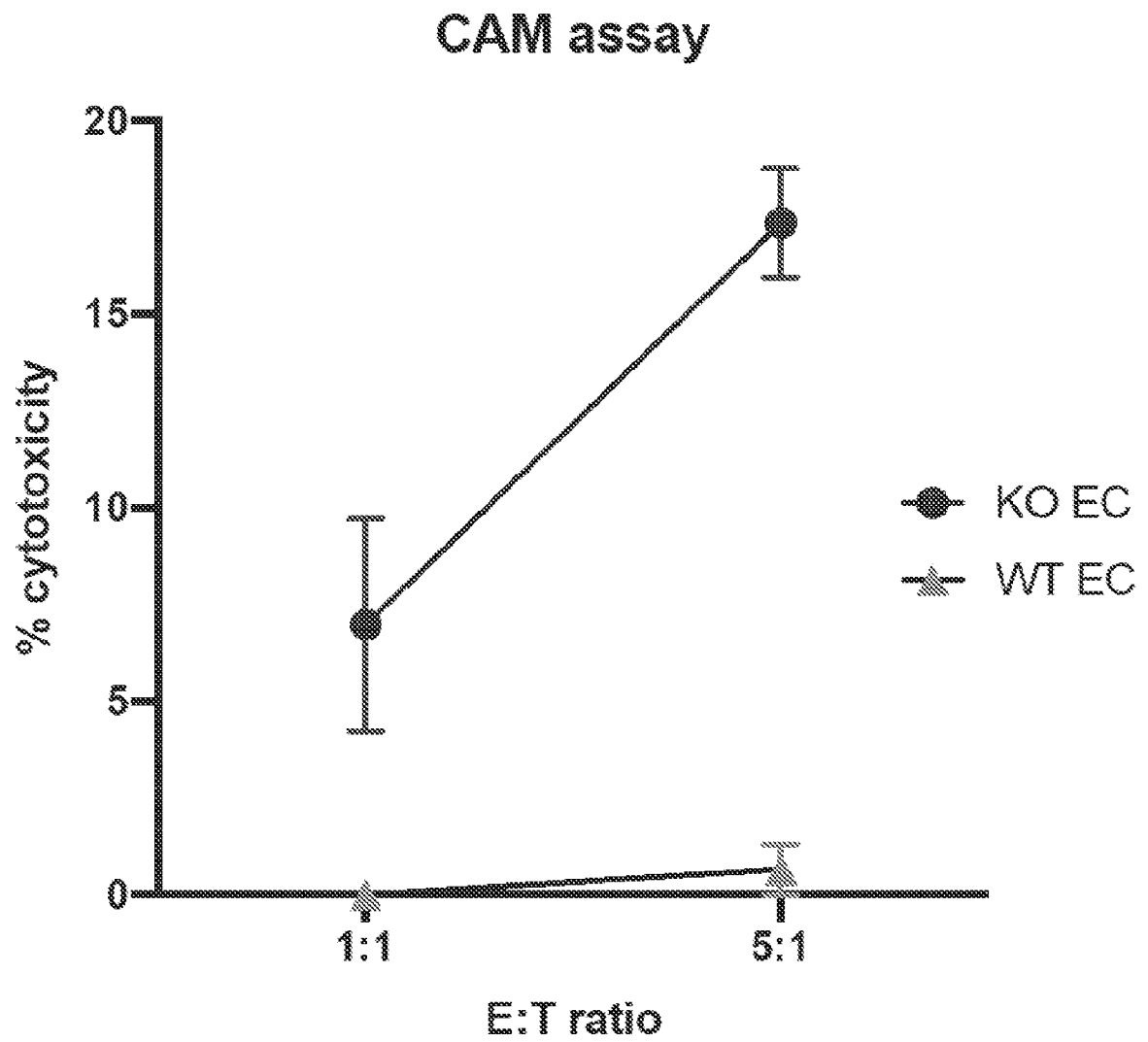


FIG. 15

**FIG. 16**

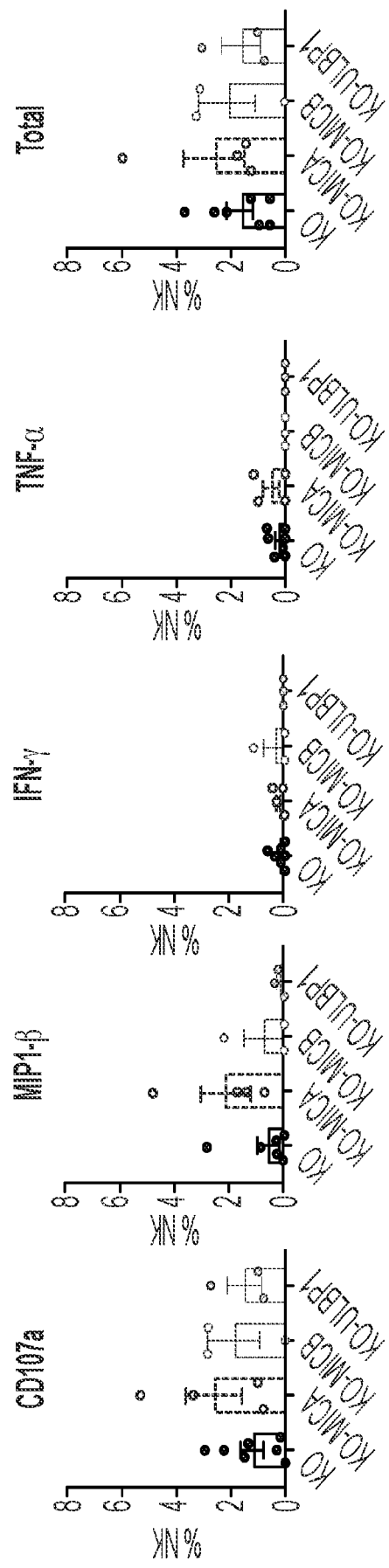


FIG. 17A

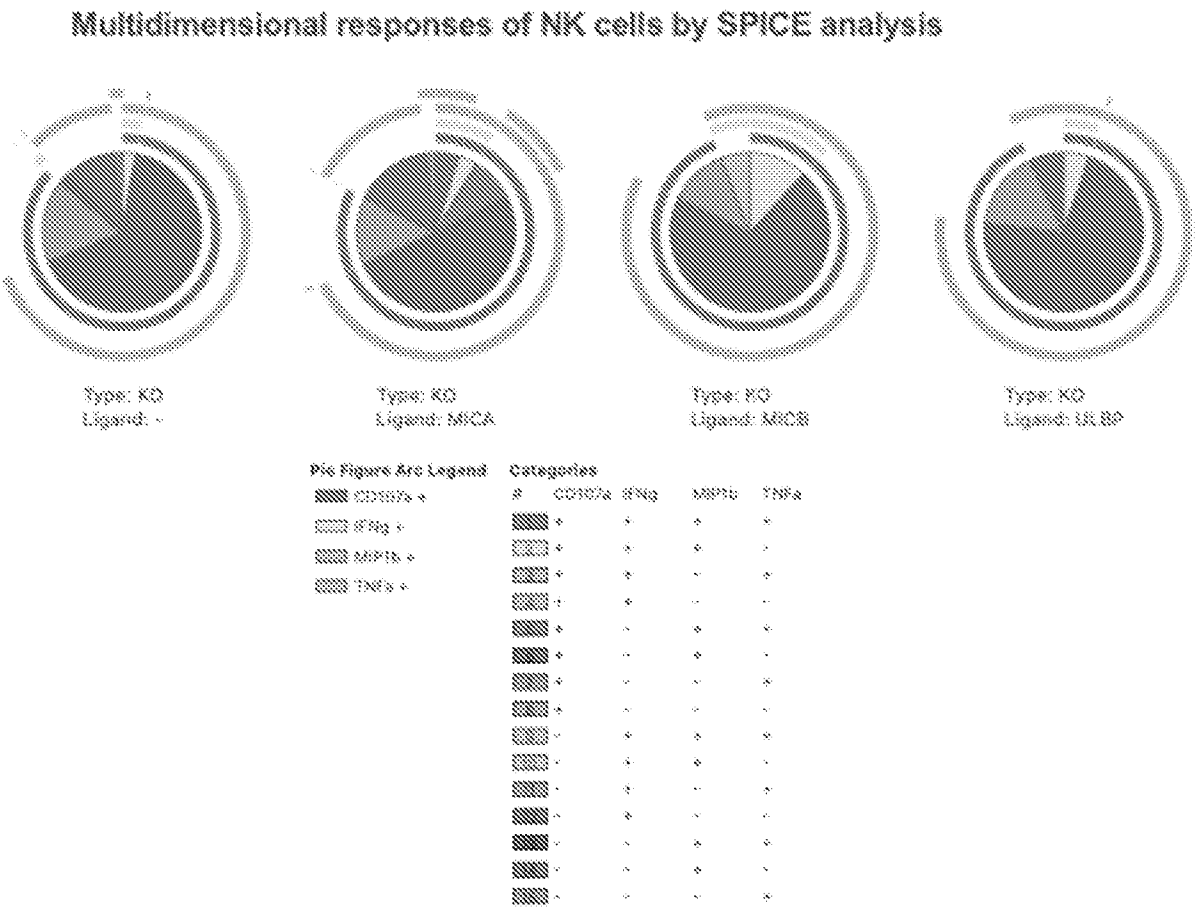


FIG. 17B

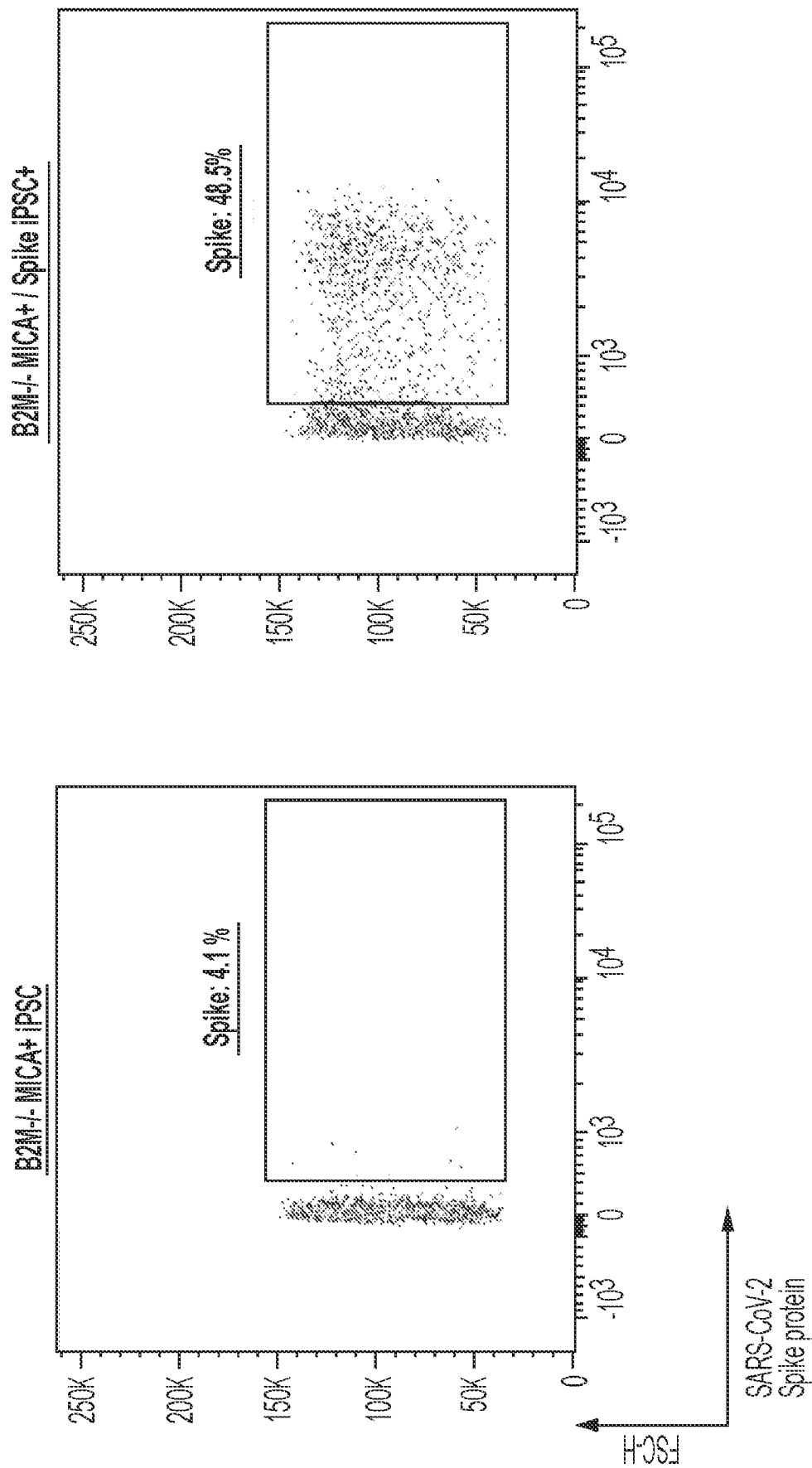


FIG. 18A

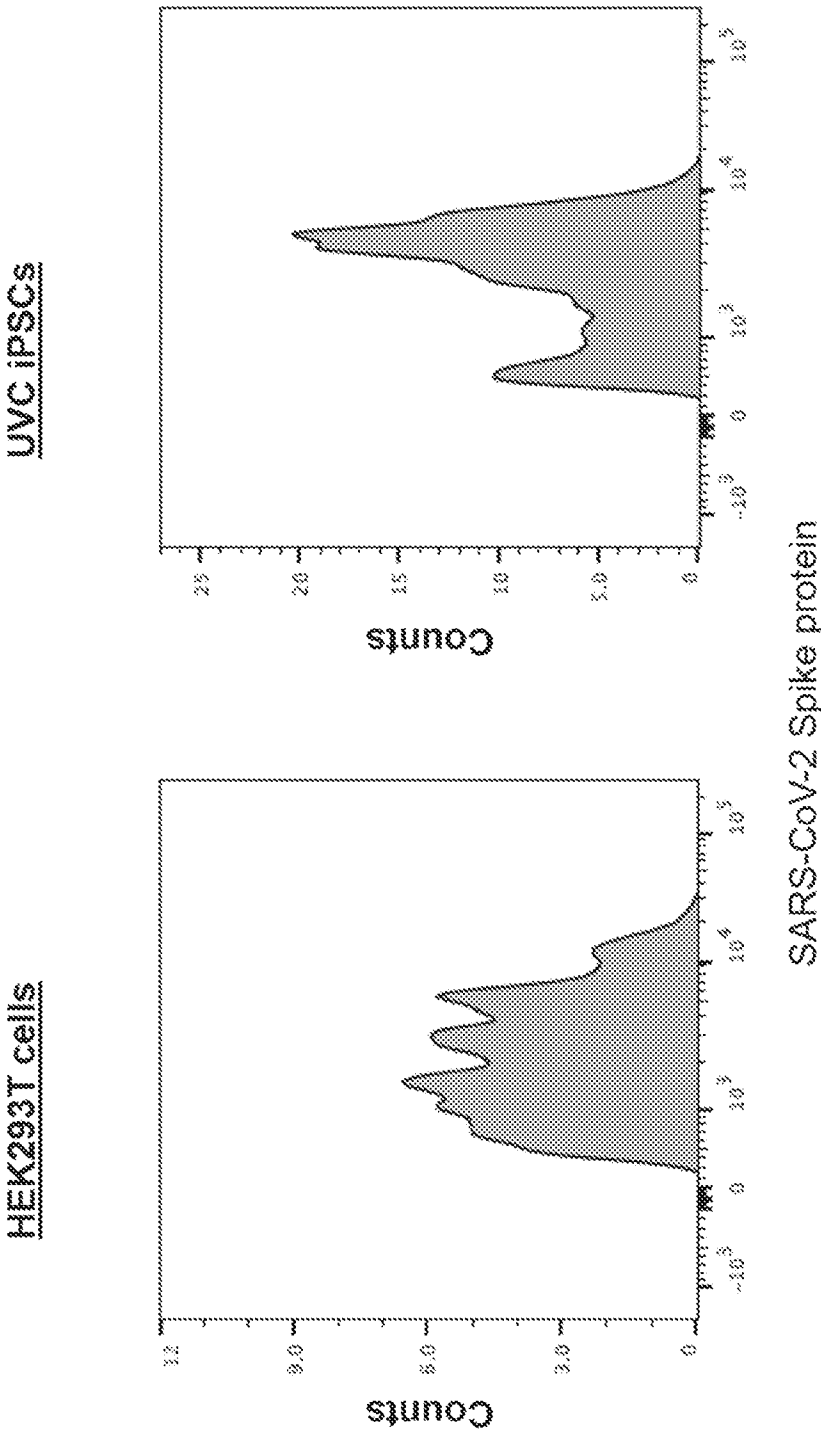


FIG. 18B

Dilution:	20-34 RBD wk2						20-34 RBD wk8					
	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD
1:25	0.0705	0.0765	0.0685	0.1975	0.064	0.0686	0.0667	0.0751	0.0648	0.1253	0.0675	0.075
1:75	0.0603	0.0626	0.0569	0.0654	0.0566	0.0581	0.058	0.0601	0.0557	0.0773	0.1272	0.0619
1:225	0.058	0.0574	0.0566	0.0635	0.0377	0.0393	0.0614	0.0584	0.0576	0.0642	0.0639	0.0778
1:675	0.0545	0.054	0.0532	0.0598	0.0601	0.0621	0.0575	0.0553	0.0537	0.0578	0.0565	0.0617
1:2025	0.0559	0.0544	0.0556	0.054	0.0585	0.06	0.0578	0.0568	0.0541	0.0592	0.057	0.0616
1:6075	0.056	0.0556	0.0573	0.0584	0.0588	0.063	0.06	0.0601	0.0597	0.0598	0.0606	0.0599
1:18225	0.059	0.0587	0.0605	0.0584	0.0628	0.0568	0.0605	0.0587	0.0609	0.0607	0.0772	0.063
