CLAIMS

~~1. A method of improving polypeptide antigen immunogenicity, the method comprising providing a polypeptide antigen to be modified, and covalently conjugating the polypeptide antigen to one or more steroid acid moieties to produce a modified polypeptide antigen, the modified polypeptide antigen being conjugated to a sufficient number of steroid acid moieties to increase endosomal escape of the modified polypeptide antigen upon intracellular delivery relative to a polypeptide antigen lacking said modification, wherein the modified polypeptide antigen triggers an improved adaptive immune response to said polypeptide antigen upon administration to a subject as compared to a corresponding unmodified polypeptide antigen.~~

~~2. The method of claim 1, wherein the modified polypeptide antigen is conjugated to a sufficient number of steroid acid moieties such that the modified polypeptide antigen exhibits greater stability (e.g., thermal stability) than that of the polypeptide antigen prior to conjugation.~~

~~3. The method of claim 1 or 2, wherein the polypeptide antigen is a protein antigen, and/or has a molecular weight of at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130, 135, 140, 145, or 150 kDa.~~

~~4. The method of any one of claims 1 to 3, wherein the polypeptide antigen comprises one or more MHC class I epitopes and/or MHC class II epitopes.~~

~~5. The method of any one of claims 1 to 4, wherein the polypeptide antigen is or comprises a tumor-associated antigen (TAA), tumor-specific antigen (TSA), a neoantigen, a viral antigen, a bacterial antigen, a fungal antigen, an antigen associated with a disease or disorder amenable to treatment by vaccination and/or immunotherapy; or any antigenic fragment thereof.~~

~~6. The method of any one of claims 1 to 5, wherein the polypeptide antigen is or comprises a corona viral antigen (e.g., SARS-CoV-2 Spike protein (**SEQ ID NO: 3**) or SARS-CoV Spike protein (**SEQ ID NO: 4**) or an antigenic fragment thereof; or a cancer antigen, such as a single-nucleotide variant antigen, a mutational frameshift antigen, splice variant antigen, a gene fusion antigen, an endogenous retroelement antigen, or another class of antigen, such as a human leukocyte antigen (HLA)-somatic mutation-derived antigen or a post-translational TSA, a viral-derived cancer antigen (e.g., from human papillomavirus (HPV), cytomegalovirus, or Epstein–Barr virus (EBV)), a cancer-testis antigen, HER2, PSA, TRP-1,~~

~~TRP-2, EpCAM, GPC3, CEA, MUC1, MAGE-A1, NY-ESO-1, SSX-2, mesothelin (MSLN), EGFR, cell lysates or other material derived from a tumor (e.g., tumor-derived exosomes).~~

~~7. The method of any one of claims 1 to 6, wherein the steroid acid triggers ceramide accumulation on the inner leaflet of endosomes, thereby destabilizing endosomal membranes and facilitating endosomal escape of the polypeptide antigen upon intracellular delivery.~~

~~8. The method of any one of claims 1 to 7, wherein the steroid acid triggers increased acid sphingomyelinase (ASM)-mediated cleavage of sphingomyelin to form ceramide.~~

~~9. The method of any one of claims 1 to 8, wherein the steroid acid is a bile acid.~~

~~10. The method of any one of claims 1 to 9, wherein the steroid acid is a primary bile acid or a secondary bile acid.~~

~~11. The method of any one of claims 1 to 10, wherein the steroid acid is or comprises:~~

- ~~(a) a bile acid which is: cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), glycodeoxycholic acid (GDCA), glycocholic acid (GCA), taurocholic acid (TCA), glycodeoxycholic acid (CDCA), glycochenodeoxycholic acid (GCDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), taurohyodeoxycholic acid (THDCA), taurochenodeoxycholic acid (TCDCA), ursocholic acid (UCA), tauroursodeoxycholic acid (TUDCA), ursodeoxycholic acid (UDCA), or glycoursodeoxycholic acid (GUDCA);~~
- ~~(b) an analog of the bile acid of (a) that: induces endocytosis; triggers ceramide accumulation on the inner leaflet of endosomes; triggers increased acid sphingomyelinase (ASM)-mediated cleavage of sphingomyelin to form ceramide; and/or has a hydrophobicity greater than that of cholic acid;~~
- ~~(c) a bile acid or bile acid analog that is more hydrophobic than cholic acid (e.g. CDCA, DCA, LCA, TCA, TDCA, TCDCA, GCA, GDCA, or GCDCA); or~~
- ~~(d) any combination of (a) to (c).~~

~~12. The method of any one of claims 1 to 11, wherein each modified polypeptide antigen molecule is conjugated to at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25,~~

26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 steroid acid moieties.

