

COMPOSITIONS AND METHODS FOR THE INHIBITION OF NATURAL KILLER CELLS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application 63/526,410, filed July 12, 2023, the entire contents of which are incorporated herein by reference.

5 SEQUENCE LISTING

This application includes a Sequence Listing in XML format that constitutes part of the disclosure. Said XML file, created June 24, 2024, is named "103783-0344_SL.xml" and is 17,669 bytes in size.

10 STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with government support under 75N91019D00024 awarded by the National Institutes of Health and under HU00012020067, HU00012120104, and HU00012120094 awarded by the Uniformed Services University of the Health Sciences. The government has certain rights in the invention.

15 FIELD OF THE DISCLOSURE

Disclosed herein are novel polynucleotides and polynucleotide constructs for inhibiting natural killer cells or for preventing other cells from being killed by natural killer cells, as well as cells capable of expressing the novel polynucleotides and polynucleotide constructs described herein. Compositions disclosed herein comprise one or more polynucleotides that encode a polypeptide
20 capable of having NK cell inhibiting activity to be used with one or more additional polynucleotides, such as, an exogenously delivered polynucleotide, and in specific instances, one or more mRNA vaccines. Methods are further disclosed relating to methods for enhancing the durability of one or more exogenously delivered polynucleotides as well as methods for reducing one or more unwanted side effects associated with the administration of exogenously delivered
25 polynucleotides.

BACKGROUND OF THE DISCLOSURE

Although initial formulations of mRNA vaccines have been tested since 1995, the Pfizer-BioNTech COVID-19 vaccine (BNT162b2) was the first United States Food and Drug Administration (FDA) approved mRNA vaccine. Phase III clinical trials demonstrated that BNT162b2 was highly
30 efficacious and safe in preventing COVID-19 (Chakraborty et al., 2021. From COVID-19 to Cancer mRNA Vaccines: Moving from Bench to Clinic in the Vaccine Landscape. *Front Immunol* 12: 679344; and Polack et al., 2020. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine.

- N Engl J Med* 383: 2603-2615). Subsequent studies have shown that while protection against infection with novel variants can be variable, the vaccine still confers significant protection against development of severe disease (Liu et al., 2021. Effectiveness and safety of SARS-CoV-2 vaccine in real-world studies: a systematic review and meta-analysis. *Infectious Diseases of Poverty* 10: 132). As of February 2023, over 400 million BNT162b2 vaccinations have been administered in the United States (Centers for Disease Control and Prevention. COVID Data Tracker. US Department of Health and Human Services, CDC, Atlanta, GA). While the basic tenets by which mRNA vaccines work are characterized, there remains many unknowns regarding the precise factors which drive immunogenicity and reactogenicity of mRNA vaccines.
- Reactogenicity to BNT162b2 vaccination is very common but does not occur in all individuals. According to the Centers for Disease Control and Prevention (CDC), 84.7% of 18-55 years old reported at least one local injection site reaction, and 77.4% reported at least one systemic reaction within 7 days of vaccination (Centers for Disease Control. 2022. Pfizer-BioNTech COVID-19 Vaccine Reactions & Adverse Events). In the Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study cohort, generally healthy adults reported substantial heterogeneity with regards to both severity and duration of local and systemic symptoms after BNT162b2 vaccination (Coggins et al., 2021. Adverse Effects and Antibody Titers in Response to the BNT162b2 mRNA COVID-19 Vaccine in a Prospective Study of Healthcare Workers. *Open Forum Infectious Diseases* 9). In addition, while most individuals develop detectable IgG antibodies to SARS-CoV-2 spike protein after BNT162b2 vaccination, there is a wide range in the levels of peak antibody titers (Moncunill et al., 2022. Determinants of early antibody responses to COVID-19 mRNA vaccines in a cohort of exposed and naïve healthcare workers. *eBioMedicine* 75; Laing et al., 2022. Durability of Antibody Response and Frequency of SARS-CoV-2 Infection 6 Months after COVID-19 Vaccination in Healthcare Workers. *Emerg Infect Dis* 28: 828-832).
- One area not yet extensively evaluated is the potential involvement of natural killer (NK) cells in the inflammatory responses to mRNA vaccination. NK cells have been found to become activated within days after mRNA vaccination in both animal and human studies (Li et al., 2022. Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine. *Nature Immunology* 23: 543-555; Kowalczyk et al., 2016). Self-adjuvanted mRNA vaccines induce local innate immune responses that lead to a potent and boostable adaptive immunity. *Vaccine* 34: 3882-3893; Cuapio et al., 2022. NK cell frequencies, function and correlates to vaccine outcome in BNT162b2 mRNA anti-SARS-CoV-2 vaccinated healthy and immunocompromised individuals. *Molecular Medicine* 28: 20; Saresella et al., 2022. Innate immune responses to three doses of the

BNT162b2 mRNA SARS-CoV-2 vaccine. *Front Immunol* 13: 947320). The exact roles NK cells play in contributing to acute inflammatory side effects or in shaping the mRNA vaccine-induced adaptive immune response have not yet been characterized.

5 NK cells are innate immune cells that make up 5-20% of peripheral blood lymphocytes (Abel et al., 2018. Natural Killer Cells: Development, Maturation, and Clinical Utilization. *Frontiers in Immunology* 9). Through a range of activating and inhibitory receptors present on their cell surface, NK cells recognize and target cells that appear transformed, infected, or stressed (Abel, 2018). Once an NK cell becomes activated it has two main effector functions: cytotoxicity and release of inflammatory cytokines. Through these functions, NK cells can play a key role in
10 inflammatory and regulatory processes. NK cells are well recognized as having the ability to both amplify and diminish adaptive immune responses elicited by vaccines (Cox et al., 2021. Targeting natural killer cells to enhance vaccine responses. *Trends in Pharmacological Sciences* 42: 789-801). After vaccination, NK cells can release cytokines and stimulate antigen-presenting cells, which enhances the adaptive response (Cox, 2021). On the other hand, cytolytic activities by NK
15 cells can serve to contain adaptive immunity by reducing the number of responding T cells, which can subsequently diminish T cell help to B cells and reduce the quantity and quality of antibodies produced (Cox, 2021; Rydzynski et al., 2015. Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. *Nature Communications* 6: 6375; and Cook et al., 2015. NK cells inhibit humoral immunity by reducing the abundance of CD4+ T follicular helper
20 cells during a chronic virus infection. *J Leukoc Biol* 98: 153-162). It is suspected that increased activation and release of pro-inflammatory molecules by NK cells could be driving some of the local and systemic symptoms commonly observed after mRNA vaccination.

Notably, there is substantial person-to-person variation in terms of frequency, phenotype, and function of NK cells at baseline (Freud et al., 2017. The Broad Spectrum of Human Natural Killer
25 Cell Diversity. *Immunity* 47: 820-833). The present disclosure seeks to reduce inflammatory side effects and/or increase the magnitude and/or duration of antibody and/or T-cell responses induced by the delivery of a polynucleotide (e.g., BNT162b2 vaccination) by modifying NK cell frequencies and/or function.

A number of patent applications and issued patents disclose vaccine technologies generally, and
30 more recently mRNA vaccines specifically.

For example, U.S. Pat. App. Pub. No.: 2020/0155671 discloses RNA decorated particles such as RNA decorated lipid particles and a method for producing the same.

U.S. Pat. App. Pub. No.: 2020/0197508 discloses immunostimulatory RNA molecules comprising sequences derived from an Influenza A virus nucleoprotein-encoding RNA molecule that act as adjuvants and/or immunostimulatory agents to enhance host immune responses.

U.S. Pat. No.: 10,898,574 discloses formulations, compositions and methods for delivering
5 biological moieties such as modified nucleic acids into cells to modulate protein expression.

U.S. Pat. No.: 10,703,789 discloses compositions, methods, processes, kits and devices for the design, preparation, manufacture and/or formulation of polynucleotides, primary constructs and modified mRNA molecules (mmRNA).

Notwithstanding the state of vaccine technology, there is an urgent need to enhance the durability
10 of existing and future vaccines. Additionally, a need exists to reduce the unwanted side effects associated with vaccines following the administration of a vaccine to a subject. The present disclosure provides one or more nucleic acids encoding for proteins having NK cell inhibitory activity as well as cells and compositions comprising the same.

SUMMARY OF THE EMBODIMENTS

15 In accordance with some aspects, there are provided isolated polynucleotide constructs comprising:

(a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity; and

(b) a second polynucleotide encoding a polypeptide capable of inducing an immune
20 response or encoding a naturally endogenously produced protein as well as compositions comprising such constructs.

In accordance with other aspects, there are provided compositions comprising:

(a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity;

(b) a second polynucleotide encoding a polypeptide capable of inducing an immune
25 response or encoding a naturally endogenously produced protein as well as compositions comprising such constructs; and

(c) a pharmaceutically acceptable carrier.

In accordance with any aspects, the polynucleotide construct or first and second polynucleotides
30 are mRNA molecules.

In accordance with any aspects, the first polynucleotide may encode a serine protease inhibitor (serpin) polypeptide or fragment thereof capable of having natural killer (NK) cell inhibiting activity, such as SERPINB9 or fragment thereof capable of having NK cell inhibiting activity. Additionally or alternatively, the first polynucleotide may encode a major histocompatibility complex (MHC) class polypeptide or fragment thereof having NK cell inhibiting activity, such as an MHC class I, MHC class II, or MHC class III polypeptide having NK cell inhibiting activity, such as an HLA-E polypeptide, such as HLA-E*0103 or HLA-E*0101, or a fragment thereof capable of having NK cell inhibiting activity. Additionally or alternatively, the first polynucleotide may encode a lectin polypeptide, such as CLEC2D, or a fragment thereof capable of having NK cell inhibiting activity. Additionally or alternatively, the first polynucleotide may encode a cadherin polypeptide, or a fragment thereof capable of having NK cell inhibiting activity. Additionally or alternatively, the first polynucleotide may encode a CD155 (poliovirus receptor) polypeptide, or a fragment thereof capable of having NK cell inhibiting activity. Additionally or alternatively, the first polynucleotide may encode a RTN4 polypeptide, or a fragment thereof capable of having NK cell inhibiting activity.

In accordance with any aspects, the second polynucleotide may encode an antigen capable of inducing an immune response, such as a COVID-19 antigen capable of inducing an immune response against COVID-19. Alternatively, the second polynucleotide may encode a normally endogenously produced protein, such as Factor VIII or a biologically active fragment thereof.

In accordance with other aspects, there are provided methods for enhancing one or both of the magnitude and durability of immune responses to a vaccine or for reducing one or more unwanted side effects associated with the administration of a vaccine, comprising the steps of:

(a) administering to a subject a pharmaceutically effective amount of a first polynucleotide that encodes a polypeptide capable of having NK cell inhibiting activity; and (b) administering to a subject a pharmaceutically effective amount of one or more vaccines;

wherein inhibition of NK cell activity enhances the magnitude and/or durability of immune responses induced by the one or more vaccines administered to the subject and/or reduces one or more unwanted side effects associated with the administration of a vaccine.

The vaccine may be a polynucleotide vaccine comprising a polynucleotide encoding a vaccine antigen, optionally wherein the polynucleotide is an mRNA molecule. The first polynucleotide and polynucleotide encoding a vaccine antigen may be present in a single polynucleotide construct.

Alternatively, the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in a single composition. Alternatively, the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in different compositions. When used, the single
5 polynucleotide construct or the single composition may be any construct or composition as described herein.

In accordance with other aspects, there are provided methods for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects associated with administration of an exogenously administered polynucleotide, comprising the
10 steps of:

(a) administering to a subject a pharmaceutically effective amount of a first polynucleotide encoding a polypeptide capable of having NK cell inhibiting activity; and

(b) administering to the subject a second exogenously administered polynucleotide;

wherein inhibition of NK cell activity enhances the durability of the exogenously
15 administered polynucleotide and/or reduces one or more unwanted side effects associated with administration of the exogenously administered polynucleotide.

The second exogenously administered polynucleotide may encode an antigen capable of inducing an immune response, such as a COVID-19 antigen capable of inducing an immune response against COVID-19. The second exogenously administered polynucleotide may encode
20 a normally endogenously produced protein, such as Factor VIII or a biologically active fragment thereof.

In accordance with any such methods where the first polynucleotide and second exogenously administered polynucleotide are provided separately, step (a) may be performed before step (b), after step (b), or simultaneously with step (b).

25 In accordance with other aspects, there are provided constructs and compositions as described herein, for enhancing one or both of the magnitude and durability of immune responses to a vaccine, such as a polynucleotide vaccine or reducing one or more unwanted side effects associated with administration of a polynucleotide vaccine, or for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects
30 associated with administration of an exogenously administered polynucleotide.

In accordance with other aspects, there are provided cells expressing a polynucleotide construct as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a strobe chart.

5 FIG. 1B is a sample questionnaire used by participants in a study.

FIG. 2 is a flow cytometry gating strategy for NK cell populations.

FIG. 3 (panels a-d) are graphs illustrating natural killer (NK) cell frequencies and functionality in relation to sex.

FIG. 4 (panels a-b) are graphs illustrating NK cell functionality in female versus male participants.

10 FIG. 5 (panels a-d) are graphs illustrating NK cell frequencies and functionality in relation to age.

FIG. 6 (panels a-e) are graphs illustrating the longitudinal frequencies of NK cells.

FIGs. 7A-7B indicate the breakdown of various local and systemic symptoms exhibited in the cohort of Example 1.

15 FIG. 8 (panels a-f) are graphs illustrating symptom scores after vaccination in relation to NK cell frequencies and functionality.

FIG. 9 (panels a-d) are graphs illustrating the functionality of NK cells on participants with low versus high symptom scores after vaccination 1 and 2.

FIG. 10 (panels a-d) are graphs illustrating IgG levels at 1 month and 6 months post 2nd vaccination in relation to the NK cell frequencies and functionality.

20 FIG. 11 (panels a-h) are graphs illustrating IgG levels at 1 month and 6 months post 2nd vaccination in relation to the NK cytotoxicity index.

FIG. 12 is a heat map illustrating correlations between NK cell receptors and post-vaccination symptom scores and IgG levels at 1 and 6 months post-vaccination.

25 FIG. 13 shows protection of leukemic 721.211 cells expressing SERPINB or SERPINB and HLA-E from killing by NK cells, in comparison to the parent cell line and cells expressing only HLA-E.

FIG. 14 shows the percentages of spike protein-specific CD8⁺ T cells activated by incubation with spike S1 peptides in splenocytes (panel A) or lymph node cells (panel B) of mice vaccinated with different mRNA LNP vaccine formulations.

DETAILED DESCRIPTION OF THE EMBODIMENTS

30 The disclosed embodiments described herein relate to polynucleotide constructs, cells expressing polynucleotide constructs, compositions comprising a nucleic acid that encodes one or more NK cell inhibitors capable of inhibiting NK cell activity, and methods for enhancing the durability of one or more vaccines (e.g., enhancing the magnitude and/or duration of immune responses

elicited by a vaccine), as well as, for reducing unwanted side effects associated with vaccines following administration to a subject. In some embodiments, the disclosed constructs, compositions, and methods enhance one or both of the magnitude and durability of one or both of antibody and T cell responses to vaccine antigens, such as vaccine antigens encoded by exogenously delivered polynucleotides, such as antigens encoded by mRNA of mRNA vaccines.

Definitions:

As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

As used herein, the term “about,” when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of plus or minus 10% around the specified value, as such variations are appropriate to perform the disclosed methods and compositions.

As used herein, “administer” and/or “administering” refers to the act of giving or providing a composition or compound to a subject (e.g., a patient) by the subject themselves or by a caregiver, such as a medical professional or the like, including the act of ingestion by or application to the subject or the like wherein the composition or compound can exert its effects.

As used herein, the term “antigen” means a substance that can generate one or more immune responses. Antigens can include, but are not limited to, peptides, proteins, glycoproteins, polysaccharides, and lipids; portions thereof and combinations thereof. As used herein, antigens can be natural or synthetic.

As used herein, the terms “carrier” and/or “pharmaceutically acceptable carrier” mean any and all carriers that are compatible with the other ingredients in the formulation and biologically acceptable for the intended route of administration.

As used herein, the term “cDNA” refers to a DNA that is complementary to, and synthesized from, a mRNA template using the enzyme reverse transcriptase.

As used herein, the term “coding sequence” means a polynucleotide, which directly specifies the amino acid sequence of a polypeptide. The boundaries of a coding sequence are generally determined by an open reading frame, which typically begins with a start codon and ends with a stop codon.

As used herein, the term “construct” is used to describe a molecule, such as a polynucleotide (e.g., a SerpinB9 polynucleotide) which may optionally be chemically bonded to

one or more additional molecular moieties (e.g., one or more additional polynucleotides).

As used herein, the term “**control sequences**” means nucleic acid sequences necessary for expression of a polynucleotide encoding a polypeptide described herein in the expression system at issue (if any). Each control sequence may be native (*i.e.*, from the same gene) or foreign (*i.e.*,
5 from a different gene) to the polynucleotide encoding the polypeptide or native or foreign to each other. Such control sequences include, but are not limited to, a leader, polyadenylation sequence, propeptide sequence, promoter, signal peptide sequence, and transcription terminator. Typically, control sequences may include at least one promoter and transcriptional and translational stop signals. When present, control sequences may be provided with linkers for the purpose of
10 introducing specific restriction sites facilitating ligation of the control sequences with the coding region of the polynucleotide encoding a polypeptide.

As used herein, the terms “**effective amount**” and/or “**pharmaceutically effective amount**” refer to an amount that when administered to a host, or to a cell, issue, or organ of a host, achieves an intended therapeutic result (e.g., inhibition of NK cell activity or induction of an immune response).

15 As used herein, the terms “**enhance**,” “**improve**,” “**increase**,” “**decrease**,” or “**reduce**,” or grammatical equivalents thereof, indicate values that are relative to a baseline measurement (e.g., with respect to natural killer cell activity, a measurement in the same subject prior to inhibiting natural killer cell activity and/or a measurement in a control individual (or multiple control individuals) in the absence of inhibiting natural killer cell activity).

20 As used herein, the term “**expression**” refers to any step involved in the production of a polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

As used herein, the term “**exogenous**” means that a molecule (e.g., a nucleic acid) or activity referred to is introduced or delivered into an organism or subject (e.g., a patient).

25 As used herein, the term “**expression vector**” refers to any means for the cloning of and/or transfer of a nucleic acid into a host cell. The term “vector” includes both viral and nonviral means for introducing the nucleic acid into a cell *in vitro*, *ex vivo* or *in vivo*. A large number of vectors known in the art may be used to manipulate nucleic acids, incorporate response elements and promoters into genes, etc. Possible vectors include, but are not limited to, plasmids or modified
30 viruses including, for example bacteriophages.

As used herein, the term “**host cell**” means any cell type that is susceptible to transformation, transfection, transduction, or the like with a nucleic acid construct or expression vector to express

one or more polypeptides. The term “host cell” encompasses any progeny of a parent cell that is not identical to the parent cell due to mutations that occur during replication.

As used herein, the term “**hybrid polypeptide**” means a polypeptide in which a region of one polypeptide is fused at the N-terminus or the C-terminus of a region of another polypeptide, directly or through a linker. A hybrid polypeptide as described herein may have at least 20% of the desired biological activity of the mature polypeptide, *e.g.*, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 100% of the desired biological activity of the mature polypeptide, such as NK cell inhibiting activity.

As used herein, the term “**isolated**” means a substance in a form or environment that does not occur in nature. Non-limiting examples of isolated substances include (1) any non-naturally occurring substance, (2) any substance including, but not limited to, any nucleic acid, variant, protein, peptide, or cofactor that is at least partially removed from one or more or all of the naturally occurring constituents with which it is associated in nature; (3) any substance modified by the hand of man relative to that substance found in nature; or (4) any substance modified by increasing the amount of the substance relative to other components with which it is naturally associated (*e.g.*, multiple copies of a gene encoding the substance; use of a stronger promoter than the promoter naturally associated with the gene encoding the substance).

As used herein, the term “**lipid**” refers to a group of organic compounds that include, but are not limited to, esters of fatty acids, and are characterized by being insoluble in water, but soluble in some organic solvents. Examples of lipids include “simple lipids” (*e.g.*, fats and oils as well as waxes), “compound lipids,” (*e.g.*, phospholipids and glycolipids), and “derived lipids” (*e.g.*, steroids).

The term “**lipid particle**” includes a lipid formulation that can be used to deliver a polynucleotide to a target site of interest (*e.g.*, cell, tissue, organ, etc.). Typically, polynucleotides are fully encapsulated within a lipid particle.

As used herein, the term “**natural killer cell inhibiting activity**” and/or “**NK cell inhibiting activity**” means a reduction in the level of natural killer cell activity in the presence of an inhibitor as described herein, as compared with the level of natural killer cell activity in the absence of the inhibitor. For the sake of clarity, any reduction in the level of natural killer cell activity does not necessarily require a reduction in the total number of natural killer cells. For example, the reduction in the level of natural killer cell activity could occur not only from a reduction in the total number of natural killer cells, but also from the inhibition of the binding of a natural killer cell to a

particular target, (e.g., a cell or a cell surface protein) and/or by protecting and/or insulating a cell from natural killer cell activity (e.g., by inhibiting cell cytolytic activity by either a cell surface or secreted molecule that binds to a receptor on the surface or in an internal compartment of a natural killer cell to attenuate the killing activity of the natural killer cell).

- 5 As used herein, the term “**nucleic acid**” means a polynucleotide and includes a single or a double-stranded polymer of deoxyribonucleotide bases (*i.e.*, guanine (G) cytosine (C) adenine (A) and thymine (T)) or ribonucleotide bases (*i.e.*, guanine (G) cytosine (C) adenine (A) and uracil (U)). Except where otherwise noted, nucleic acid sequences disclosed herein may recite “T”s in a representative DNA sequence, but where the sequence represents RNA (e.g., mRNA), the “T”s
10 would be substituted for “U”s. Nucleic acids may also include fragments and modified nucleotides. For the sake of clarity, the terms “polynucleotide”, “polynucleotide sequence”, “nucleic acid sequence”, “nucleotide sequence” and “nucleic acid fragment” are used interchangeably to denote a polymer of RNA and/or DNA that is single- or double-stranded, optionally containing synthetic, non-natural, or altered nucleotide bases.
- 15 As used herein, the term “**nucleic acid construct**” means a nucleic acid molecule, either single- or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature, or which is synthetic.