1:54675	0.0705	0.0689	0.0578	0.059	0.0597	0.0574	0.0576	0.0589	0.0577	0.0614	0.0616	0.0601
EPT:	25	25	25	25	25	25	25	25	25	25	25	25
Dilution:	20-34 RBD wk6						20-34 RBD wk8					
	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD
1:25	0.0783	0.0859	0.0769	0.0908	0.065	0.0679	0.0721	0.075	0.0648	0.0861	0.0692	0.0857
1:75	0.071	0.0687	0.0663	0.0679	0.0595	0.0671	0.0588	0.0625	0.0579	0.066	0.0592	0.0622
1:225	0.0662	0.065	0.0618	0.0641	0.0582	0.0583	0.0594	0.0587	0.0545	0.0628	0.054	0.0571
1:675	0.0677	0.0643	0.0612	0.0599	0.0575	0.0593	0.0555	0.0586	0.0546	0.0609	0.055	0.0557
1:2025	0.0671	0.0636	0.0613	0.0601	0.0595	0.0572	0.0556	0.0576	0.0539	0.0615	0.0542	0.0583
1:6075	0.07	0.0645	0.0617	0.06	0.0557	0.0597	0.0571	0.0579	0.0551	0.058	0.0566	0.0591
1:18225	0.0561	0.0628	0.0571	0.0556	0.0553	0.0576	0.0543	0.0551	0.0547	0.055	0.0569	0.0577
1:54675	0.072	0.0664	0.0676	0.0604	0.0583	0.0612	0.0547	0.0565	0.0521	0.0597	0.0591	0.0628