13. The method of any one of claims 1 to 12, wherein the modified polypeptide antigen molecule is conjugated to the steroid acid at solvent-accessible amine (e.g., primary amine) and/or sulfhydryl groups of the polypeptide antigen.

14. The method of any one of claims 1 to 13, wherein the modified polypeptide antigen molecule is conjugated to the steroid acid via a linker (e.g., bifunctional, trifunctional linker, or multi-functional linker).

15. The method of any one of claims 1 to 14, wherein the steroid acid is comprised in a steroid acid-peptide conjugate and the polypeptide antigen is conjugated to the steroid acid-peptide conjugate (e.g., via said peptide, such as at an N- or C-terminal residue).

16. The method of claim 15, wherein the peptide:

(i) comprises a protein transduction domain that stimulates endocytosis and/or endosomal formation;

(ii) comprises a subcellular targeting signal;

(iii) is a cationic peptide (e.g., a non-cell-penetrating cationic peptide);

(iv) is a non-immunogenic peptide; or

(v) any combination of (i) to (iv).

17. The method of claim 16, wherein the subcellular targeting signal is a nuclear localization signal, such as a classical NLS (e.g., NLS from SV-40 large T-antigen (e.g., PKKKRKV; **SEQ ID NO: 7**) or from other classical NLSs) or a non-classical NLS (e.g., acidic M9 domain in the hnRNP A1 protein; the sequence KIPIK in yeast transcription repressor Matα2; PY-NLS; ribosomal NLS; and the complex signals of U snRNPs).

18. The method of claim 16 or 17, wherein the nuclear localization signal is a/an: SV40 NLS (e.g., comprised in **SEQ ID NO: 1 or 7**), GWG-SV40NLS (e.g., comprised in **SEQ ID NO: 8**), hnRNPA1 M9 NLS (e.g., comprised in **SEQ ID NO: 9**), hnRNP D NLS (e.g., comprised in **SEQ ID NO: 10**), hnRNP M NLS (e.g., comprised in **SEQ ID NO: 11**), PQBP-1 NLS (e.g., comprised in **SEQ ID NO: 12**), NLS2-RG Domain RPS17 (e.g., comprised in **SEQ ID NO: 13**), NLS1 RPS17 (e.g., comprised in **SEQ ID NO: 14**),

NLS2 RPS17 (e.g., comprised in **SEQ ID NO: 15**), NLS3 RPS17 (e.g., comprised in **SEQ ID NO: 16**), cMyc NLS (e.g., comprised in **SEQ ID NO: 17**), HuR NLS (e.g., comprised in **SEQ ID NO: 18**), Tus NLS (e.g., comprised in **SEQ ID NO: 19**), or Nucleoplasmin NLS (e.g., comprised in **SEQ ID NO: 20**) ; or is a variant of an NLS having nuclear localization activity, the NLS comprising or consisting of the amino acid sequence of any one of **SEQ ID NOs: 7 to 20**.

19. The method of any one of claims 1 to 18, wherein the modified polypeptide antigen triggers:

(i) increased cytosolic delivery of the modified polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen;

(ii) increased total cellular delivery of the modified polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen;

(iii) enhanced cellular immunity against said polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen;

(iv) increased IFN-gamma production by CD8+ T cells upon exposure to said polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen;

(v) enhanced humoral immunity against said polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen;

(vi) an increased variety of antibody species against the polypeptide antigen, as compared to a corresponding unmodified polypeptide antigen; or

(vii) any combination of (i) to (vi).

20. A population of cells (e.g., *in vitro* or *ex vivo*) comprising the modified polypeptide antigen produced by the method of any one of claims 1 to 19, or the modified polypeptide antigen as defined in any one of claims 1 to 19.

21. The population of cells of claim 20, which comprises immune cells (e.g., T cells), antigen-presenting cells (e.g., dendritic cells, macrophages, engineered antigen-presenting cells), MHC class I-expressing cells, MHC class II-expressing cells, or any combination thereof.

22. An immunogenic composition comprising: the modified polypeptide antigen produced by the method of any one of claims 1 to 19, the modified polypeptide antigen as defined in any one of claims 1 to 19, the population of cells of claim 20 or 21, or any combination thereof; and a pharmaceutically acceptable excipient and/or adjuvant (e.g., an emulsion adjuvant, oil-in-water emulsion adjuvant, or a squalene-based oil-in-water emulsion adjuvant).