- As used herein, the term “**open reading frame**” and/or “**ORF**” refers to a sequence of nucleic
20 acids, either DNA or RNA, that encode a protein or polypeptide. An ORF may comprise a translation start signal or initiation codon, such as ATG or AUG, and a termination codon.

- As used herein, the term “**operably linked**” means a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence. A nucleic acid is “operably
25 linked” when it is placed into a functional relationship with another nucleic acid sequence. The coding sequences need not be contiguous to one another so long as the expressed sequences are ultimately processed to produce the desired protein.

- As used herein, term “**polynucleotide fragment**” means a nucleotide sequence of reduced length relative to the reference nucleic acid and comprising, over the common portion, a nucleotide
30 sequence identical to the reference nucleic acid. In one aspect, a fragment is at least 80% the length of the reference nucleic acid, *e.g.*, at least 85% the length of the reference nucleic acid, at least 90% the length of the reference nucleic acid, at least 95% the length of the reference nucleic

acid, at least 96% the length of the reference nucleic acid, at least 97% the length of the reference nucleic acid, at least 98% the length of the reference nucleic acid, at least 99% the length of the reference nucleic acid at least 99.9% the length of the reference nucleic acid, or at least 99.99% the length of the reference nucleic acid.

- 5 As used herein, the terms the terms “**polypeptide**” “**protein**”, and “**peptide**” are used interchangeably and may refer to a polymer of two or more amino acids.

As used herein, term “**polypeptide fragment**” means an amino acid sequence of reduced length relative to the reference polypeptide and comprising, over the common portion, an amino acid sequence identical to the reference polypeptide. In one aspect, a fragment is at least 80% the
10 length of the reference polypeptide, e.g., at least 85% the length of the reference polypeptide, at least 90% the length of the reference polypeptide, at least 95% the length of the reference polypeptide, at least 96% the length of the reference polypeptide, at least 97% the length of the reference polypeptide, at least 98% the length of the reference polypeptide, at least 99% the length of the reference polypeptide at least 99.9% the length of the reference polypeptide, or at
15 least 99.99% the length of the reference polypeptide.

As used herein, the term “**recombinant**” refers to a cell, nucleic acid, polypeptide, expression cassette or vector, refers to a material, or a material corresponding to the natural or native form of the material, that has been modified by the introduction of a new moiety or alteration of an existing moiety by recombinant techniques, or is identical thereto but produced or derived from
20 synthetic materials using recombinant techniques. For example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell (*i.e.*, “exogenous nucleic acids”) or express native genes that are otherwise expressed at a different level, typically, under-expressed or not expressed at all.

As used herein, the term “**reference sequence**” refers to a defined sequence used as a basis for
25 a sequence comparison. A reference sequence may be a subset of a larger sequence, for example, a segment of a full-length gene or polypeptide sequence. Generally, a reference sequence is at least 20 nucleotide or amino acid residues in length, at least 25 residues in length, at least 50 residues in length, or the full length of the nucleic acid or polypeptide. Since two polynucleotides or polypeptides may each (1) comprise a sequence (*i.e.*, a portion of the complete
30 sequence) that is similar between the two sequences, and (2) may further comprise a sequence that is divergent between the two sequences, sequence comparisons between two (or more) polynucleotides or polypeptides are typically performed by comparing sequences of the two

polynucleotides or polypeptides to identify and compare local regions of sequence similarity.

As used herein, the term “**RNA transcript**” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complimentary copy of the DNA sequence, it is referred to as the primary transcript or pre-mRNA. A RNA transcript is referred to as the mature RNA when it is a RNA sequence derived from post-transcriptional processing of the primary transcript pre-mRNA. “Messenger RNA” or “mRNA” refers to RNA that is without introns and can be translated into protein by a cell.

As used herein, the term “**unwanted side effect(s)**” refers to one or more effects and/or symptoms associated with administration of a substance to a subject that are not the desired and/or intended effects and/or are unpleasant to the subject.

As used herein, the term “**vaccine**” means an immunogenic composition for administration to a mammal for eliciting an immune response against a particular antigen, which may be referred to herein as a “vaccine antigen”.

As used herein, the term “**variant**” means a polypeptide having the desired biological activity of the reference polypeptide (such as NK cell inhibiting activity or ability to induce an immune response) and having an amino acid sequence comprising an alteration (*i.e.*, a substitution, insertion, and/or deletion) at one or more, namely, several, amino acid positions relative to the reference polypeptide. A substitution means replacement of the amino acid occupying a position with a different amino acid; a deletion means removal of the amino acid occupying a position; and an insertion means adding an amino acid adjacent to and immediately following the amino acid occupying a position.

As used herein, the term “**wild-type**” polynucleotide means that the polynucleotide does not comprise any mutations relative to a polynucleotide found in nature. A “wild type” protein means that the protein comprises the amino acid sequence of a protein found in nature and exhibits a level of activity of the protein found in nature.

POLYNUCLEOTIDES

In accordance with some aspects, the present disclosure relates to one or more isolated polynucleotides encoding peptides having biological activity. As described herein it is envisioned that the one or more polynucleotides are capable of encoding any polypeptide of interest. It is contemplated that in certain embodiments, one or more polynucleotides described herein will encode a polypeptide having NK cell inhibiting activity. It is further contemplated that in certain embodiments, one or more polynucleotides described herein will encode an antigen (*e.g.*, a

polynucleotide that can be administered as a vaccine). It is still further contemplated that in certain embodiments, one or more polynucleotides will encode a normally endogenously produced protein (e.g., a polynucleotide that can be administered to subjects with, e.g., genetic mutations which result in missing and/or inadequate amounts or function of certain normally endogenously produced proteins) (see, Dolgin, E., The tangled history of mRNA vaccines. *Nature*, 2021. 597(7876): p. 318-324).

The polynucleotides disclosed throughout may be individual polynucleotides or polynucleotide constructs comprising one or more polynucleotides (e.g., one polynucleotide, two polynucleotides, three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on). Further still, the polynucleotides provided herein may be one or more polynucleotides, one or more polynucleotide fragments, and/or combinations thereof. In certain embodiments, the polynucleotides described herein are wild-type polynucleotides. In other embodiments, the polynucleotides described herein are synthetic, that is, the synthetic polynucleotides described herein are not found in nature or are made of or contain one or more synthetic, non-natural, and/or altered nucleotide bases. In still other embodiments, the polynucleotides described herein are recombinant polynucleotides.

Further, it is to be understood that the one or more polynucleotides described herein may or may not comprise an ORF. In certain embodiments, the one or more polynucleotides described herein comprise an ORF. In certain embodiments, the one or more polynucleotides provided herein may or may not further comprise one or more untranslated regions (UTRs). In more specific embodiments the one or more polynucleotides described comprise a 5' UTR. In other specific embodiments, the one or more polynucleotides comprise a 3' UTR. In still other specific embodiments, the one or more polynucleotides comprise a 5' UTR and a 3' UTR. The one or more polynucleotides described herein may or may not further comprise a poly(A) tail and/or 5' cap analog. In particular embodiments, the one or more polynucleotides comprise a poly(A) tail and/or 5' cap. In another specific embodiment, the one or more polynucleotides is an RNA transcript. In an even more specific embodiment, the one or more polynucleotides is a mature RNA transcript. In another specific embodiment, the one or more polynucleotides is an RNA molecule. In an even more specific embodiment, the one or more polynucleotides is a mRNA molecule.

Polynucleotide Constructs:

In certain aspects, the present disclosure describes a polynucleotide construct. In all embodiments disclosed herein, it is to be understood that the resulting polypeptide encoded by

the polynucleotide construct can be an isolated polypeptide, an isolated hybrid polypeptide, an isolated synthetic polypeptide, or an isolated wildtype polypeptide. It is to be further understood that embodiments disclosed herein contemplate polypeptides exhibiting biological activity comprising NK cell inhibiting activity. It is to be further understood that embodiments disclosed herein contemplate polypeptides exhibiting biological activity comprising immunogenic activity.

As described herein, the present disclosure describes a polynucleotide construct comprising one or more polynucleotides (e.g., one polynucleotide, two polynucleotides, three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on).

In one aspect, the present disclosure describes a polynucleotide construct comprising one or more polynucleotides, comprising:

- (a) a first polynucleotide encoding a polypeptide capable of having NK cell inhibiting activity; and
- (b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a normally endogenously produced protein.

In this regard, it should be understood that the first and second polynucleotide may be arranged in the polynucleotide construct in any order. It also should be understood that the first polynucleotide and the second polynucleotide each independently may encode a full-length polypeptide or may encode a fragment of a full-length polypeptide having the specified biological activity (e.g., NK cell inhibiting activity or inducing an immune response or the desired biological activity of the normally endogenously produced protein).

Where more than two polynucleotides (e.g., three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on) are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides may be a polynucleotide or polynucleotide fragment.

In specific embodiments, the first polynucleotide described herein comprises an ORF. In certain embodiments, the first polynucleotide may or may not further comprise one or more untranslated regions (UTRs). In certain embodiments the first polynucleotide comprises a 5' UTR. In other certain other embodiments, the first polynucleotide comprises a 3' UTR. In still certain other embodiments, the first polynucleotide comprises a 5' UTR and a 3' UTR. The first polynucleotide may or may not further comprise a poly(A) tail and/or 5' cap analog. In particular embodiments,

the first polynucleotide comprises a poly(A) tail and/or 5' cap. In a more specific embodiment, the first polynucleotide is an RNA transcript. In an even more specific embodiment, the first polynucleotide is a mature RNA transcript. In a more specific embodiment, the first polynucleotide is an RNA molecule. In an even more specific embodiment, the first polynucleotide is a mRNA molecule.

In specific embodiments, the second polynucleotide described herein comprises an ORF. In certain embodiments, the second polynucleotide may or may not further comprise one or more untranslated regions (UTRs). In certain embodiments the second polynucleotide comprises a 5' UTR. In other certain other embodiments, the second polynucleotide comprises a 3' UTR. In still certain other embodiments, the second polynucleotide comprises a 5' UTR and a 3' UTR. The second polynucleotide may or may not further comprise a poly(A) tail and/or 5' cap analog. In particular embodiments, the second polynucleotide comprises a poly(A) tail and/or 5' cap. In a more specific embodiment, the second polynucleotide is an RNA transcript. In an even more specific embodiment, the second polynucleotide is a mature RNA (mRNA) transcript. In a more specific embodiment, the second polynucleotide is an RNA molecule. In an even more specific embodiment, the second polynucleotide is an mRNA molecule.

Where more than two polynucleotides (*e.g.*, three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on) are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides may comprise an ORF. Further, where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides may or may not further comprise one or more untranslated regions (UTRs). In certain embodiments where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides comprise a 5' UTR. In other certain other embodiments, where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides comprise a 3' UTR. In still certain other embodiments, where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides comprise a 5' UTR and a 3' UTR. In yet certain other embodiments, where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides may or may not further comprise a poly(A) tail and/or a 5' cap analog. In particular embodiments, where more than two polynucleotides are envisioned to carry out embodiments provided herein,

it is contemplated that those additional polynucleotides comprise a poly(A) tail and/or a 5' cap. In still a more specific embodiment, where more than two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides are RNA transcripts, or are RNA molecules. In an even more specific embodiment, where more than
5 two polynucleotides are envisioned to carry out embodiments provided herein, it is contemplated that those additional polynucleotides are mature RNA transcripts, or are mRNA molecules.

In a particular embodiment, the first polynucleotide is a polynucleotide that encodes a serine protease inhibitor (Serpin) polypeptide having biological activity. In still another embodiment, the first polynucleotide is a polynucleotide that encodes a major histocompatibility complex (MHC)
10 polypeptide having biological activity. In yet a more particular embodiment, the first polynucleotide is a polynucleotide that encodes a lectin polypeptide having biological activity. In still yet a more particular embodiment, the first polynucleotide is a polynucleotide that encodes a cadherin polypeptide having biological activity. In yet still a more particular embodiment, the first polynucleotide is a polynucleotide that encodes a CD155 (*i.e.*, polio virus receptor) polypeptide
15 having biological activity. In another more particular embodiment, the first polynucleotide is a polynucleotide that encodes an RTN4 polypeptide having biological activity. In a certain particular embodiment, the first polynucleotide is a polynucleotide that encodes a serpin polypeptide having NK cell inhibiting activity. In still another embodiment, the first polynucleotide is a polynucleotide that encodes an MHC polypeptide having NK cell inhibiting activity. In yet a more particular
20 embodiment, the first polynucleotide is a polynucleotide that encodes a lectin polypeptide having NK cell inhibiting activity. In still yet a more particular embodiment, the first polynucleotide is a polynucleotide that encodes a cadherin polypeptide having NK cell inhibiting activity. In yet still a more particular embodiment, the first polynucleotide is a polynucleotide that encodes a CD155 polypeptide having NK cell inhibiting activity. In another more particular embodiment, the first
25 polynucleotide is a polynucleotide that encodes an RTN4 polypeptide having NK cell inhibiting activity.

In accordance with any of these embodiments, the second polynucleotide may encode an antigen. Alternatively, in accordance with any of the embodiments pertaining to the first polynucleotide, the second polynucleotide may encode a normally endogenously produced protein.

30 Natural Killer Cell Inhibitors:

Natural killer (NK) cells are a type of cytotoxic lymphocyte critical to the innate immune system that belong to the rapidly expanding family of known innate lymphoid cells (ILC) and represent 5 to 20% of circulating lymphocytes in humans. The role of NK cells is analogous to that

of cytotoxic T cells in the vertebrate adaptive immune response. NK cells provide rapid responses to virus-infected cell and other intracellular pathogens acting at around 1–3 days after infection and respond to tumor formation. Unlike other immune cells which detect the major histocompatibility complex (MHC) presented on infected cell surfaces and effect death of the infected cell by lysis or apoptosis, NK cells are unique. NK cells have the ability to recognize and kill stressed cells in the absence of antibodies and MHC. Such a mechanism provides for a faster immune reaction. In addition to natural killer cells being effectors of innate immunity, both activating and inhibitory NK cell receptors play important functional roles (e.g., including self-tolerance and the sustaining of NK cell activity). NK cells also play a role in the adaptive immune response (e.g., experiments have demonstrated the ability of NK cells to respond to the immediate environment, formulate antigen-specific immunological memory, and respond to secondary infections with the same antigen).

In humans, NK cells are generally sorted into two classes; to wit, CD56^{bright}CD16⁻ NK cells, which are regulatory NK cells with greater cytokine-producing capabilities, and CD56^{dim}CD16⁺ NK cells, which are anti-tumor/anti-viral NK cells having enhanced cytotoxic functions (Poznanski, et al., “What defines NK cell functional fate: phenotype or metabolism.” *Frontiers in Immunology*, vol. 10, 2019, www.frontiersin.org/articles/10.3389/fimmu.2019.01414). Moreover, NK cells express a variety of additional receptors which fine tune NK cell activity (Id.). Non-limiting examples of receptors include, but are not limited to, activating Natural Cytotoxicity Receptors (NKp30, NKp44, and NKp46), activating and inhibitory CD94/NKG2 receptors that recognize non-classical MHC, and inhibitory KIR receptors that recognize classical MHC (Id.). The expression of one or more of these receptors can be a way to identify specific NK cell developmental stages, effector subsets, and memory populations (Id.).

Relevant to the subject matter provided herein are inhibitors of NK cells, e.g., polynucleotides and polynucleotide constructs encoding polypeptides that inhibit NK cells. In certain embodiments disclosed throughout, the NK inhibitors can be any NK inhibitor. In more specific embodiments provided herein the one or more NK inhibitors are selected from the group consisting of serine protease inhibitors (serpins), major histocompatibility complex (MHC) molecules, lectins, cadherins, CD155 (i.e., poliovirus receptor) proteins, RTN4 proteins, and combinations thereof.

Serpins:

Serine protease inhibitors (serpins) are a superfamily of proteins of similar structures first identified for their protease inhibition activity and are found in all kingdoms of life. Over 1000 serpins have been identified in animals, plants, fungi, bacteria, archaea, and viruses, and

over 30 human serpin proteins have been specifically identified (Law et al., 2006. An overview of the serpin superfamily. *Genome Biol* 7(5): 216). Serpins are therefore the largest and most diverse superfamily of protease inhibitors. Serpins are notable for their unusual mechanism of action, in which they inhibit their target protease by disrupting the target's active site. Protease inhibition by serpins controls an array of biological processes, including coagulation and inflammation, and consequently these proteins are the target of medical research.

Most serpins are protease inhibitors, targeting extracellular, chymotrypsin-like serine proteases. These proteases possess a nucleophilic serine residue in a catalytic triad in their active site. Serpins act as irreversible, suicide inhibitors by trapping an intermediate of the protease's catalytic mechanism. Some serpins inhibit other protease classes, typically cysteine proteases, and are termed "cross-class inhibitors". These enzymes differ from serine proteases in that they use a nucleophilic cysteine residue, rather than a serine, in their active site. Nonetheless, the enzymatic chemistry is similar, and the mechanism of inhibition by serpins is the same for both classes of protease. Although most serpins control proteolytic cascades, some proteins with a serpin structure are not enzyme inhibitors, but instead perform diverse functions such as storage, transport, and molecular chaperoning. The term serpin is used to describe these members as well, despite their non-inhibitory function, since they are evolutionarily related. Germaine to the present disclosure is the discovery that the SERPINB9 protein has been shown to protect cells from lysis by inhibiting granzyme B, the major effector protease used by both natural killer and cytolytic T-cells.

As disclosed herein, in one embodiment, the one or more polynucleotides is a polynucleotide that encodes a serpin polypeptide. In another embodiment, the one or more polynucleotides encodes one or more serpin polypeptides selected from the group consisting of SERPINA1, SERPINA2, SERPINA3, SERPINA4, SERPINA5, SERPINA6, SERPINA7, SERPINA8, SERPINA9, SERPINA10, SERPINA11, SERPINA12, SERPINA13, SERPINB1, SERPINB2, SERPINB3, SERPINB4, SERPINB5, SERPINB6, SERPINB7, SERPINB8, SERPINB9, SERPINB10, SERPINB11, SERPINB12, SERPINB13, SERPINC1, SERPIND1, SERPINE1, SERPINE2, SERPINE3, SERPINF1, SERPINF2, SERPING1, SERPINH1, SERPINI1, SERPINI2, and combinations thereof.

In still another embodiment, the one or more polynucleotides encodes one or more serpin polypeptide variants selected from the group consisting of SERPINA1, SERPINA2, SERPINA3, SERPINA4, SERPINA5, SERPINA6, SERPINA7, SERPINA8, SERPINA9, SERPINA10,

SERPINA11, SERPINA12, SERPINA13, SERPINB1, SERPINB2, SERPINB3, SERPINB4, SERPINB5, SERPINB6, SERPINB7, SERPINB8, SERPINB9, SERPINB10, SERPINB11, SERPINB12, SERPINB13, SERPINC1, SERPIND1, SERPINE1, SERPINE2, SERPINE3, SERPINF1, SERPINF2, SERPING1, SERPINH1, SERPINI1, SERPINI2, and combinations thereof.

In a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide. In another specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide variant. In another specific embodiment, the one or more polynucleotides encodes a fragment of a SERPINB9 polypeptide capable of having NK cell inhibiting activity.

10 In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide, wherein the one or more polynucleotides has at least 60% sequence identity to the mRNA sequence of SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 65% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a
15 SERPINB9 polypeptide and has at least 70% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 75% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 80% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 85% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 90% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 91% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more
25 polynucleotides encodes a SERPINB9 polypeptide and has at least 92% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 93% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 94% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 95% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 96% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9

polypeptide and has at least 97% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 98% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 99% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 99.5% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 99.9% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has at least 99.99% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide and has 100% sequence identity to SEQ ID NO:1. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the one or more polynucleotides comprises a sequence having 100% sequence identity to SEQ ID NO:1. In a particular specific embodiment, the SERPINB9 polypeptide has SEQ ID NO.: 1.

In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 60% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 65% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 70% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 75% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 80% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 85% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 90% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 91% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 92% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9

polypeptide wherein the SERPINB9 polypeptide has at least 93% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 94% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 95% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 96% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 97% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 98% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 99% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 99.5% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 99.9% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has at least 99.99% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide has 100% sequence identity to SEQ ID NO:5. In yet a more specific embodiment, the one or more polynucleotides encodes a SERPINB9 polypeptide wherein the SERPINB9 polypeptide comprises a sequence having 100% sequence identity to SEQ ID NO:5. In a particular specific embodiment, the encoded SERPINB9 polypeptide has SEQ ID NO: 5.

MHC Proteins:

The major histocompatibility complex (MHC) is a large locus containing a set of closely linked polymorphic genes that code for cell surface proteins (MHC molecules) essential for the adaptive immune system. The MHC gene family is divided into three subgroups: MHC class I, MHC class II, and MHC class III. Among all those genes present in MHC, there are two types of genes coding for the proteins MHC class I molecules and MHC class II molecules that are directly involved in antigen presentation.

MHC class I molecules:

MHC class I molecules are one of the classes of major histocompatibility complex (MHC) molecules and are found on the cell surface of nucleated cells. Their function is to display peptide fragments of proteins from within the cell to cytotoxic T cells (CTLs). Because MHC class I molecules present peptides derived from cytosolic proteins, the pathway of MHC class I presentation is often called the cytosolic or endogenous pathway. Alternatively, class I MHC itself can serve as an inhibitory ligand for natural killer cells (NKs). Reduction in the normal levels of surface class I MHC is a mechanism employed by some viruses and certain tumors to evade CTL responses and is known to activate NK cell killing. MHC-I molecules include HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, and HLA-L.