EPT:	25	25	25	25	25	25	25	25	25	25	25	25

FIG. 19A

Dilution:	20-34 FLED wk2						20-34 FLED wk0					
	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD
1:25	0.0731	0.0804	0.0714	0.0906	0.0762	0.0893	0.0687	0.0941	0.0687	0.0839	0.0673	0.1586
1:75	0.0601	0.0603	0.0571	0.0623	0.0595	0.063	0.058	0.068	0.0612	0.0648	0.0592	0.0884
1:225	0.0578	0.0574	0.0545	0.0561	0.0571	0.0595	0.0569	0.062	0.0587	0.0604	0.0573	0.0881
1:575	0.0574	0.0563	0.0531	0.0533	0.0563	0.0583	0.0533	0.0571	0.0588	0.0619	0.0811	0.0805
1:2025	0.0578	0.0557	0.0545	0.0546	0.055	0.0578	0.0559	0.0577	0.0545	0.0567	0.0562	0.0806
1:6075	0.0579	0.0549	0.0534	0.0547	0.0573	0.0577	0.0557	0.0567	0.058	0.0564	0.0562	0.0575
1:18225	0.0586	0.0583	0.1031	0.0581	0.0808	0.0823	0.050	0.0691	0.0585	0.059	0.0563	0.058
1:54675	0.0639	0.0522	0.06	0.0603	0.0819	0.0807	0.0604	0.0635	0.081	0.0611	0.0583	0.0811
EPT:	25	25	25	25	25	25	25	25	25	25	25	25
Dilution:	20-34 FLED wk6						20-34 FLED wk8					
	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD	BF842J	BF421EC	N435ME	YR352NH	BM342G	AE571JD
1:25	0.1062	0.1041	0.0955	0.1113	0.0968	0.1112	0.1059	0.1046	0.111	0.1286	0.1105	0.1278
1:75	0.0915	0.0826	0.0789	0.0843	0.0742	0.0821	0.0832	0.0806	0.0829	0.0905	0.0881	0.0986
1:225	0.0869	0.0793	0.0757	0.0773	0.0751	0.0746	0.0831	0.0797	0.0824	0.0876	0.0846	0.093
1:575	0.0782	0.0749	0.0758	0.0762	0.0715	0.0734	0.0774	0.0779	0.0816	0.0786	0.0805	0.0899
1:2025	0.0798	0.0782	0.0748	0.0735	0.0703	0.0733	0.0772	0.0787	0.0797	0.0815	0.0794	0.0869
1:6075	0.0782	0.0787	0.0764	0.0731	0.0703	0.0623	0.0735	0.0761	0.0766	0.0784	0.075	0.0843
1:18225	0.0794	0.0742	0.0722	0.0714	0.0701	0.0799	0.0758	0.0768	0.0714	0.0775	0.0722	0.0757
1:54675	0.0807	0.0927	0.0728	0.0729	0.0763	0.0799	0.0735	0.0739	0.0817	0.088	0.0798	0.0801
EPT:	25	25	25	25	25	25	25	25	25	25	25	25

FIG. 19B

SEQUENCE LISTING

<110> INTIMA BIOSCIENCE, INC.