10 MHC class II molecules:

MHC class II molecules are a class of MHC molecules normally found only on professional antigen-presenting cells such as dendritic cells, mononuclear phagocytes, some endothelial cells, thymic epithelial cells, and B cells. These cells are important in initiating immune responses. The antigens presented by class II molecules are derived from extracellular proteins. Class II molecules interact mainly with immune cells, like the T helper cell (CD4⁺). The peptide (antigen) presented regulates how T cells respond to an infection. Stable peptide binding is essential to prevent detachment and degradation of a peptide, which could occur without secure attachment to the MHC molecule. This would prevent T cell recognition of the antigen, T cell recruitment, and a proper immune response. MHC-II molecules include HLA-DM, HLA-DO, HLA-DP, HLA-DQ, and HLA-DR.

20 MHC class III molecules:

MHC class III is another class of MHC molecules. Unlike other MHC types, MHC class III molecules are poorly defined structurally and functionally. MHC class III molecules do not appear to be involved in antigen binding, and only a few appear to be involved in immunity. Others, however, appear to be signaling molecules involved in cell communication.

As disclosed herein, in one embodiment, the one or more polynucleotides encodes one or more MHC molecules (polypeptides) selected from the group consisting of MHC class I, MHC class II, and MHC class III polypeptides, and combinations thereof. In still another embodiment, the one or more polynucleotides encodes one or more MHC class I polypeptides. In yet another embodiment, the one or more polynucleotides encodes one or more MHC class I polypeptides selected from the group consisting of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, HLA-L, and combinations thereof. In another embodiment, the one or more polynucleotides

encodes one or more MHC class II polypeptides. In still another embodiment, the one or more polynucleotides encodes one or more MHC class II polypeptides selected from the group consisting of HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR, and combinations thereof. In still yet another embodiment, the one or more polynucleotides encodes one or more MHC class III polypeptides. In yet still another embodiment, the one or more polynucleotides encodes one or more MHC class polypeptides selected from the group consisting of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR, and combinations thereof. In yet still another embodiment, the one or more polynucleotides polynucleotide encodes one or more MHC class polypeptide variants selected from the group consisting of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, HLA-L, HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR, and combinations thereof.

In an even more specific embodiment, the one or more polynucleotides encodes the MHC class I polypeptide HLA-E. In still yet another specific embodiment, the one or more polynucleotides encodes an HLA-E polypeptide variant. In another specific embodiment, the one or more polynucleotides encodes a fragment of an HLA-E polypeptide capable of having NK cell inhibiting activity.

In still a more specific embodiment, the one or more polynucleotides encodes MHC class I polypeptide HLA-E*0103. In another specific embodiment, the one or more polynucleotides encodes an HLA-E*0103 polypeptide variant. In another specific embodiment, the one or more polynucleotides encodes a fragment of an HLA-E*0103 polypeptide capable of having NK cell inhibiting activity.

In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 60% sequence identity to the mRNA sequence of SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 65% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 70% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 75% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 80% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103

polypeptide wherein the one or more polynucleotides has at least 85% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 90% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a

5 HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 91% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 92% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has

10 at least 93% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 94% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 95% sequence identity to SEQ ID NO:2. In yet a more

15 specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 96% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 97% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-

20 E*0103 polypeptide wherein the one or more polynucleotides has at least 98% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 99% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 99.5%

25 sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 99.9% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has at least 99.99% sequence identity to SEQ ID NO:2. In yet a more specific

30 embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides has 100% sequence identity to SEQ ID NO:2. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the one or more polynucleotides comprises a sequence having 100% sequence identity to SEQ ID

NO:2. In a particular specific embodiment, the one or more polynucleotides encodes an HLA-E*0103 polypeptide wherein the one or more polynucleotides has SEQ ID NO:2.

In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide has at least 60% sequence identity to SEQ ID

[illegible]

least 99% sequence identity to SEQ ID NO:6. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide has at least 99.5% sequence identity to SEQ ID NO:6. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide has at least 99.9% sequence identity to SEQ ID NO:6. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide has at least 99.99% sequence identity to SEQ ID NO:6. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide has 100% sequence identity to SEQ ID NO:6. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0103 polypeptide wherein the HLA-E*0103 polypeptide comprises a sequence having 100% sequence identity to SEQ ID NO:6. In a particular specific embodiment, the one or more polynucleotides encodes an HLA-E*0103 polypeptide wherein the encoded HLA-E*0103 polypeptide has SEQ ID NO: 6.

In still a more specific embodiment, the one or more polynucleotides encodes MHC class I polypeptide HLA-E*0101. In another specific embodiment, the one or more polynucleotides encodes an HLA-E*0101 polypeptide variant. In another specific embodiment, the one or more polynucleotides encodes a fragment of an HLA-E*0101 polypeptide capable of having NK cell inhibiting activity.

In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 60% sequence identity to the mRNA sequence of SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 65% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 70% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 75% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 80% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 85% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 90% sequence identity

to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 91% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 92% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 93% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 94% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 95% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 96% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 97% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 98% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 99% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 99.5% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 99.9% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has at least 99.99% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides has 100% sequence identity to SEQ ID NO:3. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the one or more polynucleotides comprises a sequence having 100% sequence identity to SEQ ID NO:3. In a particular specific embodiment, the one or more polynucleotides encodes an HLA-E*0101 polypeptide wherein the one or more polynucleotides has SEQ ID NO:3.

In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the HLA-E*0101 polypeptide has at least 60% sequence identity to SEQ ID

[illegible]

has at least 99.9% sequence identity to SEQ ID NO:7. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the HLA-E*0101 polypeptide has at least 99.99% sequence identity to SEQ ID NO:7. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the HLA-E*0101 polypeptide has 100% sequence identity to SEQ ID NO:7. In yet a more specific embodiment, the one or more polynucleotides encodes a HLA-E*0101 polypeptide wherein the HLA-E*0101 polypeptide comprises a sequence having 100% sequence identity to SEQ ID NO:7. In a particular specific embodiment, the one or more polynucleotides encodes an HLA-E*0101 polypeptide wherein the encoded HLA-E*0101 polypeptide has SEQ ID NO:7.

10 Lectin Polypeptides:

Lectins and C-type lectins are a heterogeneous group of proteins with a variety of different functions. There are two C-type lectins found in the Natural Killer gene Complex (NKG), Killer cell lectin-like receptor subfamily B, member 1 (also known as KLRB1 or CD161) and C-type lectin domain family 2 member D (CLEC2D). LLT1 proteins are particularly germane to this disclosure for their role in NK cell and B cell regulation (Llibre et al. 2016. Multi-functional lectin-like transcript-1: A new player in human immune regulation. *Immunol Lett.* 177: 62-69). Non-limiting examples of lectins and c-type lectins polypeptides include CD161, Clec2d8 (mouse), Clec2d11 (rat), and CLEC2d (human).

As disclosed herein, in one embodiment, the one or more polynucleotides encodes one or more lectin polypeptides. In another embodiment, the one or more polynucleotides encodes one or more lectin polypeptides selected from the group consisting of Clec2d8 (mouse), Clec2d11 (rat), CLEC2D (human), and combinations thereof.

In a more specific embodiment, the one or more polynucleotides encodes one or more lectin polypeptides selected from the group consisting of Clec2d8 (mouse), Clec2d11 (rat), CLEC2D (human), and combinations thereof.

In an even more specific embodiment, the one or more polynucleotides encodes the lectin polypeptide CLEC2D. In still yet another specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide variant. In another specific embodiment, the one or more polynucleotides encodes a fragment of a CLEC2D polypeptide capable of having NK cell inhibiting activity.

In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the one or more polynucleotides has at least 60% sequence identity to the

[illegible]

- has at least 99.9% sequence identity to SEQ ID NO:4. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the one or more polynucleotides has at least 99.99% sequence identity to SEQ ID NO:4. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the one or more polynucleotides has 100% sequence identity to SEQ ID NO:4. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the one or more polynucleotides comprises a sequence having 100% sequence identity to SEQ ID NO:4. In a particular specific embodiment, the one or more polynucleotides encodes an CLEC2D polypeptide wherein the one or more polynucleotides has SEQ ID NO:4.
- 10 In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 60% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 65% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 70% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 75% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 80% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 85% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 90% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 91% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 92% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 93% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 94% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 95% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D

polypeptide wherein the CLEC2D polypeptide has at least 96% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 97% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 98% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 99% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 99.5% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 99.9% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has at least 99.99% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide has 100% sequence identity to SEQ ID NO:8. In yet a more specific embodiment, the one or more polynucleotides encodes a CLEC2D polypeptide wherein the CLEC2D polypeptide comprises a sequence having 100% sequence identity to SEQ ID NO:8. In a particular specific embodiment, the one or more polynucleotides encodes an CLEC2D polypeptide wherein the encoded CLEC2D polypeptide has SEQ ID NO:8.

20 *Cadherin Polypeptides:*

Calcium-dependent adhesion molecules, or cadherins, are cell adhesion molecules important in forming adherens junctions allowing cells to adhere to each other. Cadherins are a class of type-I transmembrane proteins and are calcium (Ca^{2+}) ion dependent. The cadherin superfamily is essential in maintaining cell-cell contact and regulating cytoskeletal complexes and includes cadherins, protocadherins, desmosomal cadherins, and more.

Classical Cadherins:

Generally, classical cadherins take a role in cell layer formation and structure formation, desmosomal cadherins focus on resisting cell damage. Classical cadherins include CDH1 (e-cadherin), CDH2, CDH3, and CDH12.

30 *Desmosomal Cadherins:*

There are two types of desmosomal cadherins, desmogleins and desmocollins, both of which play roles in maintaining the function of desmosomes by mitigating the mechanical stress of tissues.

Desmogelins include DSG1, DSG2, DSG3, and DSG4. Desmocollins include DSC1, DSC2, DSC3, and DSC4.

Protocadherins:

Protocadherins are the largest mammalian subgroup of the cadherin superfamily of homophilic cell-adhesion proteins and have been shown to mediate cell-cell adhesion, particularly in cells of the nervous system. Protocadherins include PCDH1, PCDH7, PCDH8, PCDH9, PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17, PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, and FAT4.

15 Ungrouped cadherins:

The following are ungrouped cadherins, that is, cadherins that do not group in any of the aforementioned cadherin subgroups (*i.e.*, classical cadherins, desmosomal cadherins, or protocadherins). Ungrouped cadherins include CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH23, CDH22, CDH24, CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, and RET.

As disclosed herein, in one embodiment, the one or more polynucleotides encodes one or more cadherin polypeptides. In another embodiment, the one or more polynucleotides encodes one or more cadherin polypeptides selected from the group consisting of classical cadherins, desmosomal cadherins, protocadherins, ungrouped cadherins, and combinations thereof.

In still another embodiment, the one or more polynucleotides encodes one or more classical polypeptides selected from the group consisting of CDH1 (e-cadherin), CDH2, CDH3, CDH12, and combinations thereof.

In yet another embodiment, the one or more polynucleotides encodes one or more desmosomal cadherin polypeptides selected from the group consisting of desmogleins, desmocollins and combinations thereof.

In still yet another embodiment, the one or more polynucleotides encodes one or more desmoglein cadherin polypeptides selected from the group consisting of DSG1, DSG2, DSG3, DSG4, and combinations thereof.

5 In yet still another embodiment, the one or more polynucleotides encodes one or more desmocollins cadherin polypeptides selected from the group consisting of DSC1, DSC2, DSC3, DSC4, and combinations thereof.

In another embodiment, the one or more polynucleotides encodes one or more protocadherin polypeptides selected from the group consisting of PCDH1, PCDH7, PCDH8, PCDH9, PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17, PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, 10 PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, 15 PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, FAT4, combinations thereof.

In another embodiment, the one or more polynucleotides encodes one or more ungrouped cadherin polypeptides selected from the group consisting of CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH23, 20 CDH22, CDH24, CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET, and combinations thereof.

In still another embodiment, the one or more polynucleotides encodes one or more cadherin polypeptides selected from the group consisting of CDH1 (e-cadherin), CDH2, CDH3, CDH12, DSG1, DSG2, DSG3, DSG4, DSC1, DSC2, DSC3, DSC4, PCDH1, PCDH7, PCDH8, PCDH9, 25 PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17, PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, 30 PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, FAT4, CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18,

CDH19, CDH20, CDH23, CDH22, CDH24, CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET, and combinations thereof.

In still another embodiment, the one or more polynucleotides encodes one or more cadherin polypeptide variants selected from the group consisting of CDH1 (e-cadherin), CDH2, CDH3, CDH12, DSG1, DSG2, DSG3, DSG4, DSC1, DSC2, DSC3, DSC4, PCDH1, PCDH7, PCDH8, PCDH9, PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17, PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, FAT4, CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH23, CDH22, CDH24, CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET, and combinations thereof.

In a more specific embodiment, the one or more polynucleotides encodes a CDH1 (e-cadherin) polypeptide. In a more specific embodiment, the one or more polynucleotides encodes a variant CDH1 (e-cadherin) polypeptide. In another specific embodiment, the one or more polynucleotides encodes a fragment of a CDH1 (e-cadherin) polypeptide capable of having NK cell inhibiting activity.

CD155 (Poliovirus Receptor or "PVR"):

CD155 (cluster of differentiation 155), also known as the poliovirus receptor because it is the molecule poliovirus uses to enter cells, is a protein encoded by the *CDR* gene in humans. The CD155 gene is specific to the primate lineage and encodes a Type I transmembrane glycoprotein in the immunoglobulin superfamily. Its normal cellular function is in the establishment of intercellular adherens junctions between epithelial cells. The role of CD155 in the immune system is unclear, though it may be involved in intestinal humoral immune responses. Subsequent data has also suggested that CD155 may also be used to positively select MHC-independent T cells in the thymus.

In specific embodiments provided herein, the one or more polynucleotides encodes a CD155 (poliovirus receptor) polypeptide. In a more specific embodiment, the one or more polynucleotides encodes a variant CD155 (poliovirus receptor) polypeptide. In another specific embodiment, the one or more polynucleotides encodes a fragment of a CD155 (poliovirus receptor) polypeptide capable of having NK cell inhibiting activity.

Nogo receptor 1 (also referred to as NgR1):

Nogo-66 Receptor (NgR) or Nogo receptor 1 (NgR1) is encoded by the *RTN4R* gene in humans and NgR1 plays a role in axonal growth inhibition and possibly in the regulation of axonal regeneration and plasticity in the central nervous system. NgR1 is implicated in neuronal plasticity and regeneration. Germane to this disclosure is the recent discovery that NgR1 is capable of inhibiting natural killer cell activity. Recently, it has been demonstrated that NgR1 has a role in inhibiting natural killer cell-mediated killing by destabilizing immunological synapse formation through binding to its ligand NogoA (RTN4 in humans) on target cells (Oh et al., 2023. NgR1 is an NK cell inhibitory receptor that destabilizes the immunological synapse. *Nature Immunology* 24:463-473).

In specific embodiments provided herein, the one or more polynucleotides encodes an RTN4 polypeptide. In a more specific embodiment, the one or more polynucleotides encodes a variant RTN4 polypeptide. In another specific embodiment, the one or more polynucleotides encodes a fragment of a RTN4 polypeptide capable of having NK cell inhibiting activity.

Antigens and Normally Endogenously Produced Proteins:

As noted above, the second polypeptide (e.g., one or more second polypeptides) encodes a polypeptide capable of inducing an immune response (such as an antigen) or a normally endogenously produced protein (e.g., a polynucleotide that can be administered to subjects with, e.g., genetic mutations which result in missing and/or inadequate amounts or function of certain normally endogenously produced proteins) (Dolgin, E., The tangled history of mRNA vaccines. *Nature*, 2021. 597(7876): p. 318-324).

The polypeptide capable of inducing an immune response (such as an antigen) is not particularly limited. Although the examples below illustrate embodiments with reference to vaccines against coronavirus-19 (e.g., where the second polynucleotide would encode a COVID-19 antigen) the present disclosure encompasses embodiments where the second polypeptide encodes a polypeptide capable of inducing an immune response against a different pathogen or against a tumor cell. Non-limiting examples include vaccines against influenza, coronaviruses, *Clostridium*

difficile, HIV, malaria, norovirus, etc. In each case, the second polypeptide (e.g., one or more second polypeptides) would encode a polypeptide capable of inducing an immune response against the target, e.g., an influenza antigen, a coronavirus antigen, a *Clostridium difficile* antigen, an HIV antigen, a malaria antigen, a norovirus antigen, etc.

- 5 The normally endogenously produced protein is not particularly limited. As set forth above, the normally endogenously produced protein may be any normally endogenously produced protein that is inadequately produced in the target subject (patient) such as due to a genetic mutation which results in inadequate amounts the protein or functionally deficient variants of the protein. Non-limiting examples include factor VIII, which could be used to treat hemophilia. Thus, in
10 specific embodiments, the second polynucleotide encodes factor VIII or a biologically active fragment thereof.

CELL HOSTS EXPRESSING THE DISCLOSED POLYNUCLEOTIDES AND POLYNUCLEOTIDE CONSTRUCTS

- In another aspect, the disclosure contemplates host cells expressing the polynucleotides and/or
15 polynucleotide constructs disclosed herein. In certain embodiments, the cell hosts will be genetically engineered to express the polynucleotides and/or polynucleotide constructs. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequence of the polypeptide or polypeptides of interest as well as any required and necessary transcriptional/translational control sequences and/or signals. These methods include,
20 but are not limited to, *in vitro* recombinant DNA techniques, synthetic techniques, and in vivo recombination/genetic recombination. See, for example, the techniques described in Maniatis et al., 1989, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory, N.Y. and Ausubel et al., 1989, Current Protocols in Molecular Biology, Greene Publishing Associates and Wiley Interscience, N.Y.

- 25 The host cell may be any cell useful in the production of the polypeptides and/or polypeptide constructs disclosed herein, e.g., a prokaryote or a eukaryote. More specifically, the host cell may be, but is not limited to, a microbial cell, a fungal cell, an insect cell, a mammalian cell, and/or a plant cell.

- Microbial expression hosts may be preferred because of their ease of use and the broad
30 technology platforms that are readily available for these organisms. In certain embodiments, the prokaryotic host cell may be any Gram-positive or Gram-negative bacterium. Gram-positive bacteria include, but are not limited to, *Bacillus*, *Clostridium*, *Enterococcus*, *Geobacillus*, *Lactobacillus*, *Lactococcus*, *Oceanobacillus*, *Staphylococcus*, *Streptococcus*, and *Streptomyces*.

Gram-negative bacteria include, but are not limited to, *Campylobacter*, *E. coli*, *Flavobacterium*, *Fusobacterium*, *Helicobacter*, *Ilyobacter*, *Neisseria*, *Pseudomonas*, *Salmonella*, and *Ureaplasma*.

The bacterial host cell may be any *Bacillus* cell including, but not limited to, *Bacillus alkalophilus*, *Bacillus amyloliquefaciens*, *Bacillus brevis*, *Bacillus circulans*, *Bacillus clausii*, *Bacillus coagulans*,
5 *Bacillus firmus*, *Bacillus lautus*, *Bacillus lentus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus stearothermophilus*, *Bacillus subtilis*, and/or *Bacillus thuringiensis* cells.

The bacterial host cell may also be any *Streptococcus* cell including, but not limited to, *Streptococcus equisimilis*, *Streptococcus pyogenes*, *Streptococcus uberis*, and/or *Streptococcus equi* subsp. *Zooepidemicus* cells.

10 The bacterial host cell may also be any *Streptomyces* cell including, but not limited to, *Streptomyces achromogenes*, *Streptomyces avermitilis*, *Streptomyces coelicolor*, *Streptomyces griseus*, and/or *Streptomyces lividans* cells.

In another embodiment, the host cell may also be a eukaryote, such as a fungal, insect, plant, and/or mammalian cells.

15 In a more specific embodiment, the host cell may be a fungal cell. "Fungi" as used herein includes the phyla Ascomycota, Basidiomycota, Chytridiomycota, and Zygomycota as well as the Oomycota and all mitosporic fungi (as defined by Hawksworth *et al.*, In, *Ainsworth and Bisby's Dictionary of The Fungi* 8th edition, 1995, CAB International, University Press, Cambridge, UK).

The fungal host cell may be a yeast cell. "Yeast" as used herein includes ascosporogenous yeast
20 (Endomycetales), basidiosporogenous yeast, and yeast belonging to the Fungi Imperfecti (Blastomycetes). Since the classification of yeast may change in the future, for the purposes of this disclosure, yeast shall be defined as described in *Biology and Activities of Yeast* (Skinner, Passmore, and Davenport, editors, *Soc. App. Bacteriol. Symposium Series No. 9*, 1980).

The yeast host cell may be a *Candida*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces*,
25 *Schizosaccharomyces*, or *Yarrowia* cell, such as a *Kluyveromyces lactis*, *Saccharomyces carlsbergensis*, *Saccharomyces cerevisiae*, *Saccharomyces diastaticus*, *Saccharomyces douglasii*, *Saccharomyces kluyveri*, *Saccharomyces norbensis*, *Saccharomyces oviformis*, and/or a *Yarrowia lipolytica* cell.

The fungal host cell may be a filamentous fungal cell. "Filamentous fungi" include all filamentous
30 forms of the subdivision Eumycota and Oomycota. The filamentous fungal host cell includes, but is not limited to, *Acremonium*, *Aspergillus*, *Aureobasidium*, *Bjerkandera*, *Ceriporiopsis*,

Chrysosporium, *Coprinus*, *Coriolus*, *Cryptococcus*, *Filibasidium*, *Fusarium*, *Humicola*, *Magnaporthe*, *Mucor*, *Myceliophthora*, *Neocallimastix*, *Neurospora*, *Paecilomyces*, *Penicillium*, *Phanerochaete*, *Phlebia*, *Piromyces*, *Pleurotus*, *Schizophyllum*, *Talaromyces*, *Thermoascus*, *Thielavia*, *Tolypocladium*, *Trametes*, and/or *Trichoderma* cells.