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<130> 199827-757601PCT

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<150> 63/056,460

<151> 2020-07-24

<150> 63/013,387

<151> 2020-04-21

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<170> PatentIn version 3.5

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			20					25					30		

Thr	Arg	Gly	Val	Tyr	Tyr	Pro	Asp	Lys	Val	Phe	Arg	Ser	Ser	Val	Leu
		35					40					45			

His	Ser	Thr	Gln	Asp	Leu	Phe	Leu	Pro	Phe	Phe	Ser	Asn	Val	Thr	Trp
	50					55					60				

Phe	His	Ala	Ile	His	Val	Ser	Gly	Thr	Asn	Gly	Thr	Lys	Arg	Phe	Asp
65					70					75					80

Asn	Pro	Val	Leu	Pro	Phe	Asn	Asp	Gly	Val	Tyr	Phe	Ala	Ser	Thr	Glu
			85						90					95	

Lys	Ser	Asn	Ile	Ile	Arg	Gly	Trp	Ile	Phe	Gly	Thr	Thr	Leu	Asp	Ser
			100					105					110		

Lys Thr Gln Ser Leu Leu Ile Val Asn Asn Ala Thr Asn Val Val Ile
115 120 125

Lys Val Cys Glu Phe Gln Phe Cys Asn Asp Pro Phe Leu Gly Val Tyr
130 135 140

Tyr His Lys Asn Asn Lys Ser Trp Met Glu Ser Glu Phe Arg Val Tyr
145 150 155 160

Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln Pro Phe Leu
165 170 175

Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu Arg Glu Phe
180 185 190

Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser Lys His Thr
195 200 205

Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser Ala Leu Glu
210 215 220

Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg Phe Gln Thr
225 230 235 240

Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp Ser Ser Ser
245 250 255

Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr Leu Gln Pro
260 265 270

Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile Thr Asp Ala
275 280 285

Val Asp Cys Ala Leu Asp Pro Leu Ser Glu Thr Lys Cys Thr Leu Lys
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Ser Phe Thr Val Glu Lys Gly Ile Tyr Gln Thr Ser Asn Phe Arg Val
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Gln Pro Thr Glu Ser Ile Val Arg Phe Pro Asn Ile Thr Asn Leu Cys

325

330

335

Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val Tyr Ala
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Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser Val Leu
 355 360 365

Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val Ser Pro
 370 375 380

Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp Ser Phe
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Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln Thr Gly
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Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr Gly Cys
 420 425 430

Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly Gly Asn
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Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys Pro Phe
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Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr Pro Cys
 465 470 475 480

Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser Tyr Gly
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Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val Val Val
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Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly Pro Lys
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Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe Asn Phe Asn
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580 585 590

Gly Gly Val Ser Val Ile Thr Pro Gly Thr Asn Thr Ser Asn Gln Val
595 600 605

Ala Val Leu Tyr Gln Asp Val Asn Cys Thr Glu Val Pro Val Ala Ile
610 615 620

His Ala Asp Gln Leu Thr Pro Thr Trp Arg Val Tyr Ser Thr Gly Ser
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Asn Val Phe Gln Thr Arg Ala Gly Cys Leu Ile Gly Ala Glu His Val
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Asn Asn Ser Tyr Glu Cys Asp Ile Pro Ile Gly Ala Gly Ile Cys Ala
660 665 670

Ser Tyr Gln Thr Gln Thr Asn Ser Pro Arg Arg Ala Arg Ser Val Ala
675 680 685

Ser Gln Ser Ile Ile Ala Tyr Thr Met Ser Leu Gly Ala Glu Asn Ser
690 695 700

Val Ala Tyr Ser Asn Asn Ser Ile Ala Ile Pro Thr Asn Phe Thr Ile
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Ser Val Thr Thr Glu Ile Leu Pro Val Ser Met Thr Lys Thr Ser Val
725 730 735

Asp Cys Thr Met Tyr Ile Cys Gly Asp Ser Thr Glu Cys Ser Asn Leu
740 745 750

Leu Leu Gln Tyr Gly Ser Phe Cys Thr Gln Leu Asn Arg Ala Leu Thr

755

760

765

Gly Ile Ala Val Glu Gln Asp Lys Asn Thr Gln Glu Val Phe Ala Gln
 770 775 780

Val Lys Gln Ile Tyr Lys Thr Pro Pro Ile Lys Asp Phe Gly Gly Phe
 785 790 795 800

Asn Phe Ser Gln Ile Leu Pro Asp Pro Ser Lys Pro Ser Lys Arg Ser
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Phe Ile Glu Asp Leu Leu Phe Asn Lys Val Thr Leu Ala Asp Ala Gly
 820 825 830

Phe Ile Lys Gln Tyr Gly Asp Cys Leu Gly Asp Ile Ala Ala Arg Asp
 835 840 845

Leu Ile Cys Ala Gln Lys Phe Asn Gly Leu Thr Val Leu Pro Pro Leu
 850 855 860

Leu Thr Asp Glu Met Ile Ala Gln Tyr Thr Ser Ala Leu Leu Ala Gly
 865 870 875 880

Thr Ile Thr Ser Gly Trp Thr Phe Gly Ala Gly Ala Ala Leu Gln Ile
 885 890 895

Pro Phe Ala Met Gln Met Ala Tyr Arg Phe Asn Gly Ile Gly Val Thr
 900 905 910

Gln Asn Val Leu Tyr Glu Asn Gln Lys Leu Ile Ala Asn Gln Phe Asn
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Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser Ala
 930 935 940

Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu Asn
 945 950 955 960

Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser Val
 965 970 975

Leu Asn Asp Ile Leu Ser Arg Leu Asp Lys Val Glu Ala Glu Val Gln
980 985 990

Ile Asp Arg Leu Ile Thr Gly Arg Leu Gln Ser Leu Gln Thr Tyr Val
995 1000 1005

Thr Gln Gln Leu Ile Arg Ala Ala Glu Ile Arg Ala Ser Ala Asn
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Leu Ala Ala Thr Lys Met Ser Glu Cys Val Leu Gly Gln Ser Lys
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Arg Val Asp Phe Cys Gly Lys Gly Tyr His Leu Met Ser Phe Pro
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Gln Ser Ala Pro His Gly Val Val Phe Leu His Val Thr Tyr Val
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Pro Ala Gln Glu Lys Asn Phe Thr Thr Ala Pro Ala Ile Cys His
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Asp Gly Lys Ala His Phe Pro Arg Glu Gly Val Phe Val Ser Asn
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Gly Thr His Trp Phe Val Thr Gln Arg Asn Phe Tyr Glu Pro Gln
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1145 1150 1155

His Thr Ser Pro Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn
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Ala Ser Val Val Asn Ile Gln Lys Glu Ile Asp Arg Leu Asn Glu

1175

1180

1185

Val Ala Lys Asn Leu Asn Glu Ser Leu Ile Asp Leu Gln Glu Leu
 1190 1195 1200

Gly Lys Tyr Glu Gln Tyr Ile Lys Trp Pro Trp Tyr Ile Trp Leu
 1205 1210 1215

Gly Phe Ile Ala Gly Leu Ile Ala Ile Val Met Val Thr Ile Met
 1220 1225 1230

Leu Cys Cys Met Thr Ser Cys Cys Ser Cys Leu Lys Gly Cys Cys
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Ser Thr Glu Lys Ser Asn Ile Ile Arg Gly Trp Ile Phe Gly Thr Thr

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90

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Arg Val Tyr Ser Ser Ala Asn Asn Cys Thr Phe Glu Tyr Val Ser Gln
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Pro Phe Leu Met Asp Leu Glu Gly Lys Gln Gly Asn Phe Lys Asn Leu
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Arg Glu Phe Val Phe Lys Asn Ile Asp Gly Tyr Phe Lys Ile Tyr Ser
 180 185 190

Lys His Thr Pro Ile Asn Leu Val Arg Asp Leu Pro Gln Gly Phe Ser
 195 200 205

Ala Leu Glu Pro Leu Val Asp Leu Pro Ile Gly Ile Asn Ile Thr Arg
 210 215 220

Phe Gln Thr Leu Leu Ala Leu His Arg Ser Tyr Leu Thr Pro Gly Asp
 225 230 235 240

Ser Ser Ser Gly Trp Thr Ala Gly Ala Ala Ala Tyr Tyr Val Gly Tyr
 245 250 255

Leu Gln Pro Arg Thr Phe Leu Leu Lys Tyr Asn Glu Asn Gly Thr Ile
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Thr Leu Lys Ser
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<213> Severe acute respiratory syndrome coronavirus 2

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Leu Cys Pro Phe Gly Glu Val Phe Asn Ala Thr Arg Phe Ala Ser Val
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Tyr Ala Trp Asn Arg Lys Arg Ile Ser Asn Cys Val Ala Asp Tyr Ser
35 40 45

Val Leu Tyr Asn Ser Ala Ser Phe Ser Thr Phe Lys Cys Tyr Gly Val
50 55 60

Ser Pro Thr Lys Leu Asn Asp Leu Cys Phe Thr Asn Val Tyr Ala Asp
65 70 75 80

Ser Phe Val Ile Arg Gly Asp Glu Val Arg Gln Ile Ala Pro Gly Gln
85 90 95

Thr Gly Lys Ile Ala Asp Tyr Asn Tyr Lys Leu Pro Asp Asp Phe Thr
100 105 110

Gly Cys Val Ile Ala Trp Asn Ser Asn Asn Leu Asp Ser Lys Val Gly
115 120 125

Gly Asn Tyr Asn Tyr Leu Tyr Arg Leu Phe Arg Lys Ser Asn Leu Lys
130 135 140

Pro Phe Glu Arg Asp Ile Ser Thr Glu Ile Tyr Gln Ala Gly Ser Thr
145 150 155 160

Pro Cys Asn Gly Val Glu Gly Phe Asn Cys Tyr Phe Pro Leu Gln Ser
165 170 175

Tyr Gly Phe Gln Pro Thr Asn Gly Val Gly Tyr Gln Pro Tyr Arg Val
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Val Val Leu Ser Phe Glu Leu Leu His Ala Pro Ala Thr Val Cys Gly
195 200 205

Pro Lys Lys Ser Thr Asn Leu Val Lys Asn Lys Cys Val Asn Phe
210 215 220

<210> 4
<211> 19
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

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1 5 10 15

Gln Ile Leu

<210> 5
<211> 18
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 5
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1 5 10 15

Gly Phe

<210> 6
<211> 73
<212> PRT
<213> Severe acute respiratory syndrome coronavirus 2

<400> 6
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1 5 10 15

Asn Ser Ala Ile Gly Lys Ile Gln Asp Ser Leu Ser Ser Thr Ala Ser
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Ala Leu Gly Lys Leu Gln Asp Val Val Asn Gln Asn Ala Gln Ala Leu
35 40 45

Asn Thr Leu Val Lys Gln Leu Ser Ser Asn Phe Gly Ala Ile Ser Ser
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Val Leu Asn Asp Ile Leu Ser Arg Leu
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<210> 7

<211> 50

<212> PRT

<213> Severe acute respiratory syndrome coronavirus 2

<400> 7

Lys Val Glu Ala Glu Val Gln Ile Asp Arg Leu Ile Thr Gly Arg Leu
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Gln Ser Leu Gln Thr Tyr Val Thr Gln Gln Leu Ile Arg Ala Ala Glu
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Ile Arg Ala Ser Ala Asn Leu Ala Ala Thr Lys Met Ser Glu Cys Val
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Leu Gly
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<210> 8

<211> 66

<212> PRT

<213> Severe acute respiratory syndrome coronavirus 2

<400> 8

Thr Thr Ala Pro Ala Ile Cys His Asp Gly Lys Ala His Phe Pro Arg
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Glu Gly Val Phe Val Ser Asn Gly Thr His Trp Phe Val Thr Gln Arg
20 25 30

Asn Phe Tyr Glu Pro Gln Ile Ile Thr Thr Asp Asn Thr Phe Val Ser
35 40 45

Gly Asn Cys Asp Val Val Ile Gly Ile Val Asn Asn Thr Val Tyr Asp
50 55 60

Pro Leu

65

<210> 9

<211> 51

<212> PRT

<213> Severe acute respiratory syndrome coronavirus 2

<400> 9

Asp Val Asp Leu Gly Asp Ile Ser Gly Ile Asn Ala Ser Val Val Asn
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20 25 30

Glu Ser Leu Ile Asp Leu Gln Glu Leu Gly Lys Tyr Glu Gln Tyr Ile
35 40 45

Lys Trp Pro
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<210> 10

<211> 24

<212> PRT

<213> Severe acute respiratory syndrome coronavirus 2

<400> 10

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Val Thr Ile Met Leu Cys Cys Met
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<210> 11

<211> 36

<212> PRT

<213> Severe acute respiratory syndrome coronavirus 2

<400> 11

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Cys Lys Phe Asp Glu Asp Asp Ser Glu Pro Val Leu Lys Gly Val Lys
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Leu His Tyr Thr

<210> 12
 <211> 3822
 <212> DNA
 <213> Severe acute respiratory syndrome coronavirus 2

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 <212> PRT
 <213> Human immunodeficiency virus 1

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 <212> PRT
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<400> 14
 Gly Pro Gly His Lys Ala Arg Val Leu
 1 5

<210> 15
 <211> 11
 <212> PRT
 <213> Human immunodeficiency virus 1

<400> 15
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1 5 10

<210> 16
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<400> 16
Val Leu Ala Glu Ala Met Ser Gln Val
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<210> 17
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<213> Human immunodeficiency virus 1

<400> 17
Ser Leu Phe Asn Thr Val Ala Thr Leu
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<210> 18
<211> 10
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<400> 18
Arg Tyr Pro Leu Thr Phe Gly Trp Cys Phe
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<210> 19
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<400> 19
Ala Val Asp Leu Ser His Phe Leu Lys
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Gln Val Pro Leu Arg Pro Met Thr Tyr Lys
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<210> 21
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<400> 21
Arg Tyr Pro Leu Thr Phe Gly Trp
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<210> 22
<211> 10
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<400> 22
Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe
1 5 10

<210> 23
<211> 10
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<400> 23
Arg Gly Pro Gly Arg Ala Phe Val Thr Ile
1 5 10

<210> 24
<211> 9
<212> PRT
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<400> 24
Lys Leu Thr Pro Leu Cys Val Thr Leu
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<210> 25
<211> 15
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<213> Human immunodeficiency virus 1

<400> 25
Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys
1 5 10 15

<210> 26
<211> 9

<212> PRT
<213> Human immunodeficiency virus 1

<400> 26
Arg Ala Ile Glu Ala Gln Gln His Leu
1 5

<210> 27
<211> 15
<212> PRT
<213> Human immunodeficiency virus 1

<400> 27
Arg Ile Gln Arg Gly Pro Gly Arg Ala Phe Val Thr Ile Gly Lys
1 5 10 15

<210> 28
<211> 10
<212> PRT
<213> Human immunodeficiency virus 1

<400> 28
Thr Val Tyr Tyr Gly Val Pro Val Trp Lys
1 5 10

<210> 29
<211> 11
<212> PRT
<213> Human immunodeficiency virus 1

<400> 29
Arg Leu Arg Asp Leu Leu Leu Ile Val Thr Arg
1 5 10

<210> 30
<211> 9
<212> PRT
<213> Zaire ebolavirus

<400> 30
Phe Leu Ser Phe Ala Ser Leu Phe Leu
1 5

<210> 31
<211> 9
<212> PRT
<213> Zaire ebolavirus

<400> 31

Phe Gln Gln Thr Asn Ala Met Val Thr
1 5

<210> 32
<211> 9
<212> PRT
<213> Zaire ebolavirus

<400> 32
Lys Leu Thr Glu Ala Ile Thr Ala Ala
1 5

<210> 33
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<400> 33
Arg Leu Met Arg Thr Asn Phe Leu Ile
1 5

<210> 34
<211> 8
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1 5

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1 5

<210> 36
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<400> 36
Arg Leu Ala Ser Thr Val Ile Tyr Arg
1 5

<210> 37
<211> 9
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Thr Thr Ile Gly Glu Trp Ala Phe Trp
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<210> 38
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<210> 39
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<212> PRT
<213> Zika virus