- 5 For example, the filamentous fungal host cell may be an *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus fumigatus*, *Aspergillus japonicus*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Bjerkandera adusta*, *Ceriporiopsis aneirina*, *Ceriporiopsis caregiea*, *Ceriporiopsis gilvescens*, *Ceriporiopsis pannocinta*, *Ceriporiopsis rivulosa*, *Ceriporiopsis subrufa*, *Ceriporiopsis subvermispora*, *Chrysosporium inops*, *Chrysosporium keratinophilum*,
 10 *Chrysosporium lucknowense*, *Chrysosporium merdarium*, *Chrysosporium pannicola*, *Chrysosporium queenslandicum*, *Chrysosporium tropicum*, *Chrysosporium zonatum*, *Coprinus cinereus*, *Coriolus hirsutus*, *Fusarium bactridioides*, *Fusarium cerealis*, *Fusarium crookwellense*, *Fusarium culmorum*, *Fusarium graminearum*, *Fusarium graminum*, *Fusarium heterosporum*, *Fusarium negundi*, *Fusarium oxysporum*, *Fusarium reticulatum*, *Fusarium roseum*, *Fusarium sambucinum*, *Fusarium sarcochroum*, *Fusarium sporotrichioides*, *Fusarium sulphureum*,
 15 *Fusarium torulosum*, *Fusarium trichothecioides*, *Fusarium venenatum*, *Humicola insolens*, *Humico40estertii40osa*, *Mucor miehei*, *Myceliophthora thermophila*, *Neurospora crassa*, *Penicillium purpurogenum*, *Phanerochaete chrysosporium*, *Phlebia radiata*, *Pleurotus eryngii*, *Thielavia terrestris*, *Trametes villosa*, *Trametes versicolor*, *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma reesei*, and/or a *Trichoderma viride* cell.

In still another embodiment, the host cell may be an insect cell.

In a more specific embodiment, the host cell may include, but are not limited to, those cells derived from lepidopterans, hymenopterans, dipterans, and/or coleopterans.

- The insect host cell includes, but is not limited to, cells derived from insects from *Bombyx*,
 25 *Drosophila*, *Spodoptera*, and/or *Trichiplusia*.

- For example, the insect host cell may be a *Bombyx horsfieldi*, *Bombyx huttoni*, *Bombyx incomposita*, *Bombyx lemeepauli*, *Bombyx mandarina*, *Bombyx mori*, *Bombyx rotundapex*, *Bombyx shini*, *Drosophila ananassae*, *Drosophila erecta*, *Drosophila grimshawi*, *Drosophila mauritiana*, *Drosophila melanogaster*, *Drosophila mojavensis*, *Drosophila persimilis*, *Drosophila pseudoobscura*, *Drosophila sechellia*, *Drosophila simulans*, *Drosophila virilis*, *Drosophila willistoni*, *Drosophila yakuba*, *Spodopte estertiinia*, *Spodopte esterula*, *Spodoptera androgea*,
 30 *Spodopte estertiata*, *Spodoptera apertura*, *Spodoptera cilium*, *Spodoptera compta*, *Spodoptera*

connexa, *Spodoptera depravata*, *Spodoptera dolichos*, *Spodoptera eridania*, *Spodoptera evanida*, *Spodoptera excelsa*, *Spodoptera exempta*, *Spodoptera exigua*, *Spodoptera fasciculata*, *Spodoptera frugiperda*, *Spodoptera hipparis*, *Spodoptera latifascia*, *Spodoptera littoralis*, *Spodoptera litura*, *Spodopte estertiasy*, *Spodoptera marima*, *Spodopte estertiia*, *Spodoptera ochrea*, *Spodoptera ornithogalli*, *Spodoptera pecten*, *Spodoptera pectinicornis*, *Spodoptera peruviana*, *Spodoptera picta*, *Spodoptera praefica*, *Spodoptera pulchella*, *Spodoptera roseae*, *Spodopte41estertiuna*, *Spodoptera teferii*, *Spodoptera triturate*, *Spodoptera umbraculata*, *Trichoplusia arachnoides*, *Trichoplus esternea*, *Trichoplusia callista*, *Trichoplusia cinnabarina*, *Trichoplusia cupreomicans*, *Trichoplusia elacheia*, *Trichoplusia epicharis*, *Trichoplusia glyceia*, *Trichoplusia gromieri*, *Trichoplusia lectula*, *Trichoplusia lampra*, *Trichoplusia ni*, *Trichoplusia obtusisigna*, *Trichoplusia orichalcea*, *Trichoplusia photeina*, *Trichoplusia roseofasciata*, *Trichoplus estertiitia*, *Trichoplusia sogai*, *Trichoplusia telaugae*, and/or *Trichoplusia tetrastigma* cell.

In a specific embodiment, the host cell may be a *Bombyx mori* (i.e., silkworm), *Drosophila melanogaster* (i.e., fruit fly), *Spodoptera frugiperda* (i.e., army worm), *Trichoplusia ni* (i.e., cabbage looper), and/or a *Aedes* sp (i.e., any species of mosquito, more specifically, *aedes aegypti*, or yellow fever mosquito).

In a still more specific embodiment, the host cell may be the larva of *Bombyx mori* (i.e., silkworm), *Drosophila melanogaster* (i.e., fruit fly), *Spodoptera frugiperda* (i.e., army worm), *Trichoplusia ni* (i.e., cabbage looper), and/or a *Aedes* sp. (i.e., any species of mosquito, more specifically, *aedes aegypti*, or yellow fever mosquito).

In yet a more specific embodiment, the host cell are those cells derived from moth (ATCC CCL 80), army worm (ATCC CRL 1711), mosquito larvae (ATCC lines CCL 125, CCL 126, CRL 1660, CRL 1591, CRL 6585, CRL 6586) and silkworm (ATCC CRL 8851). In especially preferred embodiments, the cell line is a *Drosophila* cell line, such as a Schneider 2 cell line (see, e.g., Schneider, 1972, *J. Embryol. Exp. Morph.*, Vol 27, pp. 353-365), a cell line derived from *Spodoptera*, such as Sf9 cells, Sf21 cells, or expressSF⁺, or a cell line derived from *Trichoplusia*, such as Tn5 cells, H5 cells, and High-Five™ (Invitrogen) cells.

The insect cells expressing the polypeptides disclosed herein may be constructed in accordance with methods known in the art (Lindskog, et al., *Biopharmaceutical Processing*, Elsevier, 2018, Pages 111-130, ISBN 9780081006238, doi: 10.1016/B978-0-08-100623-8.00006-2.).

In specific embodiments, mammalian cells are the hosts for the production of the polypeptides encoded from the polynucleotide constructs described herein, due to their capability to glycosylate proteins in the most compatible form for human application.

In a specific embodiment, the mammalian host cell is a human cell and/or a non-human cell.

- 5 In an even more specific embodiment, the host cell is a hamster cell, a murine cell, or a human cell.

In still an even more specific embodiment, the host cell is a Chinese hamster cell (CHO) cell a baby hamster kidney (BHK) cell, and/or a mouse myeloma cell (*e.g.*, NS0, SP2/0, etc.).

- 10 Hosts contemplated herein also include plants, *e.g.*, a transgenic plant, plant part, or plant cell, comprising a polynucleotide of the present disclosure so as to express and produce the polypeptides disclosed herein in recoverable quantities. The transgenic plant can be dicotyledonous (a dicot) or monocotyledonous (a monocot). Examples of monocot plants include, but are not limited to, grasses, such as meadow grass (blue grass, *Poa*), forage grass such as *Festuca*, *Lolium*, temperate grass, such as *Agrostis*, and cereals, *e.g.*, wheat, oats, rye, barley, 15 rice, sorghum, and maize (corn).

Examples of dicot plants include, but are not limited to, tobacco, legumes, such as lupins, potato, sugar beet, pea, bean and soybean, and cruciferous plants (family *Brassicaceae*), such as cauliflower, rape seed, and the closely related model organism *Arabidopsis thaliana*.

- 20 Examples of plant parts are stem, callus, leaves, root, fruits, seeds, and tubers as well as the individual tissues comprising these parts, *e.g.*, epidermis, mesophyll, parenchyme, vascular tissues, meristems. Specific plant cell compartments, such as chloroplasts, apoplasts, mitochondria, vacuoles, peroxisomes and cytoplasm are also considered to be a plant part. Furthermore, any plant cell, whatever the tissue origin, is considered to be a plant part. Likewise, plant parts such as specific tissues and cells isolated to produce the polypeptides disclosed herein 25 are also considered plant parts, *e.g.*, embryos, endosperms, aleurone and seed coats.

Also included within the scope of the present disclosure are the progeny of such plants, plant parts, and plant cells.

- The transgenic plant or plant cell expressing the polypeptides disclosed herein may be constructed in accordance with methods known in the art. In short, the plant or plant cell is 30 constructed by incorporating one or more expression constructs encoding the hybrid polypeptide

into the plant host genome or chloroplast genome and propagating the resulting modified plant or plant cell into a transgenic plant or plant cell.

Useful methods for measuring the expression of the polypeptides described herein are well known in the art (e.g., Southern blots (DNA detection), dot or slot blots (DNA, RNA), Northern blots (RNA), and RT-PCR (RNA) analyses). Other useful methods include determining the translation state of the cell by measuring the abundances of the constituent protein species present in the cell using processes well known in the art (e.g., Western blots (protein detection)).

COMPOSITIONS:

In another aspect, the disclosure contemplates compositions comprising one or more of the polynucleotide sequences described herein and a carrier. The compositions described herein may be prepared in accordance with acceptable pharmaceutical procedures, such as described in *Remington's Pharmaceutical Sciences*, 17th edition, ed. Alfonso R. Gennaro, Mack Publishing Company, Easton, Pa. (1985).

In one embodiment, the present disclosure describes a composition comprising:

- (a) one or more of the polynucleotide constructs described herein; and
- (b) a pharmaceutically acceptable carrier.

In another embodiment, the present disclosure describes a composition comprising:

- (a) one or more of the polynucleotide constructs described herein;
- (b) one or more of the polynucleotides described herein (e.g., one polynucleotide, two polynucleotides, three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on); and
- (c) a pharmaceutically acceptable carrier.

In still another embodiment, the present disclosure describes a composition comprising one or more polynucleotides (e.g., one polynucleotide, two polynucleotides, three polynucleotides, four polynucleotides, five polynucleotides, six polynucleotides, seven polynucleotides, eight polynucleotides, nine polynucleotides, ten polynucleotides, and so on), comprising:

- (a) a first polynucleotide encoding a polypeptide capable of having NK cell inhibiting activity, and

(b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a normally endogenously produced protein; and

(c) a pharmaceutically acceptable carrier.

It is to be understood that the ratio of first polynucleotide to second polynucleotide can be any ratio effective to achieve desired effect (e.g., enhancing the durability of one or more exogenously delivered polynucleotides or reducing the unwanted side effects of one or more exogenously delivered polynucleotides). For example the molar ratio of first polynucleotide to second polynucleotide may be from about 100:1 to 1:100 (e.g., the molar ratio of first polynucleotide to second polynucleotide may be from 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, :5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, or 1:100, or the molar ratio of second polynucleotide to first polynucleotide may be from 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, :5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, or 1:100). Additionally or alternatively, the weight ratio of first polynucleotide to second polynucleotide may be from about 100:1 to 1:100, and about 1:100 (e.g., the weight ratio of first polynucleotide to second polynucleotide may be from 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, :5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, or 1:100 or the weight ratio of second polynucleotide to first polynucleotide may be from 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:3.5, 1:4, 1:4.5, :5, 1:5.5, 1:6, 1:6.5, 1:7, 1:7.5, 1:8, 1:8.5, 1:9, 1:9.5, 1:10, 1:15, 1:20, 1:25, 1:30, 1:35, 1:40, 1:45, 1:50, 1:55, 1:60, 1:65, 1:70, 1:75, 1:80, 1:85, 1:90, 1:95, or 1:100).

In a particular embodiment, the molar ratio of first polypeptide to second polypeptide is, or is about, 1:1.

In a particular embodiment, the molar ratio of first polypeptide to second polypeptide is, or is about, 1:100.

In a particular embodiment, the molar ratio of first polypeptide capable of having NK cell inhibiting activity to second polypeptide encoding an antigen is, or is about, 1:1.

In a particular embodiment, the molar ratio of first polypeptide capable of having NK cell inhibiting activity to second polypeptide encoding an antigen is, or is about, 1:100.

In a particular embodiment, the molar ratio of first polypeptide capable of having NK cell inhibiting activity to second polypeptide encoding a normally endogenously produced protein is, or is about, 1:1.

In a particular embodiment, the molar ratio of first polypeptide capable of having NK cell inhibiting activity to second polypeptide encoding a normally endogenously produced protein is, or is about, 1:100.

Carriers:

- 5 The carrier (*i.e.*, the pharmaceutically acceptable carrier) typically will have properties (*e.g.*, viscosity, yield value, shear stress, shear rate, etc.) that allow the compositions disclosed herein to remain efficacious (*e.g.*, capable of enhancing the durability of a vaccine, capable of reducing the unwanted side effects of a vaccine, etc.) once formulated. The carrier typically will be compatible with the polynucleotides described herein and optionally will be capable of stabilizing
- 10 them. One or more solubilizing agents can be utilized as or present in a carrier suitable for the polynucleotides described herein. Examples of acceptable carriers include, but are not limited to, biocompatible vehicles, adjuvants, additives, and diluents to achieve a composition usable as a dosage form for the intended route of administration. Examples of other carriers include, but are not limited to, colloidal silicon oxide, magnesium stearate, cellulose, and sodium lauryl sulfate.
- 15 Additional suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences.

In a specific embodiment, the carrier is a solid carrier. In a more specific embodiment, suitable solid carriers include, but are not limited to, calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, methyl cellulose, sodium carboxymethyl cellulose,

20 polyvinylpyrrolidone, low melting waxes, ion exchange resins, and combinations thereof.

The solid carriers described herein may further contain one or more substances, including, but not limited to, flavoring agents, lubricants, solubilizers, suspending agents, fillers, glidants, compression aids, binders or tablet-disintegrating agents or an encapsulating material.

In a specific embodiment, the carrier is a liquid carrier. In more a specific embodiment, the liquid carrier is water, an organic solvent, an oil or fat, and combinations thereof. In an even more specific embodiment, suitable liquid carriers include, but are not limited to, water, alcohols (*e.g.*, monohydric alcohols and polyhydric alcohols, *e.g.*, glycols) and their derivatives, sugar alcohols (*e.g.*, erythritol, arabitol, ribitol, isomalt, polyglycitol, maltitol, dulcitol, iditol, mannitol, xylitol, lactitol, sorbitol, etc.), and oils and fats (*e.g.*, short-chain triglycerides, medium-chain triglycerides,

30 long-chain triglycerides, saturated, monounsaturated, and/or polyunsaturated oils, soybean oil, coconut oil, canola oil, safflower oil, olive oil, corn oil, cottonseed oil, linseed oil, safflower oil, palm oil, peanut oil, flaxseed oil, sunflower oil, rice bran oil, sesame oil, rapeseed oil, cocoa butter,

almond oil, cashew oil, hazelnut oil, macadamia oil, mongongo nut oil, pecan oil, pine nut oil, pistachio oil, sachainchi oil, walnut oil, bottle gourd oil, buffalo gourd oil, butternut squash seed oil, pumpkin seed oil, watermelon seed oil, acai oil, blackcurrant seed oil, borage seed oil, evening primrose oil, carob pod oil, amaranth oil, apricot oil, apricot kernel oil, apple seed oil, argan oil, artichoke oil, avocado oil, babassu oil, ben oil, borneo tallow nut oil, cape chestnut oil, *cassia* oil, 5 cocoa butter, cocklebur oil, cohune oil, coriander seed oil, dika oil, grape seed oil, hemp oil, kapok seed oil, kenaf seed oil, lallemantia oil, marula oil, meadowfoam seed oil, mustard oil, nutmeg butter, okra seed oil, papaya seed oil, perilla seed oil, pequi oil, poppyseed oil, prune kernel oil, quinoa oil, ramtil oil, royle oil, tea seed oil, thistle oil, tigernut oil, tomato seed oil, wheat germ oil, radish oil, salicornia oil, tung oil, algae oil, copaiba oil, honge oil, jatropha oil, petroleum nut oil, 10 WL 1349 oil, a silicone oil, a mineral oil, a lauroyl macrogol-6 glyceride, a lauroyl polyoxyl-6 glyceride, an oleoyl macrogol-6 glyceride, an oleoyl polyoxyl-6 glyceride, a linoleoyl macrogol-6 glyceride, a linoleoyl polyoxyl-6 glyceride, propylene glycol monocaprylate, propylene glycol monolaurate, propylene glycol monolaurate, polglyceryl-3 dioleate, propylene glycol dicaprylocaprate, diethylene glycol monethyl ether, a caprylocaproyl macrogol-8 glyceride, a 15 caprylocaproyl polyoxyl-8 glyceride, bergamot, cade, camomile, caraway, carnauba, castor, cinnamon, cod liver, coffee, emu, eucalyptus, fish, geraniol, hyssop, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, mallow, mango seed, mink, orange, orange roughy, palm kernel, peach kernel, rosemary, sandalwood, sasquana, savoury, sea buckthorn, rhea butter, tea tree, tsubaki, vetiver, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl 20 sebacate, dimethicone 360, isopropyl myristate, octyldodecanol, oleyl alcohol, etc.), oily esters (e.g., ethyl oleate, etc.), and combinations thereof.

The liquid carriers described herein may further contain one or more suitable pharmaceutical excipients such as solubilizers, emulsifiers, buffers, preservatives, sweeteners, flavoring agents, 25 suspending agents, thickening agents, colors, viscosity regulators, stabilizers, osmo-regulators, surfactants, and combinations thereof.

In a particular embodiment, the carrier comprises a lipid particle. It is envisioned that in certain particular embodiments, the polynucleotide constructs and/or polynucleotides described herein are formulated into a lipid particle. In more specific embodiments the polynucleotides and/or 30 polynucleotide constructs described herein may be fully encapsulated within lipid particle carriers. Those skilled in the formulation art will appreciate how to formulate lipid particles for compositions as disclosed herein (Musielak et al., Synthesis and Potential Applications of Lipid Nanoparticles in Medicine. Materials (Basel). 2022 Jan 17;15(2):682. doi: 10.3390/ma15020682).

In particular embodiments, the lipid particles used in compositions as described herein are formed from a lipid selected from the group consisting of a cationic lipid a non-cationic lipid, and combinations thereof.

In an embodiment, the lipid particle is formed of a cationic lipid. Suitable cationic lipids include, but are not limited to, N,N-dioleoyl-N,N-dimethylammonium chloride ("DODAC"); N-(2,3-dioleoyloxy)propyl-N,N,N-triethylammonium chloride ("DOTMA"); N,N-distearoyl-N,N-dimethylammonium bromide ("DDAB"); N-(2,3-dioleoyloxy)propyl-N,N,N-trimethylammonium chloride ("DOTAP"); 1,2-Dioleoyloxy-3-trimethylaminopropane chloride salt ("DOTAP.C1"); 313-(N-(N',N'-dimethylaminoethane)-carbonyl)cholesterol ("DC-Chol"), N-(1-(2,3-dioleoyloxy)propyl)-N-2-(sperminecarboxamido)ethyl-N,N-dimethylammonium trifluoroacetate ("DOSPA"), dioctadecylamidoglycyl carboxyspermine ("DOGS"), 1,2-dioleoyl-sn-3-phosphoethanol amine ("DOPE"), 1,2-dioleoyl-3-dimethylammonium propane ("DODAP"), N, N-dimethyl-1,2,3-dioleoyloxypropylamine ("DODMA"), and N-(1,2-dimyristyloxyprop-3-yl)-N,N-dimethyl-N-hydroxyethyl ammonium bromide ("DMRIE"), and combinations thereof. Additionally, a number of commercial preparations of cationic lipids can be used. Non-limiting examples include LIPOFECTIN (including DOTMA and DOPE, available from GIBCO/BRL), and LIPOFECTAMINE (comprising DOSPA and DOPE, available from GIBCO/BRL).

In an embodiment, the lipid particle is formed of a non-cationic lipid. Suitable non-cationic lipids include, but are not limited to, lecithin, phosphatidylethanolamine, lysolecithin, lysophosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, sphingomyelin, cephalin, cardiolipin, phosphatidic acid, cerebrosides, dicetylphosphate, distearoylphosphatidylcholine (DSPC), dioleoylphosphatidylcholine (DOPC), dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylglycerol (DOPG), dipalmitoylphosphatidylglycerol (DPPG), dioleoyl-phosphatidylethanolamine (DOPE), palmitoyloleoylphosphatidylcholine (POPC), palmitoyloleoyl-phosphatidylethanolamine (POPE) and dioleoyl-phosphatidylethanolamine 4-(N-maleimidomethyl)-cyclohexane-1-carboxylate (DOPE-mal), dipalmitoyl phosphatidyl ethanolamine (DPPE), dimyristoylphosphoethanolamine (DMPE), distearoyl-phosphatidyl-ethanolamine (DSPE), 16-O-monomethyl PE, 16-O-dimethyl PE, 18-1-trans PE, 1-stearoyl-2-oleoyl-phosphatidylethanolamine (SOPE).

The amount of carrier present in a composition as disclosed herein, and may vary depending on the formulation, amount and type of polynucleotides being formulated, intended route of administration, etc. In an embodiment, the compositions disclosed herein may be formed of about 0.01 wt. % to 99.99 wt. % of carrier (e.g., 0.01 wt. % to 99.99 wt. %, 0.1 wt. % to 99.99 wt. %, 1.0

wt. % to 99.99 wt. %, 5.0 wt. % to 99.99 wt. %, 10.0 wt. % to 99.99 wt. %, 15.0 wt. % to 99.99 wt. %, 20.0 wt. % to 99.99 wt. %, 25.0 wt. % to 99.99 wt. %, 30.0 wt. % to 99.99 wt. %, 35.0 wt. % to 99.99 wt. %, 40.0 wt. % to 99.99 wt. %, 45.0 wt. % to 99.99 wt. %, 50.0 wt. % to 99.99 wt. %, 55.0 wt. % to 99.99 wt. %, 60.0 wt. % to 99.99 wt. %, 65.0 wt. % to 99.99 wt. %, 70.0 wt. % to 99.99 wt. %, 75.0 wt. % to 99.99 wt. %, 80.0 wt. % to 99.99 wt. %, 85.0 wt. % to 99.99 wt. %, 90.0 wt. % to 99.99 wt. %, 91.0 wt. % to 99.99 wt. %, 92.0 wt. % to 99.99 wt. %, 93.0 wt. % to 99.99 wt. %, 94.0 wt. % to 99.99 wt. %, 95.0 wt. % to 99.99 wt. %, 96.0 wt. % to 99.99 wt. %, 97.0 wt. % to 99.99 wt. %, 98.0 wt. % to 99.99 wt. %, 99.0 wt. % to 99.99 wt. %, 99.10 wt. % to 99.99 wt. %, 99.20 wt. % to 99.99 wt. %, 99.30 wt. % to 99.99 wt. %, 99.40 wt. % to 99.99 wt. %, 99.50 wt. % to 99.99 wt. %, 99.60 wt. % to 99.99 wt. %, 99.70 wt. % to 99.99 wt. %, 99.80 wt. % to 99.99 wt. %, 99.90 wt. % to 99.99 wt. %, 99.91 wt. % to 99.99 wt. %, 99.92 wt. % to 99.99 wt. %, 99.93 wt. % to 99.99 wt. %, 99.94 wt. % to 99.99 wt. %, 99.95 wt. % to 99.99 wt. %, 99.96 wt. % to 99.99 wt. %, 99.97 wt. % to 99.99 wt. %, 99.98 wt. % to 99.99 wt. %).

METHODS:

15 The present disclosure also relates to methods for enhancing the durability of one or more exogenously delivered polynucleotides, as well as methods for reducing one or more unwanted side effects associated with the administration of exogenously delivered polynucleotides (e.g., polynucleotides encoding an antigen or a normally endogenously produced protein). The present disclosure also relates to methods for enhancing one or both of the magnitude and durability of
20 immune responses induced by a vaccine, such as a vaccine comprising one or more exogenously delivered polynucleotides (e.g., an mRNA vaccine), such as the magnitude and/or durability of one or both of antibody and T cell responses to a vaccine antigen present in or encoded by an exogenously polynucleotide of the vaccine (e.g., antibody and T cell responses to an antigen encoded by mRNA of an mRNA vaccine). Additionally or alternatively, the methods disclosed
25 herein may reduce one or more unwanted side effects associated with administration of a vaccine, such as a vaccine comprising one or more exogenously delivered polynucleotides (e.g., an mRNA vaccine),

As used herein, “enhancing the durability” of an exogenously delivered polynucleotide includes increasing the duration of time during which the polynucleotide is expressed and/or during which
30 the encoded polypeptide exhibits its intended biological effect, including enhancing the magnitude and/or duration of immune responses elicited by a vaccine antigen encoded by the polynucleotide, or enhancing the magnitude and/or duration of the intended biological effect of a normally endogenously produced protein encoded by the polynucleotide. As used herein, “enhancing the

durability of a vaccine” or “enhancing the durability of immune responses induced by a vaccine” include increasing the duration of time during which the vaccine exhibits its intended biological effect, including the magnitude and duration of the intended immune response to the vaccine.

The methods disclosed herein can be applied to any animal subject. In particular embodiments, the subject is a mammal. In a more specific embodiment, the subject is a human. In still other specific embodiments, the subject is a non-human subject. Non-limiting examples of non-human subjects include, non-human primates (*e.g.*, prosimians (*e.g.*, lemurs), simians (*e.g.*, old world monkeys such as baboons, macaques, and rhesus monkeys, new world monkeys such as marmosets, capuchins, howlers, and squirrel monkeys, and apes such as gibbons, orangutans, chimpanzees, and gorillas)), mice, rats, ferrets, hamsters, gerbils, guinea pigs, rabbits, pigs, raccoons, possums, opossums, skunks, bovine animals (*e.g.*, cows, bulls, buffalos), horses, zebras, deer, sheep, goats, birds, cats, dogs, foxes, coyotes, wolves, bats, and the like, including, but not limited to animals found in zoos and in captivity for research purposes.

A particular embodiment comprises methods for enhancing the durability of one or more exogenously delivered polynucleotides (*e.g.*, a polynucleotide that encodes an antigen capable of inducing an immune response or a polynucleotide that encodes a normally endogenously produced protein to be administered to subjects with, *e.g.*, genetic mutations which result in missing and/or inadequate amounts or function of the protein, etc.) comprising administering one or more polynucleotides that encode a polypeptide capable of having NK cell inhibiting activity in conjunction with a vaccine or normally endogenously produced protein, including in conjunction with a polynucleotide encoding a vaccine antigen or normally endogenously produced protein. In particular aspects pertaining to vaccines, methods described herein may be effective for increasing one or both of the magnitude and durability of immune responses induced by one or more exogenous polynucleotides of the vaccine (*e.g.*, of an mRNA vaccine), such as the magnitude and/or durability of one or both of antibody and T cell responses to one or more vaccine antigens encoded by one or more exogenous exogenously polynucleotides of the vaccine (*e.g.*, vaccine antigens encoded by mRNA of an mRNA vaccine), such as the magnitude and/or durability of one or both of antibody and T cell responses to the vaccine antigen.. Another particular embodiment contemplates methods for reducing one or more unwanted side effects associated with the administration of one or more exogenously delivered polynucleotides comprising administering one or more polynucleotides that encode a polypeptide capable of having NK cell inhibiting activity in conjunction with a vaccine or normally endogenously produced

protein, including in conjunction with a polynucleotide encoding a vaccine antigen or normally endogenously produced protein.

Other aspects of the present disclosure also include methods for enhancing the durability of one or more exogenously delivered polynucleotides comprising administering a pharmaceutically acceptable amount of at least one of the compositions described herein (e.g., compositions comprising polynucleotide constructs and/or polynucleotides that encode one or more NK cell inhibitors capable of having NK cell inhibiting activity as described herein and a vaccine antigen or normally endogenously produced protein) to a subject (e.g., a patient). In particular aspects, such methods may be effective for increasing one or both of the magnitude and durability of immune responses induced by the vaccine antigen, such as the magnitude and/or durability of one or both of antibody and T cell responses to the vaccine antigen.

Thus, there is provided a method for enhancing one of both of the magnitude and durability of immune responses induced by a vaccine or for reducing one or more unwanted side effects associated with the administration of a vaccine, comprising the steps of:

- (a) administering to a subject a pharmaceutically effective amount of a first polynucleotide as described herein that encodes a polypeptide capable of having NK cell inhibiting activity; and
 - (b) administering to a subject a pharmaceutically effective amount of one or more vaccines,
- wherein inhibition of NK cell activity enhances the magnitude and/or durability of immune responses induced by the one or more vaccines administered to the subject and/or reduces one or more unwanted side effects associated with the administration of a vaccine.

In specific embodiments, the method enhances one or both of antibody and T cell responses to a vaccine antigen of the vaccine.

- The vaccine may be a polynucleotide vaccine comprising a polynucleotide encoding a vaccine antigen as described herein, optionally wherein the polynucleotide is an mRNA molecule. The first polynucleotide and polynucleotide encoding a vaccine antigen may be present in a single polynucleotide construct as described herein. The first polynucleotide and polynucleotide encoding a vaccine antigen may be present in separate polynucleotide constructs formulated in a single composition. The first polynucleotide and polynucleotide encoding a vaccine antigen may be present in separate polynucleotide constructs formulated in separate compositions.

In another specific embodiment, there is provided a method for enhancing the durability of one or more exogenously delivered polynucleotide comprising the steps of:

- 5 (a) administering to a subject a pharmaceutically effective amount of a pharmaceutical composition comprising a first polynucleotide that encodes one or more Natural Killer (NK cell) inhibitors capable of having NK cell inhibiting activity; and
- (b) administering to a subject a pharmaceutically effective amount of a pharmaceutical composition comprising one or more second exogenous polynucleotides (*e.g.*, a polynucleotide that encodes an antigen capable of inducing an immune response or a polynucleotide that encodes a normally endogenously produced protein;
- 10 wherein the inhibition of NK cell activity enhances the durability of the one or more exogenous polynucleotides administered to the subject.

The first polynucleotide may be administered in the same composition as the second exogenous polynucleotide(s), or the first polynucleotide and the second exogenous polynucleotide(s) may be administered in separate compositions, simultaneously or sequentially in any order, as discussed
15 in more detail below.

Further aspects of the present disclosure further include methods for reducing one or more unwanted side effects associated with the administration of one or more exogenously delivered polynucleotides to a subject, comprising administering a pharmaceutically acceptable amount of at least one of the compositions described herein (*e.g.*, compositions comprising polynucleotide
20 constructs and/or polynucleotides that encode one or more NK cell inhibitors capable of having NK cell inhibiting activity as described herein) to a subject in conjunction with a vaccine or normally endogenously produced protein, including in conjunction with a polynucleotide encoding a vaccine antigen or normally endogenously produced protein..

In another specific embodiment there is provided a method for reducing one or more unwanted
25 side effects associated with the administration of one or more exogenously delivered polynucleotide comprising the steps of:

- (a) administering to a subject a pharmaceutically effective amount of a pharmaceutical composition comprising a polynucleotide that encodes one or more Natural Killer (NK cell) inhibitors capable of having NK cell inhibiting activity; and
- 30 (b) administering to a subject a pharmaceutically effective amount of a pharmaceutical composition comprising one or more exogenous polynucleotides (*e.g.*, a polynucleotide

that encodes an antigen capable of inducing an immune response or a polynucleotide that encodes a normally endogenously produced protein;

wherein the inhibition of NK cell activity reduces one or more unwanted side effects associated with the administration of one or more exogenous polynucleotides.

5 The first polynucleotide may be administered in the same composition as the second exogenous polynucleotide(s), or the first polynucleotide and the second exogenous polynucleotide(s) may be administered in separate compositions, simultaneously or sequentially in any order, as discussed in more detail below.

It is contemplated that the compositions described herein comprising a polynucleotide that
10 encodes one or more NK cell inhibitors capable of having NK cell inhibiting activity can be administered before, after, or simultaneously with the administration of one or more exogenous polynucleotides.

In a more particular embodiment is a method for enhancing one or both of the magnitude and the durability of immune responses induced by a vaccine comprising administering to a subject a
15 pharmaceutically effective amount of any of the compositions described herein in conjunction with administration of a vaccine.

In a specific embodiment is a method for enhancing the durability of a vaccine comprising the steps of:

(a) administering to a subject a pharmaceutically effective amount of a pharmaceutical
20 composition comprising a polynucleotide that encodes one or more Natural Killer (NK cell) inhibitors capable of having NK cell inhibiting activity; and

(b) administering to a subject a pharmaceutically effective amount of one or more vaccines;

wherein the inhibition of NK cell activity enhances one or both of the magnitude and the
25 durability of immune responses induced by the one or more vaccines administered to the subject.

In specific embodiments, the method enhances one or both of antibody and T cell responses to a vaccine antigen of the vaccine.

In particularly specific embodiments, the vaccine is a polynucleotide vaccine (e.g., an RNA
30 vaccine, such as an mRNA vaccine).

In still another more particular embodiment, is a method for reducing one or more side effects associated with the administration of a vaccine to a subject comprising administering a pharmaceutically effective amount of any of the compositions described herein to a subject.

In another specific embodiment is a method for reducing one or more unwanted side effects associated with the administration of a vaccine comprising the steps of:

(a) administering to a subject a pharmaceutically effective amount of a pharmaceutical composition comprising a polynucleotide that encodes one or more Natural Killer (NK cell) inhibitors capable of having NK cell inhibiting activity; and

(b) administering to a subject a pharmaceutically effective amount of one or more vaccines;

wherein the inhibition of NK cell activity reduces one or more unwanted side effects associated with the administration of a vaccine.

In particularly specific embodiments, the vaccine is a polynucleotide vaccine (e.g., an RNA vaccine, such as an mRNA vaccine).

It is contemplated that the compositions described herein comprising a polynucleotide that encodes one or more NK cell inhibitors capable of having NK cell inhibiting activity can be administered before, after, or simultaneously with the administration of one or more vaccines.

In a particular embodiment, the compositions described herein comprising polynucleotide constructs and/or polynucleotides that encodes one or more NK cell inhibitors capable of having NK cell inhibiting activity as described herein are administered to a subject before the administration of one or more vaccines (or one or more polynucleotides encoding a polypeptide capable of inducing an immune response or one or more polynucleotides encoding a normally endogenously produced polypeptide) are administered to the subject. Such embodiments may be advantageous to deplete NK cells or NK cell activity prior to vaccination (or prior to administration of the one or more polynucleotides encoding a polypeptide capable of inducing an immune response or one or more polynucleotides encoding a normally endogenously produced polypeptide).

In another embodiment, the compositions described herein are administered to a subject simultaneously with the administration of one or more vaccines (i.e., "simultaneous administration). Simultaneous administration shall mean that the compositions described herein comprising a polynucleotide that encodes one or more NK cell inhibitors capable of having NK

cell inhibiting activity and the one or more vaccines are administered substantially at the same time (e.g., as simultaneously as practical) or at exactly at the same time.

In another particular embodiment, the compositions described herein comprising a polynucleotide that encodes one or more NK cell inhibitors capable of having NK cell inhibiting activity are administered to a subject after the administration of one or more vaccines are administered to the subject.

It is to be understood that any of the method steps disclosed herein (*i.e.*, administering the compositions comprising one or more polynucleotide constructs and/or one or more polynucleotides capable of having NK cell inhibiting activity as described herein before, after, or simultaneously with the administration of one or more vaccines) can be repeated as many times as necessary to achieve the desired effects of enhancing the durability of one or more vaccines and/or reducing one or more unwanted side effects associated with the administration of a vaccine.

It is to also be understood that all of the methods described herein contemplate that delivery of the compositions comprising one or more polynucleotide constructs and/or one or more polynucleotides capable of having NK cell inhibiting activity as described herein can be administered to a subject in accordance with any of the methods of administration commonly known in the art. Non-limiting examples include delivery of the compositions herein via, oral (PO), intravenous (IV), intramuscular (IM), intra-arterial, intramedullary, intrathecal, subcutaneous (SQ), intraventricular, transdermal, interdermal, intradermal, rectal (PR), vaginal, intraperitoneal (IP), intragastric (IG), topical and/or transdermal (*e.g.*, by lotions, creams, powders, ointments, liniments, gels, drops, etc.), mucosal, intranasal, buccal, enteral, vitreal, and/or sublingual administration, by intratracheal instillation, bronchial instillation, and/or inhalation, as an oral spray, nasal spray, and/or aerosol, and/or through a portal vein catheter and combinations thereof.

In particular embodiments, the compositions comprising one or more polynucleotide constructs and/or one or more polynucleotides capable of having NK cell inhibiting activity as described herein are administered to a subject via intravenous methods.

It is further understood that any of the method steps disclosed herein (*i.e.*, administering the compositions comprising one or more polynucleotide constructs and/or one or more polynucleotides capable of having NK cell inhibiting activity as described herein) are administered to a subject in an amount effective (*i.e.*, an effective amount) to achieve a particular result (*i.e.*, a therapeutic result).

In certain embodiments, the effective amount of the polynucleotide constructs and/or polynucleotides contained in the compositions described herein or administered to a subject as described herein (polynucleotides encoding polypeptides capable of having NK cell inhibiting activity and/or polynucleotides encoding polypeptides capable of inducing an immune response or encoding normally endogenously produced proteins) may range from 0.5 μ g to 300 μ g, administered as a single dose or as two or more divided doses. In some embodiments, the effective amount of a single polynucleotide as described herein (encoding either a polypeptide capable of having NK cell inhibiting activity or a polypeptide capable of inducing an immune response or a normally endogenously produced proteins) is, or is about, 0.5 μ g to 150 μ g, or any value therebetween. In some embodiments, the effective amount of a polynucleotide construct or composition comprising two polynucleotides as described herein is, or is about, 0.5 μ g to 300 μ g, or any value therebetween. For example an effective dose of an mRNA vaccine polynucleotide may be about 3 μ g for children or 30 μ g for adults, or from 10-25 μ g for children or 100 μ g for adults. Thus, a total dose of polynucleotides or polynucleotide constructs as described herein may be, or may be about, .5 μ g, 1 μ g, 2 μ g, 3 μ g, 4 μ g, 5 μ g, 6 μ g, 7 μ g, 8 μ g, 9 μ g, 10 μ g, 11 μ g, 12 μ g, 13 μ g, 14 μ g, 15 μ g, 16 μ g, 17 μ g, 18 μ g, 19 μ g, 20 μ g, 25 μ g, 30 μ g, 35 μ g, 40 μ g, 45 μ g, 50 μ g, 55 μ g, 60 μ g, 65 μ g, 70 μ g, 75 μ g, 80 μ g, 85 μ g, 90 μ g, 95 μ g, 100 μ g, 110 μ g, 120 μ g, 130 μ g, 140 μ g, 150 μ g, 160 μ g, 170 μ g, 180 μ g, 190 μ g, 200 μ g, 250 μ g, or 300 μ g, or any value therebetween. When additional polynucleotides are present (either individually or as part of a construct), such as three, four, or more, polynucleotides, the effective amount may be greater than these values, such as proportionally greater based on the number of polynucleotides.

EMBODIMENTS

The following embodiments are non-limiting and illustrative of the subject matter of the present disclosure:

Embodiment 1. An isolated polynucleotide construct comprising: (a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity; and (b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a naturally endogenously produced protein.

Embodiment 2. A composition comprising: (a) one or more polynucleotide constructs of Embodiment 1; and (b) pharmaceutically acceptable carrier.

Embodiment 3. A composition comprising: (a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity; (b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a naturally endogenously produced protein; and (c) a pharmaceutically acceptable carrier.

Embodiment 4. The construct or composition of any one of Embodiments 1-3, wherein the polynucleotide construct or first and second polynucleotides are mRNA molecules.

Embodiment 5. The construct or composition of any one of the preceding Embodiments, wherein the first polynucleotide encodes a serine protease inhibitor (serpin) polypeptide or fragment thereof capable of having natural killer (NK) cell inhibiting activity.

Embodiment 6. The construct or composition of Embodiment 5, wherein the serpin polypeptide is a serpin polypeptide selected from the group consisting of SERPINA1, SERPINA2, SERPINA3, SERPINA4, SERPINA5, SERPINA6, SERPINA7, SERPINA8, SERPINA9, SERPINA10, SERPINA11, SERPINA12, SERPINA13, SERPINB1, SERPINB2, SERPINB3, SERPINB4, SERPINB5, SERPINB6, SERPINB7, SERPINB8, SERPINB9, SERPINB10, SERPINB11, SERPINB12, SERPINB13, SERPINC1, SERPIND1, SERPINE1, SERPINE2, SERPINE3, SERPINF1, SERPINF2, SERPING1, SERPINH1, SERPINI1, SERPINI2, and combinations thereof.

Embodiment 7. The construct or composition of any one of Embodiments 5-6, wherein the serpin polypeptide is SERPINB9.

Embodiment 8. The construct or composition of Embodiment 7, where the first polynucleotide encodes a serpin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein: (a) the first polynucleotide comprises SEQ ID NO:1 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:1; or (b) the first polynucleotide encodes a serpin polypeptide that comprises SEQ ID NO:5 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:5.

Embodiment 9. The construct or composition of any one of c Embodiments 1-4, wherein the first polynucleotide encodes a major histocompatibility complex (MHC) class polypeptide or fragment thereof having NK cell inhibiting activity.

Embodiment 10. The construct or composition of Embodiment 9, wherein the MHC class polypeptide having NK cell inhibiting activity is selected from the group consisting of MHC class I polypeptides, MHC class II polypeptides, MHC class III polypeptides, and combinations thereof.

Embodiment 11. The construct or composition of any one of Embodiments 9-10, wherein the first polynucleotide encodes an MHC class I polypeptide or fragment thereof capable of having NK

cell inhibiting activity, optionally wherein the MHC class I polypeptide is selected from the group consisting of HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G, HLA-K, HLA-L, and combinations thereof.

Embodiment 12. The construct or composition of Embodiment 11, wherein the MHC class I polypeptide is an HLA-E polypeptide.

Embodiment 13. The construct or composition of Embodiment 12, wherein the HLA-E polypeptide is HLA-E*0103.

Embodiment 14. The construct or composition of Embodiment 13, where the first polynucleotide encodes an HLA-E*0103 polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein: (a) the first polynucleotide comprises SEQ ID NO:2 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:2; or (b) the first polynucleotide encodes an HLA-E*0103 polypeptide that comprises SEQ ID NO:6 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:6.

Embodiment 15. The construct or composition of Embodiment 12, wherein the HLA-E polypeptide is HLA E*0101.

Embodiment 16. The construct or composition of Embodiment 15, where the first polynucleotide encodes an HLA-E*0101 polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein: (a) the first polynucleotide comprises SEQ ID NO:3 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:3; or (b) the first polynucleotide encodes an HLA-E*0101 polypeptide that comprises SEQ ID NO:7 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:7.

Embodiment 17. The construct or composition of any one of Embodiments 9-10, wherein the first polynucleotide encodes an MHC class II polypeptide or fragment thereof capable of having NK

cell inhibiting activity, wherein the MHC class II polypeptide is selected from the group consisting of HLA-DM, HLA-DO, HLA-DP, HLA-DQ, HLA-DR, and combinations thereof.

Embodiment 18. The construct or composition of any one of Embodiments 1-4, wherein the first polynucleotide encodes a lectin polypeptide or fragment thereof capable of having NK cell inhibiting activity.

Embodiment 19. The construct or composition of Embodiment 18, wherein the lectin polypeptide is selected from the group consisting of Clec2d8 (mouse), Clec2d11 (rat), CLEC2D (human), and combinations thereof.

Embodiment 20. The construct or composition of any one of Embodiments 18-19, wherein the lectin polypeptide is CLEC2D.

Embodiment 21. The construct or composition of Embodiment 20, where the first polynucleotide encodes a CLEC2D or a fragment thereof capable of having NK cell inhibiting activity, wherein: (a) the first polynucleotide comprises SEQ ID NO:4 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:4; or (b) the first polynucleotide encodes a CLEC2D polypeptide that comprises SEQ ID NO:8 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:8.

Embodiment 22. The construct or composition of any one of Embodiments 1-4, wherein the first polynucleotide encodes a cadherin polypeptide or fragment thereof capable of having NK cell inhibiting activity.

Embodiment 23. The construct or composition of Embodiment 22, wherein the cadherin polypeptide is selected from the group consisting of classical cadherins, desmosomal cadherins, protocadherins, ungrouped cadherins, and combinations thereof.