<400> 40
Ile Gly Val Ser Asn Arg Asp Phe Val
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<210> 41
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<400> 41
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Gly Gly Thr Trp
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<210> 42
<211> 20
<212> PRT
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<400> 42
Gln Pro Glu Asn Leu Glu Tyr Arg Ile Met Leu Ser Val His Gly Ser
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Gln His Ser Gly
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<210> 43
<211> 20
<212> PRT
<213> Zika virus

<400> 43
Lys Gly Val Ser Tyr Ser Leu Cys Thr Ala Ala Phe Thr Phe Thr Lys
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Ile Pro Ala Glu
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<210> 44
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<213> Zika virus

<400> 44
Phe Glu Ala Thr Val Arg Gly Ala Lys Arg Met Ala Val Leu Gly Asp
1 5 10 15

Thr Ala Trp Asp
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<210> 45
<211> 20
<212> PRT
<213> Zika virus

<400> 45
His Arg Ser Gly Ser Thr Ile Gly Lys Ala Phe Glu Ala Thr Val Arg
1 5 10 15

Gly Ala Lys Arg
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<210> 46
<211> 17
<212> PRT
<213> Influenza A virus

<400> 46
Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu Asp Tyr His Asp
1 5 10 15

Ser

<210> 47
<211> 17
<212> PRT
<213> Influenza A virus

<400> 47
Glu Leu Leu Val Leu Leu Glu Asn Glu Arg Thr Leu Asp Tyr His Asp
1 5 10 15

Ser

<210> 48
<211> 20
<212> PRT
<213> Influenza A virus

<400> 48
Thr Tyr Val Leu Ser Ile Ile Pro Ser Gly Pro Leu Lys Ala Glu Ile
1 5 10 15

Ala Gln Arg Leu
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<210> 49
<211> 11
<212> PRT
<213> Influenza A virus

<400> 49
Leu Tyr Lys Lys Leu Lys Arg Glu Ile Thr Phe
1 5 10

<210> 50
<211> 16
<212> PRT
<213> Influenza A virus

<400> 50
Gly Phe Glu Met Ile Trp Asp Pro Asn Gly Trp Thr Gly Thr Asp Asn
1 5 10 15

<210> 51
<211> 17
<212> PRT
<213> Influenza A virus

<400> 51
Gly Gln Ala Ser Tyr Lys Ile Phe Arg Ile Glu Lys Gly Lys Ile Val
1 5 10 15

Lys

<210> 52
<211> 17
<212> PRT
<213> Influenza A virus

<400> 52
Gly Trp Ala Ile Tyr Ser Lys Asp Asn Ser Val Arg Ile Gly Ser Lys
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Gly

<210> 53
<211> 3822
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic
polynucleotide

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<213> Severe acute respiratory syndrome coronavirus 2

<400> 54

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 35 40 45

Thr Ala Ser Trp Phe Thr Ala Leu Thr Gln His Gly Lys Glu Asp Leu
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Lys Phe Pro Arg Gly Gln Gly Val Pro Ile Asn Thr Asn Ser Ser Pro
 65 70 75 80

Asp Asp Gln Ile Gly Tyr Tyr Arg Arg Ala Thr Arg Arg Ile Arg Gly
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Gly Asp Gly Lys Met Lys Asp Leu Ser Pro Arg Trp Tyr Phe Tyr Tyr
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Leu Gly Thr Gly Pro Glu Ala Gly Leu Pro Tyr Gly Ala Asn Lys Asp
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Gly Ile Ile Trp Val Ala Thr Glu Gly Ala Leu Asn Thr Pro Lys Asp

130

135

140

His Ile Gly Thr Arg Asn Pro Ala Asn Asn Ala Ala Ile Val Leu Gln
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Leu Pro Gln Gly Thr Thr Leu Pro Lys Gly Phe Tyr Ala Glu Gly Ser
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Arg Gly Gly Ser Gln Ala Ser Ser Arg Ser Ser Ser Arg Ser Arg Asn
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Ser Ser Arg Asn Ser Thr Pro Gly Ser Ser Arg Gly Thr Ser Pro Ala
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Arg Met Ala Gly Asn Gly Gly Asp Ala Ala Leu Ala Leu Leu Leu Leu
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Asp Arg Leu Asn Gln Leu Glu Ser Lys Met Ser Gly Lys Gly Gln Gln
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Gln Gln Gly Gln Thr Val Thr Lys Lys Ser Ala Ala Glu Ala Ser Lys
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Lys Pro Arg Gln Lys Arg Thr Ala Thr Lys Ala Tyr Asn Val Thr Gln
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Ala Phe Gly Arg Arg Gly Pro Glu Gln Thr Gln Gly Asn Phe Gly Asp
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Gln Glu Leu Ile Arg Gln Gly Thr Asp Tyr Lys His Trp Pro Gln Ile
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Ala Gln Phe Ala Pro Ser Ala Ser Ala Phe Phe Gly Met Ser Arg Ile
 305 310 315 320

Gly Met Glu Val Thr Pro Ser Gly Thr Trp Leu Thr Tyr Thr Gly Ala
 325 330 335

Ile Lys Leu Asp Asp Lys Asp Pro Asn Phe Lys Asp Gln Val Ile Leu
 340 345 350

Leu Asn Lys His Ile Asp Ala Tyr Lys Thr Phe Pro Pro Thr Glu Pro
355 360 365

Lys Lys Asp Lys Lys Lys Lys Ala Asp Glu Thr Gln Ala Leu Pro Gln
370 375 380

Arg Gln Lys Lys Gln Gln Thr Val Thr Leu Leu Pro Ala Ala Asp Leu
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<212> PRT

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