Embodiment 24. The construct or composition of any one of Embodiments 22-23, wherein the first polynucleotide encodes a classical cadherin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein the classical cadherin polypeptide is selected from the group consisting of CDH1 (e-cadherin), CDH2, CDH3, CDH12, and combinations thereof.

Embodiment 25. The construct or composition of any one of Embodiments 22-23, wherein the first polynucleotide encodes desmosomal cadherin polypeptide or fragment thereof capable of

having NK cell inhibiting activity, wherein the desmosomal cadherin polypeptide is selected from the group consisting of desmogleins, desmocollins and combinations thereof.

Embodiment 26. The construct or composition of Embodiment 25, wherein the first polynucleotide encodes a desmoglein polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein the desmoglein polypeptide is selected from the group consisting of DSG1, DSG2, DSG3, DSG4, and combinations thereof.

Embodiment 27. The construct or composition of Embodiment 25, wherein the first polynucleotide encodes a desmocollin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein the desmocollin polypeptide is selected from the group consisting of DSC1, DSC2, DSC3, DSC4, and combinations thereof.

Embodiment 28. The construct or composition of any one of Embodiments 22-23, wherein the first polynucleotide encodes a protocadherin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein the protocadherin polypeptide is selected from the group consisting of PCDH1, PCDH7, PCDH8, PCDH9, PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17, PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, FAT4, and combinations thereof.

Embodiment 29. The construct or composition of any one of Embodiments 22-23, wherein the first polynucleotide encodes an ungrouped cadherin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein the ungrouped cadherin polypeptide is selected from the group consisting of CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH23, CDH22, CDH24, CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET, and combinations thereof.

Embodiment 30. The construct or composition of Embodiment 22, wherein the first polynucleotide encodes a cadherin polypeptide variant or fragment thereof capable of having NK cell inhibiting activity, wherein the cadherin polypeptide is selected from the group consisting of CDH1 (e-cadherin), CDH2, CDH3, CDH12, DSG1, DSG2, DSG3, DSG4, DSC1, DSC2, DSC3, DSC4, PCDH1, PCDH7, PCDH8, PCDH9, PCDH10, PCDH11X/11Y, PCDH12, PCDH15, PCDH17,

PCDH18, PCDH19, PCDH20, PCDHA1, PCDHA2, PCDHA3, PCDHA4, PCDHA5, PCDHA6, PCDHA7, PCDHA8, PCDHA9, PCDHA10, PCDHA11, PCDHA12, PCDHA13, PCDHAC1, PCDHAC2, PCDHB1, PCDHB2, PCDHB3, PCDHB4, PCDHB5, PCDHB6, PCDHB7, PCDHB8, PCDHB9, PCDHB10, PCDHB11, PCDHB12, PCDHB13, PCDHB14, PCDHB15, PCDHB16, 5 PCDHB17, PCDHB18, PCDHGA1, PCDHGA2, PCDHGA3, PCDHGA4, PCDHGA5, PCDHGA6, PCDHGA7, PCDHGA8, PCDHGA9, PCDHGA10, PCDHGA11, PCDHGA12, PCDHGB1, PCDHGB2, PCDHGB3, PCDHGB4, PCDHGB5, PCDHGB6, PCDHGB7, PCDHGC3, PCDHGC4, PCDHGC5, FAT, FAT2, FAT4, CDH4, CDH5, CDH6, CDH7, CDH8, CDH9, CDH10, CDH11, CDH13, CDH15, CDH16, CDH17, CDH18, CDH19, CDH20, CDH23, CDH22, CDH24, 10 CDH26, CDH28, CELSR1, CELSR2, CELSR3, CLSTN1, CLSTN2, CLSTN3, DCHS1, DCHS2, LOC389118, PCLKC, RESDA1, RET, and combinations thereof.

Embodiment 31. The construct or composition of any one of Embodiments 1-4, wherein the first polynucleotide encodes CDH1 (e-cadherin) or a fragment thereof capable of having NK cell inhibiting activity, or a variant of CDH1 (e-cadherin) or a fragment thereof capable of having NK 15 cell inhibiting activity.

Embodiment 32. The construct or composition of any one of Embodiments 1-4, wherein the first polynucleotide encodes CD155 (poliovirus receptor) or a fragment thereof capable of having NK cell inhibiting activity, or a variant of CD 155 or a fragment thereof capable of having NK cell inhibiting activity.

20 Embodiment 33. The construct or composition of any one of Embodiments 1-4, wherein the first polynucleotide encodes RTN4 or a fragment thereof capable of having NK cell inhibiting activity, or a variant of RTN4 or a fragment thereof capable of having NK cell inhibiting activity.

Embodiment 34. The construct or composition of any one of the preceding Embodiments, wherein the second polynucleotide encodes an antigen capable of inducing an immune response.

25 Embodiment 35. The construct or composition of Embodiment 34, wherein the antigen is a COVID-19 antigen capable of inducing an immune response against COVID-19.

Embodiment 36. The construct or composition of any one of Embodiments 1-33, wherein the second polynucleotide encodes a normally endogenously produced protein.

30 Embodiment 37. The construct or composition of Embodiment 36, wherein the normally endogenously produced protein is Factor VIII or a biologically active fragment thereof.

Embodiment 38. A method for enhancing durability of a vaccine or for reducing one or more unwanted side effects associated with the administration of a vaccine, comprising the steps of: (a) administering to a subject a pharmaceutically effective amount of a first polynucleotide that encodes a polypeptide capable of having NK cell inhibiting activity; and (b) administering to a

subject a pharmaceutically effective amount of one or more vaccines; wherein inhibition of NK cell activity enhances the durability of the one or more vaccines administered to the subject and/or reduces one or more unwanted side effects associated with the administration of a vaccine.

Embodiment 39. The method of Embodiment 38, wherein the vaccine is a polynucleotide vaccine comprising a polynucleotide encoding a vaccine antigen, optionally wherein the polynucleotide is an mRNA molecule.

Embodiment 40. The method of Embodiment 39, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in a single polynucleotide construct.

Embodiment 41. The method of Embodiment 39, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in a single composition.

Embodiment 42. The method of any one of Embodiments 40 or 41, wherein the single polynucleotide construct or the single composition are a construct or composition according to any one of Embodiments 1-35.

Embodiment 43. The method of Embodiment 39, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in separate compositions.

Embodiment 44. A method for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects associated with administration of an exogenously administered polynucleotide, comprising the steps of: (a) administering to a subject a pharmaceutically effective amount of a first polynucleotide encoding a polypeptide capable of having NK cell inhibiting activity; and (b) administering to the subject a second exogenously administered polynucleotide; wherein inhibition of NK cell activity enhances the durability of the exogenously administered polynucleotide and/or reduces one or more unwanted side effects associated with administration of the exogenously administered polynucleotide.

Embodiment 45. The method of Embodiment 44, wherein the second exogenously administered polynucleotide encodes an antigen capable of inducing an immune response.

Embodiment 46. The method of Embodiment 45, wherein the antigen is a COVID-19 antigen capable of inducing an immune response against COVID-19.

Embodiment 47. The method of Embodiment 44, wherein the second exogenously administered polynucleotide encodes a normally endogenously produced protein.

Embodiment 48. The method of Embodiment 47, wherein the normally endogenously produced protein is Factor VIII or a biologically active fragment thereof.

Embodiment 49. The method of any one of Embodiments 38-39 or 43-48, wherein step (a) is performed before step (b).

Embodiment 50. The method of any one of Embodiments 38-39 or 43-48, wherein step (a) is performed after step (b).

- 5 Embodiment 51. The method of any one of Embodiments 38-39 or 43-48, wherein step (a) is performed simultaneously with step (b).

Embodiment 52. A construct or composition according to any one of Embodiments 1-35, for enhancing durability of a polynucleotide vaccine or reducing one or more unwanted side effects associated with administration of a polynucleotide vaccine.

- 10 Embodiment 53. A construct or composition according to any one of Embodiments 36-37, for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects associated with administration of an exogenously administered polynucleotide.

Embodiment 54. A cell expressing a polynucleotide construct of any one of Embodiments 1-37.

15 **EXAMPLES:**

The following examples are provided for illustrative purposes and are not intended to limit the scope of the disclosure as provided herein. Any variations in the exemplified examples which occur to the skilled artisan are intended to fall within the scope of the present disclosure.

EXAMPLE 1

20 **Materials & Methods**

Study Participants:

- Participants were enrolled in the PASS Study, an observational, longitudinal cohort study of healthcare workers (HCWs) that is evaluating clinical and immunological responses to SARS-CoV-2 infection and vaccination. The PASS study was initiated in August of 2020 with participants
25 seen monthly during the first year of the study and then quarterly during the second year of the study at either the Naval Medical Research Center (NMRC) Clinical Trials Center or the Uniformed Services University (USU) Translational Medicine Unit. The study protocol was approved by the USU Institutional Review Board. The cohort consists of generally healthy adults who are ≥ 18 years old, work at WRNMMC, are not severely immunocompromised, and were seronegative for
30 SARS-CoV-2 at time of study enrollment (Jackson-Thompson et al., 2021. Prospective Assessment of SARS-CoV-2 Seroconversion (PASS) study: an observational cohort study of SARS-CoV-2 infection and vaccination in healthcare workers. *BMC Infect Dis* 21: 544). The

subset of PASS participants included for analysis in this study also met the following criteria (see FIG. 1A):

1. no history of COVID-19 diagnosis prior to vaccination;
2. remained seronegative for SARS-CoV-2 spike-specific IgG before vaccination during monthly
5 testing;
3. received 2 vaccinations with the Pfizer/BioNTech BNT162b2 vaccine;
4. completed 2 vaccine-associated symptoms questionnaires by March 30, 2021; and
5. provided serum samples between 20-50 days and 150-200 days post-2nd vaccination.

Post-Vaccine Symptom Assessment:

- 10 Participants completed a structured vaccine-associated symptoms questionnaire at the first monthly visit after each vaccination dose as depicted in FIG. 1B. Questionnaires asked about the presence and severity of 12 symptoms (8 categorized as systemic, 3 categorized as localized to the vaccine site, and 1 categorized as non-local and non-systemic). Severity of each symptom was defined as symptom intensity and measured on a scale of 0–4 (0 = “not at all”, 1 = “a little
15 bit”, 2 = “somewhat”, 3 = “quite a bit”, 4 = “a lot”), with scores for each symptom summed for a total symptom severity score of 0–48.

Antibody testing:

- Binding IgG antibodies against vaccine strain (D614G) SARS-CoV-2 spike protein and receptor-binding domain (RBD) were measured using a microsphere-based multiplex immunoassay
20 (MMIAs) built using Luminex xMAP-based technology as previously described (Laing et al., 2022).

PBMC Isolation and Purification:

PBMCs were isolated and cryopreserved from PASS participants at baseline and at various time points following COVID-19 vaccinations as previously described (Jackson-Thompson et al., 2021).

- 25 Cell Preparation and Flow Cytometry for NK Cell Phenotyping:

- Frozen PBMC samples were thawed and then washed with pre-warmed complete RPMI media (RPMI, 10% FBS, and 1% Penicillin/Streptomycin). Cells were resuspended in pre-warmed complete RPMI with 50 U/ml DNase (Invitrogen). Cells were then counted and transferred to FACS tubes at 1×10^6 cells per tube in 100 μ l cold 1X PBS. A viability control sample was prepared
30 (5×10^5 live cells and 5×10^5 heat-killed cells) by heating 50% of the cells to 70°C for 10 minutes.

All cell samples, except for the unstained control sample, were then incubated with 1 μ L of LIVE/DEAD™ Fixable Blue (Invitrogen) viability dye for 30 minutes on ice in the dark. Cells were then washed, resuspended in PBS/0.5% BSA, and incubated for 5 minutes with BD Horizon™ Brilliant Stain Buffer. Cells were then incubated for 30 minutes on ice in the dark with
5 fluorochrome-conjugated antibodies to CD16 (BUV496), CD3 (BUV737), NKG2D (Super Bright 436), CD14 (BV510), CD19 (BV510), KIR3DL1 (BV711), CD57 (BV785), CD56 (FITC), KIR2DL2/L3/S2 (PE-Cy5.5), NKG2A (PE-Vio770), NKG2C (APC), and KIR2DL1 (APC-Vio770). The cells were washed, suspended in fixation buffer (BD Biosciences), and then incubated for 15 minutes at room temperature in the dark. After fixation, the cells were washed and resuspended
10 in PBS/0.5% bovine serum albumin (BSA) and stored at 4° C until flow cytometry analysis. Single color reference controls were prepared using UltraComp eBeads™ compensation beads (Invitrogen). All antibodies were titrated prior to use to determine optimal staining concentrations. Fluorescence minus one (FMO) controls were used to establish positivity cut-offs for CD56, CD16, CD57, NKG2A, NKG2C, NKG2D, KIR2DL1, KIR2DL2/L3/S2, and KIR3DL1. All samples were
15 analyzed using a Cytex Aurora spectral cytometer (Cytex Biosciences) at the USU Biomedical Instrumentation Center Flow Cytometry facility, and the generated data was analyzed using the FlowJo Software v10 (BD Biosciences).

The gating strategy to identify the NK cells and NK cell subsets is depicted in FIG. 2. Percent NK cells were calculated as the number of CD56+ NK cells ("Total NK Cells" gate) divided by the
20 number of PBMCs ("Total Cells" gate) and then multiplied by 100. Absolute NK cell frequencies were calculated by multiplying the number of PBMCs/ μ L of blood by the percent NK cells previously calculated. Frequencies of the NK subsets were calculated as the number of the cells from the specific subset divided by the number of NK cells ("Total NK Cells" gate) and then multiplied by 100.

25 NK Cell Functional Assay:

PBMC samples were thawed and rested overnight at 37°C in 5% CO₂ in pre-warmed complete RPMI-1640 medium, supplemented with 10% FBS, 100U/mL penicillin, 100ug/mL streptomycin and 2mM L-glutamine (assay medium). The following morning, the PBMC were collected from
wells of 6-well plates and counted. 50,000 PBMC were stained to assess the percentage of live
30 CD3-/CD56+/CD16+ NK cells using LIVE/DEAD Fixable Near-IR Dead Cell Stain (Invitrogen) for 30 minutes at room temperature in the dark. Cells were washed twice and then incubated 30 minutes at 4°C in the dark with CD56 (PE), CD16 (PE), and CD3 (FITC). All samples were analyzed using BD FACSCanto II and the generated data was analyzed using FCS Express (v6)

(De Novo Software). A total of 1×10^6 K562 target cells were labeled with 1 μ L Human TVA™ dye containing calcein-AM (Immunospot, CTL Inc.) in 1mL PBS for 20 minutes at 37°C. After this incubation and two washes in PBS, the K562 were resuspended at 1×10^5 /mL in assay medium. PBMC and K562 were then plated into a U bottom 96-well plate at NK cell: K562 (E:T) ratios of 10:1, 5:1, 2.5:1, and 1.25:1 in 200 μ L assay medium. Labeled K562 alone were also plated. The plate was centrifuged at 200xg for 1 minute and then incubated at 37°C in 5% CO₂ for 4 hours. After the 4-hour incubation, each well was mixed and 50 μ L of the cell suspension was transferred to a flat bottom 96-well plate in triplicate. The plate images were acquired on a S6 Universal Analyzer (Immunospot, CTL Inc.), available at Frederick National Laboratory for Cancer Research, and live cell counts and the percent killing of target cells was determined using NK-TVA software (Welter et al, 2018. High-Throughput GLP-Capable Target Cell Visualization Assay for Measuring Cell-Mediated Cytotoxicity. *Cells* 7(5):35. doi: 10.3390/cells7050035). To obtain a relative measure of the magnitude of highly cytotoxic NK cells for each individual, an NK cell cytotoxicity index was calculated by multiplying the percentage of target cells killed at specific E:T ratios by the absolute number of NK cells/ μ L of blood.

Statistical Analysis:

Normality tests were run on each data set. If the data had a normal distribution, an unpaired t-test was used to compare two groups. If data were not normally distributed, a Mann-Whitney U test was performed for comparisons between unpaired groups. Spearman's correlation was performed to evaluate relationships between factors, and one-way repeated measures ANOVA followed by Dunnett's multiple comparisons test was used for comparisons between multiple timepoints. Statistical analyses were performed using GraphPad 9.

For the data showing percent killing or NK cytotoxicity index at multiple effector-to-target cell ratios, area under the curve (AUC) was calculated. AUCs were calculated in GraphPad 9 by calculating the area under each data point; those areas were then compared between each group and analyzed with either a t-test or Mann-Whitney U test depending on normality. Exploratory analysis of associations between NK cell receptor expression and symptom scores after vaccination 1 and 2, and IgG levels at 1- and 6-months post-vaccination, was performed using Spearman's correlation with a Bonferroni adjustment for multiple comparisons

Analysis 1: Participant selection and demographics

By March 30th, 2021, the PASS study had enrolled 271 individuals. Fifteen of these participants were excluded from this study because they had evidence of SARS-CoV-2 infection before

vaccination and the goal of this analysis was to evaluate the impact of NK cells on vaccine responses in COVID-naïve individuals. Of the remaining 256 participants, 188 completed symptom questionnaires after both 1st and 2nd vaccinations and had provided post-2nd vaccination serum samples at time points between 20-50 and 150-200 days post-2nd vaccination (FIG. 1A)

5 Participants self-reported demographic characteristics including sex, race, and ethnicity. Of the 188 individuals included in this study, 67.5% identified as female and 32.5% as male (Table 1). 171 individuals were Non-Hispanic, 12 were Hispanic, and 5 did not specify ethnicity. Participants identified their race as 72.3% White, 10.6% Black, 9.0% Asian, 2.1% another race, 5.3% multi-racial, and 0.7% did not specify their race. The average age was 42.4 years (range 20 - 69).

10 **Table 1: Study participant demographics**

	All n/N (%)
Sex	
Female	127/188 (67.5)
Male	61/188 (32.5)
Ethnicity	
Non-Hispanic	171/188 (90.9)
Hispanic	12/188 (6.3)
Non-Specified	5/188 (2.8)
Race	
White	136/188 (72.3)
Black	20/188 (10.6)
Asian	17/188 (9.0)
Other	4/188 (2.1)
Multiracial	10/188 (5.3)
Non-Specified	1/188 (0.7)
Age	
Mean age (range)	42.4 (20-69)

Analysis 2: Evaluating the relationship between NK cell characteristics and sex or age

Flow cytometry was performed on PBMCs collected from individuals at baseline, an average of 71.2 (SD 31.9) days before vaccination with BNT162b2. Given the relatively large size of our cohort, we initially evaluated whether NK cell frequencies, absolute numbers, or functionality were

different in study participants based on either sex or age. As seen in **FIG. 3** (panels a and b), no statistically significant differences were observed between the frequencies or absolute numbers of NK cells in females compared to males (frequencies as a % of total cells: 5.9 [SD 2.9] vs 6.9 [SD 3.7]; absolute NK cell numbers/ μ l of blood 101.6 [SD 65.2] versus 106.9 [SD 63.0]).

5 Functional cytotoxicity of NK cells was assessed on PBMCs from a subset of participants. No differences were found between AUCs of percent killing (**FIG. 3**, panel c) or NK cytotoxicity index (**FIG. 3**, panel d) in males versus females. Individual results at each effector to target (E:T) ratio for NK killing and cytotoxicity indices are shown in **FIG. 4** (panels a and b). There was no relationship between age and percentage of PBMCs that are NK cells (**FIG. 5**, panel a), or age

10 and absolute NK cell numbers/ μ l of blood (**FIG. 5**, panel b). There was also no significant correlation between age and AUCs of NK cell functionality or NK cytotoxicity index (**FIG. 5**, panels c and d). Results indicate the relationship between NK cell characteristics and sex or age.

Analysis 3: Frequencies of NK cells at baseline, within one week after 1st vaccine, and one month after 2nd vaccine

15 A subset (n=18) of participants had PBMC samples obtained within one week of their first BNT162b2 vaccine dose. For these participants we conducted NK cell flow cytometry analyses at baseline, an average of 4.2 (range 1-7) days after 1st BNT162b2 vaccine, and an average of 25 (range 14-41) days after 2nd BNT162b2 vaccine to assess for any potential short-term changes in circulating NK cells following mRNA vaccination (**FIG. 6**, panels a-e). Frequencies of total NK

20 cells as a percentage of all PBMCs (**FIG. 6**, panel a), and absolute NK cell numbers/ μ l of blood (**FIG. 6**, panel b) did not differ significantly from baseline levels for the two post-vaccination time points we analyzed. While frequencies of immature CD56^{bright} CD16⁻ NK cells did not fluctuate substantially after vaccination (**FIG. 6**, panel c), frequencies of CD56^{dim} CD16⁺ mature NK cells declined in some individuals during the first week post-vaccine 1, but this difference was not

25 significantly significant (**FIG. 6**, panel d). Interestingly, percentages of CD56^{dim} CD16⁻ mature NK cells were significantly higher during the first week post-vaccine 1 compared to baseline (11.51% [SD 3.8] vs 20.65% [SD 17]; p=0.02) (**FIG. 6**, panel e). As mature NK cells are known to lose CD16 expression upon activation (Romee et al., 2013. NK cell CD16 surface expression and function is regulated by a disintegrin and metalloprotease-17 (ADAM17). *Blood* 121: 3599-3608),

30 the decrease in CD16⁺ mature NK cells and commensurate increase in CD16⁻ mature NK cells suggests early NK cell activation after vaccination. Results indicate the frequencies of NK cells at baseline, within one week after 1st vaccine, and one month after 2nd vaccine.

Analysis 4: Association between NK cell characteristics and symptoms after COVID-19 vaccination

As NK cell activation by mRNA vaccination could potentially be an important contributor to vaccine-associated inflammatory symptoms, we evaluated baseline NK cell frequencies with symptom severity scores measured by structured questionnaires collected following both BNT162b2 vaccinations. After the first vaccination, participants reported an average symptom score of 7.45 (SD 6.19) and after the second vaccination an average symptom score of 11.0 (SD 9.22). The breakdown of the various local and systemic symptoms exhibited in the cohort is summarized in **FIGs. 7A-7B**. As seen in **FIG. 8** (panels a and b), no significant associations were observed between absolute NK cell numbers/ μ l of blood and post-vaccination symptoms.

Due to constraints on conducting NK cell functionality studies on all samples, baseline functionality of NK cells based on target cell-killing was examined in subsets of individuals that had reported high (9 or higher) or low symptom (3 or lower) scores after the first vaccine dose. After the first vaccination, individuals in the low symptom score group had an average symptom score of 1.2 (range 0-3) while those with a high symptom score had an average symptom score of 16.8 (range 9-33) (data not shown). Following the second vaccination all individuals that had baseline NK functionality testing were regrouped into low and high 2nd dose symptom score groups. Individuals in the low symptom score group after 2nd dose had an average symptom score of 3.7 (range 0-9) while the high symptom score group had an average symptom score of 19.4 (range 11-43) (data not shown). The AUC for percentage of killing was not significantly different between the low and high symptom score groups post-vaccination 1 (**FIG. 8**, panel c). However, there was a significant difference between the AUCs of the NK Cytotoxicity Index measured in low versus high symptom score groups after vaccination 1 (118.5 [SD 77.5] vs 195.7 [SD 170.1], $p=0.04$) (**FIG. 8**, panel d). The higher NK Cytotoxicity Index values in highly symptomatic individuals suggests NK cells may be contributing to vaccine-related inflammatory symptoms. Baseline NK percent killing (**FIG. 8**, panel e) and NK Cytotoxicity Indices (**FIG. 8**, panel f) were not statistically different between low versus high symptom groups after 2nd vaccine dose. Individual results at each E:T ratio for NK killing and cytotoxicity indices after vaccination 1 and 2 are shown in **FIG. 9** (panels a-d). Results indicate an association between NK cell characteristics and symptoms after COVID-19 vaccination.

Analysis 5: Relationship between NK cell frequency and functionality and spike-specific IgG levels after two BNT162b2 vaccinations

As NK cells have the ability to abrogate or augment the development of adaptive immune responses, we next evaluated whether baseline NK cell frequencies and functionality are associated with higher or lower antibody levels post-vaccination. SARS-CoV-2 spike-specific IgG levels were measured from serum samples collected approximately 1 and 6 months after 2nd BNT162b2 vaccination. While no statistically significant correlation was observed between absolute NK cell numbers pre-vaccination and spike-specific IgG levels at 1 month ($Rho = -0.019$, $p = 0.79$) (FIG. 10, panel a), there was a statistically significant negative correlation between absolute NK cell numbers pre-vaccination and spike-specific IgG levels at 6 months ($Rho = -0.14$, $p = 0.04$) (FIG. 10, panel b). This finding suggests that high numbers of NK cells prior to vaccination may impede durability of mRNA vaccine-induced antibody responses. This negative correlation was also seen between the number of mature NK cells and spike-specific IgG levels at 6 months (data not shown). There were no significant correlations between NK cell killing or NK cytotoxicity indices and IgG levels at 1 and 6 months post-vaccination (FIG. 10, panels c-d; FIG. 11, panels a-h). Although there were no significant correlations between spike-specific IgG levels 6 months post-vaccination and NK cytotoxicity at various E:T ratios, there was a trend of negative correlation between these two factors (FIG. 11, panels e-h). Results indicate a relationship between NK cell frequency and functionality and spike-specific IgG levels after two BNT162b2 vaccinations.

Analysis 6: Immature NK cells expressing NKG2A are positively associated with IgG levels at 1 and 6 months post-vaccination

The associations between NK cell receptor expression and symptom scores after vaccination 1 and 2, and IgG levels at 1 and 6 months post-vaccination were examined in a series of exploratory secondary analyses (FIG. 12). Surface receptors examined included the NK activating receptors NKG2C and NKG2D, and the NK inhibitory receptors NKG2A, KIR2DL1, KIR2DL2/L3, and KIR3DL1. Receptor expression was assessed on total NK cells ($CD3^+ CD56^+$), immature NK cells ($CD56^{bright} CD16^-$), and mature ($CD56^{dim} CD16^+$) NK cells. A negative correlation was found between expression of NKG2D on NK cells and spike-specific IgG levels 6 months post vaccination ($\rho = -0.15$, $p = 0.04$). Positive correlations were found between NKG2A expression on immature NK cells and spike-specific IgG levels at 1 ($\rho = 0.26$, $p = 0.0003$) and 6 months ($\rho = 0.15$, $p = 0.03$) post-vaccination. NKG2A expression was also positively but not significantly correlated with higher spike-specific IgG levels at 1 and 6 months post-vaccination in total and

mature NK cells as well. Statistical significance remained for the positive association between NKG2A expression on immature NK cells and spike-specific IgG levels at 1 month post-vaccination even after applying a Bonferroni correction for 76 comparisons.

EXAMPLE 2

5 Protection of leukemic cells from killing by NK cells

Natural Killer (NK) cells inject packets containing an array of proteins (cytolytic granules) into aberrant cell types in order to kill them. One of the key proteins involved in killing target cells, granzyme B, is a protein-digesting enzyme. A naturally occurring inhibitor of this enzyme (SERPINB9) is expressed by some immune cells to protect them from killing by NK cells. The
10 recognition and triggering of NK cells to kill targets can also be prevented by a protein on the surface of the NK cell (NKG2A) that recognizes a protein expressed on the surface of healthy cells (HLA-E).

To assess the ability of SERPINB9 and/or HLA-E proteins to protect a leukemic cell line (721.221) that is normally a target for killing by NK cells, the killing of the unmodified cell line was compared
15 with that of cell lines engineered to contain the SERPINB9 protein, HLA-E protein, or both.

In particular, to obtain NK cells, PBMC were obtained by density gradient centrifugation of whole blood obtained from six different human donors. After isolation, the cells were counted using Muse® Count and Viability Kit (Cytex®, MCH600103) on a Muse® Cell Analyzer (Cytex®) and incubated overnight at $10\text{--}15 \times 10^6$ per well in 6-well plates in assay medium RPMI 1640 media
20 (Gibco, 21870-084), 10% heat-inactivated fetal bovine serum (Sigma, 12306C), and 1% Pen Strep Glutamine (Gibco, 10378-016). After an overnight incubation, NK cells were isolated with the human NK Cell Isolation Kit (Miltenyi Biotec, 130-092-657) according to manufacturer instructions.

To prepare the test cells, 721.221 cells (parent cell line or expressing SERPINB, HLA-E, or both,
25 or GFP) were labeled with Calcein Violet dye and resuspended at 2×10^5 cell/mL in assay medium. One hundred microliters of 721.221 cells were cultured in a U-bottom 96-well plate with NK cells in assay media at effector:target ratios of 10:1, 5:1, 2.5:1, and 1.25:1, in a final volume of 200 μ L/well. Labeled 721.221 cells alone were also plated as negative controls. The plates were centrifuged at 200 $\times$ g for 1 minute and then incubated at 37°C in 5% CO₂ for 4 hours. After the 4-
30 hour incubation, each well was mixed and 50 μ L of the cell suspension was transferred to a 96-well plate in triplicate for imaging. The percent killing of target cells was determined using the following formula: (Control count-Experimental count)/Control count * 100.

Results for the 5:1 effector:target ratio samples are reported in **FIG. 13** for each of the six donors, with the killing of the parent 721.211 cell line set to 1 for each donor. The black line represents the average level of lysis for all six donors combined. As seen in the **FIG. 13**, cells containing the SERPINB9 protein were protected from killing to a greater extent than cells with HLA-E protein on their surface. Cells with both SERPINB9 and HLA-E were protected to the same degree as cells with only SERPINB9, indicating a dominant protective effect of the SERPINB9 protein.

EXAMPLE 3

Immunization with Covid Spike Protein and SERPINB9 enhances immune response

In this animal model study, 8 week old female C57BL/6 mice were vaccinated intramuscularly in the right hind leg with lipid nanoparticle (LNP) vaccine formulations (1 µg total) in one of three groups (with 5 mice per group), where each group included 0.5 µg of LNP comprising mRNA encoding SARS-CoV-2 spike protein, one group additionally included 0.5 µg empty LNP, one group additionally included 0.5 µg of LNP comprising mRNA encoding SERPINB9 protein, and one group included 0.5 µg of LNP comprising mRNA encoding Qa1 (a murine equivalent of HLA-E) (see SEQ ID NOs. 9 and 10):

0.5 µg spike LNP + 0.5 µg empty LNP

0.5 µg spike LNP + 0.5 µg Serpin LNP

0.5 µg spike LNP + 0.5 µg Qa1 LNP

The LNP was the SM-102 LNP (used in the Moderna COVID vaccine).

Three weeks after vaccination, mice were euthanized and single cell suspensions of spleen and draining lymph node cells were prepared. Lymph node cells were pooled from five mice, and the experiment was repeated once. Splenocytes and lymph node cells were cultured at 5×10^6 cells/ml and then stimulated with two pools of spike protein peptides in DMSO (1.1 mcg/ml of S1 peptide pool, 1.1 mcg/ml of S2 peptide pool) or DMSO alone, with a final DMSO concentration of 0.2% by volume of media. Each peptide pool (obtained from Stemcell Technologies) comprised 158 15-mer peptides with 11 amino acid overlaps which, between the two peptide pools, covered the entirety of SARS-CoV-2 spike protein. The following day, cells were stained and fixed for flow cytometry and the percentage of all live, CD3+, CD8+ T-cells that were also CD25+ were assessed as for determination of the percentage of activated (CD25+) CD8 cells. Results are shown in **FIG. 14** (panels a and b). As shown in the figure, significantly greater percentages of spike protein-specific CD8+ T cells became activated by incubation with the spike S1 peptide pool

in splenocytes of mice vaccinated with LNPs containing mRNA encoding spike protein and SERPINB9 or Qa1 mRNA than mice vaccinated only with LNPs containing mRNA encoding spike mRNA (panel A). Similar results were also observed in lymph node cells (panel B), but statistical significance was not observed in lymph node cells likely because of only having two data points for each condition due to the need to pool lymph node cells from 5 mice for each experiment. Thus, these results show that mice immunized with mRNA LNP vaccines in accordance with the present disclosure exhibited a greater CD25+ response to the vaccine antigen (the spike protein in this experiment), indicating that the approach of the present disclosure enhances the immune response to the vaccine antigen (the spike protein in this experiment).

10 PROPHETIC EXAMPLE 1

Test whether NK cell depletion alters clinical symptoms, acute inflammation, and/or levels of antigen-specific antibody and T-cell responses induced by initial BNT162b2 vaccination

To determine the role NK cells play in driving inflammatory side effects and pathogen-specific adaptive immune responses induced by mRNA vaccination, we will evaluate acute inflammatory cytokines, signs of inflammatory side effects, spike protein specific antibody levels, and spike protein specific T cell responses in C57BL/6 mice in the presence or absence of NK cell depletion.

mRNA vaccine: For these studies, we will plan to use bivalent BNT162b2 + BNT162b2 Omi (BA.1), a bivalent mRNA COVID vaccine that encodes for pre-fusion SARS-CoV-2 spike proteins of D614G and BA.1 SARS-CoV-2 variants or monovalent BNT162b2 (which simply codes for ancestral D614G spike protein). Vaccine doses will be obtained from multiuse vials with remaining/leftover doses that are about to be discarded at the end of the day from the Walter Reed National Military Medical Center (WRNMMC) COVID vaccine center.

Vaccinations will consist of intramuscular (IM) injections of 50 µl of mRNA vaccine (0.2 or 5 µg per mouse). These doses have been shown to induce robust spike-specific antibody levels and modest levels of spike-specific T-cells in the spleen and lungs of C57BL/6 mice three weeks after vaccination. Mice will receive a single dose of mRNA vaccine given by intramuscular injection into the thigh muscle of a hind limb. NK cell depletion will start one day prior to vaccination by intraperitoneal injection of 200 µg of anti-NK1.1 antibody (clone PK136; Bio X Cell) every three days for a total of five doses, an approach which results in persistent depletion of over 90% of NK cells in C57BL/6 mice for a two week time period and which will be confirmed in Aim 1 (Schmitt, D.M., et al., Role of NK cells in host defense against pulmonary type A *Francisella tularensis* infection. *Microbes Infect*, 2013. 15(3): p. 201-11). We are choosing this duration of NK cell

depletion because most adverse reactions that develop in response to mRNA vaccination occur over the first several days and because antigen expression by somatic cells that take up mRNA from mRNA vaccines express vaccine-coded protein for about 10 days (Cagigi, A. and K. Lore, Immune Responses Induced by mRNA Vaccination in Mice, Monkeys and Humans. *Vaccines* (Basel), 2021. 9(1)). Control mice not depleted of NK cells will be given the same dose and frequency of isotype antibody (clone C1.18.4;Bio X Cell).

To assess the effects of NK cell depletion on vaccine induced production of inflammatory cytokines, 50 µl of blood will be obtained from mice by tail vein or submandibular bleed (depending on the proficiency of the techniques by lab personnel) at 6 hours and 24 hours after vaccination and serum then measured for inflammatory cytokines including IFN-γ, TNF-α, IL-1, IL-6, and IFN-α using a microsphere (Luminex- based) multiplex assay (R&D systems).

To assess for clinical signs of inflammatory side effects, mice will be monitored by an observer blinded to treatment group daily for 5 days after vaccination for presence or absence of fur ruffling, hunched posture, and lethargy. Additionally, rectal temperatures will be taken twice a day during the first 5 days after vaccination.

At day 21, mice will be euthanized as per V.4.6. Draining inguinal and iliac lymph nodes as well as spleen cells will be isolated for enumeration of SARS-CoV-2 spike-specific CD4 and CD8 T cells by flow cytometry and/or ELISPOT after in vitro stimulation with pools of peptides from one or both vaccine strains of SARS-CoV-2. Serum obtained from terminal bleeds will be measured for spike-specific antibody levels using a highly sensitive and specific microsphere immunoassay developed by the Broder/Laing lab in the Department of Microbiology.

Outcomes and numbers of mice to be used: The primary outcome is spike-specific antibody levels. We hypothesize that NK cell depletion will enable an increase in spike specific antibody levels of 30%. A sample size of 13 mice in each group will have 80% power to detect a 30% difference in antibody levels with a 0.05 two-sided significance level, assuming a standard deviation of 0.25 (25% from mean) in each group. Thus, we will use 15 animals per group. 15 animals per group will allow for loss of up to two animals per group from causes such as inability to obtain blood draw or removal of animal from study due to illness. As there will be two principal experimental groups (vaccinated and NK depleted vs vaccinated and not NK depleted) this will require a total of 30 mice. Additionally, to ensure that mice do not have baseline antibody and T cell responses that are cross reactive with spike protein, we will include a third set of 15 unvaccinated mice to enable comparisons between the vaccinated not NK cell depleted mice to unvaccinated mice. Total mice = 45.

PROPHETIC EXAMPLE 2

Test whether NK cell depletion alters acute inflammation and/or levels of antigen-specific antibody and T-cell responses induced by booster BNT162b2 vaccination

For this experiment, mice will receive an initial dose of vaccine on day 0 and a booster dose on day 21, with NK cell depletion starting one day prior to the booster. A prime/boost regimen with vaccine doses given at days 0 and 21 results in higher levels of spike-specific antibodies as well as high frequencies of spike specific CD4 and CD8 T-cells in the spleen three weeks after the booster vaccination.

As in Prophetic Example 1, vaccinations will consist of intramuscular injections of 50 µl of mRNA vaccine (0.2 or 5 µg per mouse). Anti-NK1.1 antibody injections (or isotype control injections for control group mice) will be continued every three days for 5 doses.

To assess the effects of NK cell depletion on booster vaccination induced production of inflammatory cytokines, 50 µl of blood will be obtained from mice by tail vein or submandibular bleed at 6 and 24 hours after the booster vaccination and serum then measured for inflammatory cytokines including IFN-γ, TNF-α, IL-1, IL-6, and IFN-α using a microsphere (Luminex-based) multiplex assay (R&D systems).

To assess for clinical signs of inflammatory side effects after booster vaccination, mice will be monitored by an observer blinded to treatment group daily for 5 days after booster vaccination for presence or absence of fur ruffling, hunched posture, and lethargy. Additionally, rectal temperatures will be taken twice a day during the first 5 days after booster vaccination

Mice will be euthanized at day 42 (21 days after booster vaccination). Draining inguinal and iliac lymph nodes as well as spleen cells will be isolated for enumeration of SARS-CoV-2 spike-specific CD4 and CD8 T cells by flow cytometry and/or ELISPOT after in vitro stimulation with pools of peptides from one or both vaccine strains of SARS-CoV-2. Serum obtained from terminal bleeds will be measured for spike-specific antibody levels using a highly sensitive and specific microsphere immunoassay developed by the Broder/Laing lab in the Department of Microbiology.

Outcomes and numbers of mice to be used: The primary outcome is spike-specific antibody levels. We hypothesize that NK cell depletion during the time period of mRNA boosting vaccination will enable an increase in spike specific antibody levels of 30%. A sample size of 13 mice in each group will have 80% power to detect a 30% difference in antibody levels with a 0.05 two-sided significance level, assuming a standard deviation of 0.25 (25% from mean) in each group. Thus, we will use 15 animals per group. 15 animals per group will allow for loss of up to

two animals per group from causes such as inability to obtain blood draw or removal of animal from study due to illness. As there will be two principal experimental groups (vaccinated and NK depleted vs vaccinated and not NK depleted) this will require a total of 30 mice.

PROPHETIC EXAMPLE 3

5 Test whether NK cell depletion prolongs duration of expression of spike-specific mRNA after COVID mRNA vaccination in the setting of primary or booster vaccination

Our central hypothesis is that NK cells deplete cells expressing vaccine-delivered spike mRNA. To directly test this, we will assess duration of spike mRNA expression in muscle and lymph node cells after initial or booster vaccination in the presence or absence of NK cell depletion.

10 Groups will include:

A. Single COVID mRNA vaccination and NK depleted with euthanasia days 1, 3, and 7 after vaccination

B. Single COVID mRNA vaccination and isotype injections with euthanasia days 1, 3, and 7

C. COVID mRNA vaccination days 0 and 21 and NK depleted with euthanasia days 1, 3, and 7

15 D. COVID mRNA vaccination days 0 and 21 and isotype injections with euthanasia days 1, 3, and 7

Mice will be vaccinated as detailed with intramuscular injection(s) of monovalent or bivalent BNT162b2 at 0.5 or 5 µg /mouse.

20 For group A, NK cell depletions will start one day prior to vaccination by intraperitoneal injection of 200 µg of anti-NK1.1 antibody (clone PK136; Bio X Cell) every three days for a total of five doses. For group C, NK cell depletions will start on day 20, one day prior to booster vaccination on day 21. Control mice in groups B and D not depleted of NK cells will be given the same dose and frequency of isotype antibody (clone C1.18.4; Bio X Cell) as groups A and C receive of depleting Ab.

25 Five mice per each mouse group will be euthanized on days 1, 3, and 7 after single mRNA vaccination for groups A and B and on days 22, 23, 25, and 28 for the booster group (*i.e.*, days 1, 3, and 7 after the booster vaccination). After euthanasia, lymph node and muscle tissues will be assessed for presence and relative quantities of spike mRNA expression by RT PCR, determination of the cells expressing spike mRNA will be determined by single cell RNA
30 sequencing, and blood collected from terminal bleed will be assessed for circulating levels of spike protein by ELISA. Flow cytometry may also be conducted on draining lymph node cells to

identify frequencies of NK cells in the lymph nodes at the various timepoints and to assess the activation status of these NK cells (this information will also be evaluated with the transcriptomic data obtained from the single cell sequencing studies performed).

Outcomes and numbers of mice to be used: The primary outcome is duration of spike mRNA expression in lymph nodes. We hypothesize that spike-specific mRNA will in NK depleted mice will be present at levels of 55% or greater than that in non-depleted mice 7 days after vaccination or booster (prior studies have shown that most mice do not have detectable mRNA at 7 days after 5 µg vaccination with BNT162b2). A sample size of 5 mice in each group will have 80% power to detect a 55% difference quantitative spike mRNA levels with a 0.05 two-sided significance level, assuming a standard deviation of 0.3 within each group, by Mann-Whitney rank-sum test. As there are four groups, each of which has 3 timepoints for euthanasia, in total this will require 5 mice/group x 4 groups x 3 timepoints per group = 60 mice.

PROPHETIC EXAMPLE 4

Test whether vaccination with mRNA vaccine containing both mRNA for SARS-CoV-2 spike protein as well as mRNA for an inhibitory ligand of Natural Killer(NK) cells results in greater immune responses and/or fewer side effects than vaccination with mRNA vaccine containing only mRNA for spike protein

As NK cell function can be inhibited by inhibitory ligands expressed on host cell surfaces, we hypothesize that a bivalent mRNA vaccine in which a lipid nanoparticle (LNP) containing mRNA sequences that encode both SARS-CoV-2 spike protein AND a protein that inhibits NK cell function will induce higher antibody and T cell responses than a vaccine that only contains mRNA encoding spike protein. The encoded NK cell inhibiting proteins may include one or more selected from HLA-E, CD155, PI-9 (also known as protease inhibitor 9 or SERPINB9), and CEACAM-1, all of which have inhibitory effects on NK cell function or of the effects of NK cells on a target cell.

Groups of age-matched C57BL/6 mice will include:

A.COVID mRNA vaccination with euthanasia days 3, 7, and 21

B.COVID+NK inhibitory ligand vaccination with euthanasia days 3, 7, and 21

C.COVID mRNA vaccination and booster dose day 21 with euthanasia days 3, 7, and 21 post-boost

D.COVID+NK inhibitory ligand vaccination with euthanasia days 3, 7, and 21

Mice will be assessed for symptoms (all groups) as detailed in experiment 2, release of acute inflammatory cytokines into serum by obtaining blood at 6 and 24 hours after vaccination or booster dose, duration of spike protein expression in serum and spike mRNA expression in lymph nodes measured after euthanasia (day 3 and 7 groups), and antibody and T-cell responses (day 5 21 groups). Blood will be obtained by either tail vein or submandibular bleed methods, depending on the technical expertise and experience of the lab personnel conducting the experiment.

Outcomes and number of mice to be used: The primary outcome is spike-specific antibody levels. We hypothesize that mRNA vaccine containing mRNA expressing an NK inhibitory ligand will enable an increase in spike specific antibody levels of 30%. A sample size of 13 mice in each 10 group will have 80% power to detect a 30% difference in antibody levels with a 0.05 two-sided significance level, assuming a standard deviation of 0.25 (25% from mean) in each group. Thus, we will use 15 animals per group. 15 animals per group will allow for loss of up to two animals per group from causes such as inability to obtain blood draw or removal of animal from study due to illness. There will be four principal experimental groups (single vaccination BNT162b2 versus 15 single vaccination BNT162b2+NK inhibitory ligand, booster dose BNT162b2 versus booster dose BNT162b2+NK inhibitory ligand) as well as a control unvaccinated group. The control unvaccinated group, which is present just to check for baseline levels of cross-reactive immune responses to spike protein, will be the same as used for Prophetic Examples 2 and 5. Thus, this experiment will require 60 mice.

20 PROPHETIC EXAMPLE 5

Test whether NK cell depletion in age-matched mice alters acute inflammation and/or levels of antigen-specific antibody and T-cell responses induced by mRNA vaccine against intestinal filarial antigens.

This experiment will be an exact replicate of Prophetic Examples 2 and 3, with the exception that 25 instead of using bivalent BNT162b2 vaccine we will utilize an mRNA vaccine expressing mRNA for BMA-LAD-2 and/or BmUDP-GT. The goal of this study is to determine if the effects observed by NK cell depletion on a COVID mRNA vaccine also occur with mRNA vaccines that express different antigens. BMA-LAD-2 is an adhesion molecule that is essential to *Brugia malayi* worms and functions to maintain tight junctions between intestinal epithelial cells of *B. malayi* adult 30 worms. BmUDP-GT is a transmembrane protein with a large extracellular portion residing in the lumen of the intestinal tract of adult *B. malayi* worms. BmUDP-GT functions as a UDP-glucuronosyl transferase and has been demonstrated to be an essential protein of *B. malayi* adult worms.

Outcomes and numbers of mice to be used: The primary outcome is filarial antigen-specific antibody levels. We hypothesize that NK cell depletion during the time period of mRNA initial vaccination or boosting vaccination will enable an increase in spike specific antibody levels of 30%. A sample size of 13 mice in each group will have 80% power to detect a 30% difference in antibody levels with a 0.05 two-sided significance level, assuming a standard deviation of 0.25 (25% from mean) in each group. Thus, we will use 15 animals per group. There will be four principal experimental groups (vaccinated and NK depleted versus vaccinated and not NK depleted, boosted and NK depleted versus boosted and not NK depleted) plus an unvaccinated group. Thus, this experiment will require 60 mice.

10 PROPHETIC EXAMPLE 6

Test whether vaccination with mRNA vaccine containing both mRNA for a vaccine antigen as well as mRNA for an inhibitory ligand of Natural Killer(NK) cells will prolong the time during which the vaccine antigen protein is expressed

To show that addition of NK inhibitors to mRNA vaccines will increase the time during which the vaccine antigen protein is expressed, mice will be vaccinated with one of the following mRNA vaccines, each containing the same amount of LNP loaded with mRNA encoding SARS-CoV-2 spike protein (as vaccine antigen), one additionally containing 0.5 µg LNP loaded with mRNA encoding green fluorescent protein (as a control), one additionally containing 0.5 µg LNP loaded with mRNA encoding a SERPIN protein (e.g., SERPIN B9 NK inhibitor), and one additionally containing 0.5 µg of LNP loaded with mRNA encoding Qa1 (NK inhibitor):

0.5 µg spike LNP + 0.5 µg GFP LNP (S+GFP)

0.5 µg spike LNP + 0.5 µg Serpin LNP (S+Serpin)

0.5 µg spike LNP + 0.5 µg Qa1 LNP (S+Qa1).

Mice will be euthanized at days 1, 3, and 7 post-vaccination and levels of spike protein mRNA present in lymph nodes and spleens will be quantified by qPCR. Additionally, serum levels of spike protein will be measured at days 1, 3, and 7 post-vaccination by ELISA. Results will show whether vaccination with the (S+Serpin) or (S+Qa1) mRNA LNP vaccine formulations prolong the time during which the vaccine antigen (spike protein) is expressed.

PROPHETIC EXAMPLE 7

30 Test whether vaccination with mRNA vaccine containing both mRNA for a vaccine antigen as well as mRNA for an inhibitory ligand of Natural Killer(NK) cells will enhance the durability of the antibody response

One of the major limitations of current mRNA vaccines is low durability of antibody responses. To show that addition of NK inhibitors to mRNA vaccines will enhance the durability of circulating antibodies, three groups of mice will be vaccinated as described above. Serum will be obtained at 4 weeks, 8 weeks, 12 weeks, and 16 weeks after vaccination, and tested for levels of anti-spike
5 IgG antibodies. Results will show whether vaccination with the (S+Serp) or (S+Qa1) mRNA LNP vaccine formulations enhances the durability of the antibody response.

It will be understood that the Specification and Examples are illustrative of the present embodiments and that other embodiments within the spirit and scope of the described embodiments will suggest themselves to those skilled in the art. Although this disclosure has been
10 described in connection with specific forms and embodiments thereof, it would be appreciated that various modifications other than those discussed above may be resorted to without departing from the spirit or scope of the disclosure. For example, equivalents may be substituted for those specifically described, and in certain cases, particular applications of steps may be reversed or interposed all without departing from the spirit or scope of the disclosure.

15

SEQUENCES

SerpB9 (SEQ ID NO. 1) (Natural human sequence from GenBank RefSeq file NM_004155)

5
120 auggaacucu uucuaugca agugguacuu uugccauacg ccuuuuuag auacuguguc
181 aagauaacc uucgcacaac guuuucuguu cuccugugag caucuccucu gccugggcca
241 ugguuucucu aggggcaaag ggaacacccg caaccagau gggccaggca cugucuuuaa
301 acacagagga agacauuau cgggcuuucc agucgcuucu cacugaagug aacaaggcug
10 361 gcacacagua ccugcugaga acggccaaca ggcucuugg agagaaaacu ugucaguucc
421 ucuaacguu uaaggaucc ugucuucau ucuaccaugc ugagcugaag gagcuuuccu
481 uuaucagagc ugcagaagag uccaggaaac acaucaacac cugggucua aaaaagaccg
541 aagguaaaau ugaagaguug uugccgggua gcuaauuga ugcagaaacc aggcugguuc
601 uugucaaugc caucuacuuc aaaggaaagu ggaugaacc guuugacgaa acauacacaa
15 661 gggaaugcc cuuuuuuuu aaccaggagg agcaaaggcc agugcagaug auguacagg
721 aggccacguu uaagcugcc cagcugggcg aggugcgcg gcagcugcug gagcugcccu
781 acgcaggaa ggagcugagc cugcugugc ugcugccuga cgacggcgug gagcucagca
841 cgguggaaaa aagucucacu uuugagaac ucacagccug gaccaagcca gacuguaug
901 agaguacuga gguugaagu cuccuuccaa aauuuuacu acaaggagau uaugacaug
20 961 aaucugugc ucggaauug ggaauuguu augccuucca acagggaag gcugacuugu
1021 cggcaaugc agcggagaga gaccugugc uguccaagu cgugcacaag aguuuugug
1081 agguagauga agaaggcacc gaggcagcg cagcgucgag cugcuuugua guugcagagu
1141 gcugcaugga aucuggcccc agguucugug cugaccacc uuuccuuuc uucaucaggc
1201 acaacagagc caacagcau cuguucugug gcagguucuc aucgccaua

25

HLA-E*0103 (SEQ ID NO. 2) (Natural human sequence from GenBank RefSeq file AY216681)

30 1 augguagaug gaaccuccu uuuaucucc ucggaggccc uggccuuac ccagaccug
61 gcgggcuccc acuccuuga guuuuuccac acuccgugu ccgggcccg ccgcggggag
121 cccgcuuca ucucugugg cuacugggac gacaccagu ucgugcgcu cgacaacgac
181 gccgcaguc cgaggauugu gccgcggcg ccguggaug agcaggagg gucagaguau
241 ugggaccgg agacacggag cgcaggggac accgcacaga uuuccgagu gaaccugcg
35 301 acgugcgcg gcuacuaca ucagagcgag gccgggucuc acaccugca guggaugcau
361 ggcugcgagc uggggccga cggcgcuuc cuccgcggg augaacagu cgcuaacgac
421 ggcaaggau aucucacccu gaauaggag cugcgcuuc ggaccgggu ggacacggcg
481 gcucagauc cagagcaaaa gucaauugau gccucgagg cggagacca gagagccuac
541 cuggaagaca caugcugga guggcuccac aaauaccug agaagggga ggagacgug
40 601 cuacaccug agccccaaa gacacacgug acucaccacc ccaucucuga ccaugaggcc
661 accugagggu gcugggccc gggcuucua ccugcgga uacacugac cuggcagcag
721 gauggggagg gccauacca ggacacggag cucguggaga ccaggccugc aggggaugga
781 accuuccaga agugggcagc uguggugug ccuucggag aggagcagag auacacgugc
841 caugucgagc augaggggcu acccgagccc gacaccuga gauggaagcc ggcuuuccag
45 901 cccaccaucc ccaucgugg caucauugcu ggccugguuc uccuuggauc uguggucucu
961 ggagcugug uugcugcugu gauauggag aagaagagc cagguggaaa aggagggagc
1021 uacucuaagg cugaguggag cgacagugc caggggucug agucacacag cuuguaa

50

HLA-E*0101 (SEQ ID NO. 3)

1 augguagaug gaaccuccu uuuacuccuc ucggaggccc uggcccuuac ccagaccugg
61 gcgggcuccc acuccuugaa guauuuccac acuuccgugu cccggcccgg ccgcggggag
5 121 ccccgcuua ucucuguggg cuacguggac gacaccagu ucgugcgcuu cgacaacgac
181 gccgcgaguc cgaggauugu gccgcgggcg ccguggaugg agcaggaggg gucagaguau
241 ugggaccggg agacacggag cgcaggggac accgcacaga uuuuccgagu gaaccugcgg
301 acgcugcgcg gcuacuacaa ucagagcgag gccgggucuc acaccucgca guggaugcau
361 ggucgcgagc uggggcccg caggcgcuuc cuccgcgggu augaacaguu cgccuacgac
10 421 ggcaaggauu aucucacccu gaaugaggac cugcgcuccu ggaccgcggg ggacacggcg
481 gcucagauu cagagcaaaa gucaaaugau gccucugagg cggagcacca gagagccuac
541 cuggaagaca caugcugga guggcuccac aaauaccugg agaaggggaa ggagacgcug
601 cuuaccugg agccccaaa gacacacgug acucaccacc ccaucucuga ccaugaggcc
661 acccugaggu gcugggccc ggguucucac ccugcgga ucacacugac cuggcagcag
15 721 gauggggagg gccauacca ggacacggag cucguggaga ccaggccugc aggggaugga
781 accuaccaga agugggcagc uguggugug ccuucuggag aggagcagag auacacgugc
841 caugucagc augaggggcu acccgagccc guacccuga gauggaagcc ggcuucccag
901 cccaccaucc ccaucgugg caucauugcu ggccugguuc uccuuggauc uguggucucu
961 ggagcugugg uugcugcugu gauauggagg aagaagagcu cagguggaaa aggagggagc
20 1021 uacucuaagg cugaguggag cgacagugcc caggggucug agucucacag cuuguaa

CLEC2D (SEQ ID NO. 4)

1 augcaugaca guacaaugu ggagaaagac auuacaccau cugaauugcc ugcaaaccca
61 gguugucugc auucaaaa gcauucuuu aaagcuaccu uauuuuggcg cuuauuuuuc
121 uuaaucaugu uucugacaau cauagugugu ggaauuguug cugcuuuuag cgcaauaaga
181 gcuaacugcc aucaagagcc aucagauugu cuucaagcug caugcccaga aagcuggauu
30 241 gguuuucaa gaaaguguu cuuuuuuuc gaugacacca agaacuggac aucaagucag
301 agguuuugug acucacaaga ugcugaucuu gcucagguug aaagcuucca ggaacugaau
361 uuccuguuga gauauaaagg ccaucugau cacuggauug ggucgagcag agaacaaggc
421 caaccaugga aauggauaaa ugguacugaa uggacaagac aguuuccuau ccugggagca
481 ggagagugug ccuauuugaa ugacaaaggu gccaguagug ccaggcacua cacagagagg
35 541 aaguggauu guuccaaauc agauauacau gucuag

SerpB9 (SEQ ID NO. 5)

40 METLSNASGTF AIRLLKLCQDNPSHNVFCSPVSISSALAMVLLGAKGNTATQMAQALSLNTEEDI
HRAFQSLLEVNKAGTQYLLRTANRLFGEKTCQFLSTFKESCLQFYHAELKELSFIRAAEESRKHI
NTWWSKKTEGKIEELPGSSIDAETRLVLVNAIFYKGKWNPFDETYTREMPFKINQEEQRPVQM
MYQEATFKLAHVGEVRAQLLELPYARKELSLVLLPDDGVELSTVEKSLTFEKLTAWTKPDCMKS
TEVEVLLPKFKLQEDYDMESVLRHLGIVDAFQQGKADLSAMSAERDLCLSKFVHKSFEVNEEG
45 TAAAAASSCFVVAECMESGPRFCADHPFLFFIRHNANSILFCGRFSSP

HLA-E*0103 (SEQ ID NO. 6)

50 MVDGTLLELSEALALTQTWAGSHSLKYFHTSVSRPGRGEPFISVG YVDDTQFVRFDNDAAASP
RMVPRAPWMEQEGSEYWDRETRSARDTAQIFRVNLR LTRGYYNQSEAGSHTLQWMHGCELG
PDGRFLRGYEQFAYDGKDYLTNEDLRSWTAVDTAAQISEQKSNDA SEAEHQRAYLEDTCVEW
LHKYLEKGKETLLHLEPPKTHVTHHPISDHEATLRCWALGFYPAEITLTWQQDGEGHTQDTELVE
TRPAGDGTGFKWAAVVVPSGEEQRYTCHVQHEGLPEPVTLRWKPASQPTIPVIGIAGLVLLGSV
55 VSGAVVAAVIWRKKSSGGKGSYSKAEWSDSAQGSSESHL

HLA-E*0101 (SEQ ID NO. 7)

5 MVDGTL L L L L L S E A L A L T Q T W A G S H S L K Y F H T S V S R P G R G E P R F I S V G Y V D D T Q F V R F D N D A A S P
R M V P R A P W M E Q E G S E Y W D R E T R S A R D T A Q I F R V N L R T L R G Y Y N Q S E A G S H T L Q W M H G C E L G
P D R R F L R G Y E Q F A Y D G K D Y L T L N E D L R S W T A V D T A A Q I S E Q K S N D A S E A E H Q R A Y L E D T C V E W
L H K Y L E K G K E T L L H L E P P K T H V T H H P I S D H E A T L R C W A L G F Y P A E I T L T W Q Q D G E G H T Q D T E L V E
10 T R P A G D G T F Q K W A A V V V P S G E E Q R Y T C H V Q H E G L P E P V T L R W K P A S Q P T I P I V G I I A G L V L L G S V
V S G A V V A A V I W R K K S S G G K G G S Y S K A E W S D S A Q G S E S H S L

CLEC2D (SEQ ID NO. 8)

15 M H D S N N V E K D I T P S E L P A N P G C L H S K E H S I K A T L I W R L F F L I M F L T I I V C G M V A A L S A I R A N C H Q E P
S V C L Q A A C P E S W I G F Q R K C F Y F S D D T K N W T S S Q R F C D S Q D A D L A Q V E S F Q E L N F L L R Y K G P S D
H W I G L S R E Q G Q P W K W I N G T E W T R Q F P I L G A G E C A Y L N D K G A S S A R H Y T E R K W I C S K S D I H V

20

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CLAIMS:

1. An isolated polynucleotide construct comprising:
 - (a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity; and
 - (b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a naturally endogenously produced protein.
2. A composition comprising:
 - (a) one or more polynucleotide constructs of claim 1; and
 - (b) a pharmaceutically acceptable carrier.
3. A composition comprising:
 - (a) a first polynucleotide encoding a polypeptide capable of having natural killer (NK) cell inhibiting activity;
 - (b) a second polynucleotide encoding a polypeptide capable of inducing an immune response or encoding a naturally endogenously produced protein; and
 - (c) a pharmaceutically acceptable carrier.
4. The construct or composition of any one of claims 1-3, wherein the polynucleotide construct or first and second polynucleotides are mRNA molecules.
5. The construct or composition of any one of the preceding claims, wherein the first polynucleotide encodes a serine protease inhibitor (serpin) polypeptide or fragment thereof capable of having natural killer (NK) cell inhibiting activity.
6. The construct or composition of claim 5, wherein the serpin polypeptide is a serpin polypeptide selected from the group consisting of SERPINA1, SERPINA2, SERPINA3, SERPINA4, SERPINA5, SERPINA6, SERPINA7, SERPINA8, SERPINA9, SERPINA10, SERPINA11, SERPINA12, SERPINA13, SERPINB1, SERPINB2, SERPINB3, SERPINB4, SERPINB5, SERPINB6, SERPINB7, SERPINB8, SERPINB9, SERPINB10, SERPINB11, SERPINB12, SERPINB13, SERPINC1, SERPIND1, SERPINE1, SERPINE2, SERPINE3, SERPINF1, SERPINF2, SERPING1, SERPINH1, SERPINI1, SERPINI2, and combinations thereof.

7. The construct or composition of any one of claims 5-6, wherein the serpin polypeptide is SERPINB9.
8. The construct or composition of claim 7, where the first polynucleotide encodes a serpin polypeptide or fragment thereof capable of having NK cell inhibiting activity, wherein:
- 5 (a) the first polynucleotide comprises SEQ ID NO:1 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:1; or
- 10 (b) the first polynucleotide encodes a serpin polypeptide that comprises SEQ ID NO:5 or a sequence having at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.9%, or at least 99.99% sequence identity to SEQ ID NO:5.
- 15 9. The construct or composition of any one of claims 1-4, wherein the first polynucleotide encodes:
- a major histocompatibility complex (MHC) class polypeptide or fragment thereof having NK cell inhibiting activity; or
- a lectin polypeptide or fragment thereof capable of having NK cell inhibiting activity; or
- 20 a cadherin polypeptide or fragment thereof capable of having NK cell inhibiting activity; or
- CDH1 (e-cadherin) or a fragment thereof capable of having NK cell inhibiting activity, or a variant of CDH1 (e-cadherin) or a fragment thereof capable of having NK cell inhibiting activity;
- or
- CD155 (poliovirus receptor) or a fragment thereof capable of having NK cell inhibiting
- 25 activity, or a variant of CD 155 or a fragment thereof capable of having NK cell inhibiting activity;
- or
- RTN4 or a fragment thereof capable of having NK cell inhibiting activity, or a variant of RTN4 or a fragment thereof capable of having NK cell inhibiting activity.
- 30 10. The construct or composition of any one of the preceding claims, wherein the second polynucleotide encodes an antigen capable of inducing an immune response, optionally wherein the antigen is a COVID-19 antigen capable of inducing an immune response against COVID-19.

11. The construct or composition of any one of claims 1-9, wherein the second polynucleotide encodes a normally endogenously produced protein, optionally wherein the normally endogenously produced protein is Factor VIII or a biologically active fragment thereof.
- 5 12. A method for enhancing one or both of the magnitude and durability of immune responses induced by a vaccine or for reducing one or more unwanted side effects associated with the administration of a vaccine, comprising the steps of:
- (a) administering to a subject a pharmaceutically effective amount of a first polynucleotide that encodes a polypeptide capable of having NK cell inhibiting activity; and
- 10 (b) administering to a subject a pharmaceutically effective amount of one or more vaccines;
- wherein inhibition of NK cell activity enhances the magnitude and/or durability of immune responses induced by the one or more vaccines administered to the subject and/or reduces one or more unwanted side effects associated with the administration of a vaccine,
- 15 optionally wherein the method enhances one or both of antibody and T cell responses to a vaccine antigen of the vaccine,
- optionally wherein the vaccine is a polynucleotide vaccine comprising a polynucleotide encoding a vaccine antigen, optionally wherein the polynucleotide is an mRNA molecule.
- 20 13. The method of claim 12, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in a single polynucleotide construct, optionally wherein the single polynucleotide construct is a construct according to any one of claims 1-11.
14. The method of claim 12, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in a single composition, optionally wherein the single composition is a composition according to any one of claims 1-11.
- 25 15. The method of claim 12, wherein the first polynucleotide and polynucleotide encoding a vaccine antigen are present in separate polynucleotide constructs formulated in separate compositions.
- 30

16. A method for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects associated with administration of an exogenously administered polynucleotide, comprising the steps of:
- (a) administering to a subject a pharmaceutically effective amount of a first polynucleotide encoding a polypeptide capable of having NK cell inhibiting activity; and
 - (b) administering to the subject a second exogenously administered polynucleotide, optionally wherein the second exogenously administered polynucleotide encodes a normally endogenously produced protein, further optionally wherein the normally endogenously produced protein is Factor VIII or a biologically active fragment thereof;
- wherein inhibition of NK cell activity enhances the durability of the exogenously administered polynucleotide and/or reduces one or more unwanted side effects associated with administration of the exogenously administered polynucleotide.
17. The method of claim 16, wherein the second exogenously administered polynucleotide encodes an antigen capable of inducing an immune response, optionally wherein the antigen is a COVID-19 antigen capable of inducing an immune response against COVID-19.
18. A construct or composition according to any one of claims 1-11, for enhancing one or both of the magnitude and durability of immune responses induced by a vaccine or reducing one or more unwanted side effects associated with administration of a vaccine, optionally wherein the vaccine is a polynucleotide vaccine comprising a polynucleotide encoding a vaccine antigen, further optionally wherein the polynucleotide is an mRNA molecule.
19. A construct or composition according to claim 11, for enhancing durability of an exogenously administered polynucleotide or reducing one or more unwanted side effects associated with administration of an exogenously administered polynucleotide.
20. A cell expressing a polynucleotide construct of any one of claims 1-11.