~~23. The immunogenic composition of claim 22, which is a therapeutic or prophylactic vaccine (e.g., anti-cancer vaccine, anti-viral vaccine, or anti-bacterial vaccine).~~

~~24. A method for triggering an enhanced adaptive immune response in a subject against an unmodified polypeptide antigen of interest, the method comprising administering the immunogenic composition of claim 22 or 23 to the subject.~~

~~25. A method for vaccinating a subject against an infectious disease, the method comprising administering the immunogenic composition of claim 22 or 23 to the subject, wherein the polypeptide antigen comprises an antigenic fragment of a pathogen (e.g., virus, bacteria, fungus) causing the infectious disease.~~

~~26. A method for treating cancer in a subject, the method comprising administering the immunogenic composition of claim 22 or 23 to the subject.~~

~~27. The method of claim 26, wherein the method is combined with immune-checkpoint inhibitor therapy.~~

~~28. A modified polypeptide antigen as defined in any one of claims 1 to 19, or produced by the method of any one of claims 1 to 19, for use in generating an immune response in a subject or for the manufacture of an immunogenic composition for generating an immune response in a subject.~~

~~29. Use of the modified polypeptide antigen as defined in any one of claims 1 to 19, the modified polypeptide antigen produced by the method of any one of claims 1 to 19, the population of cells as defined in claim 20 or 21, or the immunogenic composition as defined in claim 22 or 23, for generating an immune response in a subject or for the manufacture of a medicament (e.g., vaccine) for generating an immune response in a subject.~~

~~30. The modified polypeptide antigen for the use of claim 28, or the use of claim 29, wherein the immune response comprises enhanced cellular immunity against said polypeptide antigen, increased IFN-gamma production by CD8+ T cells upon exposure to said polypeptide antigen, enhanced humoral immunity against said polypeptide antigen, or any combination thereof, as compared to that generated from a corresponding unmodified polypeptide antigen.~~

~~31. A method for preparing a polypeptide antigen, the method comprising conjugating an unmodified polypeptide antigen to a sufficient number of steroid acid moieties to produce a modified polypeptide antigen that exhibits greater stability (e.g., thermal stability) than that of the polypeptide antigen prior to conjugation.~~

~~32. The method of claim 31, wherein the number of steroid acid moieties conjugated to the polypeptide antigen is sufficient to increase endosomal escape of the modified polypeptide antigen upon intracellular delivery relative to a polypeptide antigen lacking said modification.~~

~~33. The method of claim 31 or 32, wherein the modified polypeptide antigen is as defined in any one of claims 3 to 19.~~

1.~~34.~~ A method of improving polypeptide antigen immunogenicity, the method comprising providing a polypeptide antigen to be modified, and covalently conjugating the polypeptide antigen to one or more bile acid-peptide moieties to produce a modified polypeptide antigen, the modified polypeptide antigen being conjugated to a sufficient number of bile acid-peptide moieties to trigger an improved adaptive immune response to said polypeptide antigen upon administration to a subject as compared to a corresponding unmodified polypeptide antigen, wherein the peptide comprised in the bile acid-peptide moiety comprises a nuclear localization signal (NLS).

2.~~35.~~ The method of claim 1,~~34,~~ wherein the modified polypeptide antigen is conjugated to a sufficient number of bile acid-peptide moieties to increase antigen presentation of the modified polypeptide antigen upon intracellular delivery relative to a corresponding unmodified polypeptide antigen.

3.~~36.~~ The method of claim 1 or 2,~~34 or 35,~~ wherein the modified polypeptide antigen is conjugated to a sufficient number of bile acid-peptide moieties such that the modified polypeptide antigen exhibits greater thermal stability relative to a corresponding unmodified polypeptide antigen.

4.~~37.~~ The method of any one of claims 1 to 3,~~34 to 36,~~ wherein covalently conjugating the polypeptide antigen to one or more bile acid-peptide moieties is performed by reacting the polypeptide antigen with a molar excess of the bile acid-peptide moiety.

5.~~38.~~ The method of claim 4,~~37,~~ wherein the polypeptide antigen is reacted with between a 2-fold and 100-fold molar excess of the bile acid-peptide moiety.

6.~~39.~~ The method of claim 4,~~37,~~ wherein the polypeptide antigen is reacted with between a 2-fold and 50-fold molar excess of the bile acid-peptide moiety.

7.~~40.~~ The method of claim 4,~~37,~~ wherein the polypeptide antigen is reacted with between a 5-fold and 25-fold molar excess of the bile acid-peptide moiety.

8.~~41.~~ The method of any one of claims 1 to 7,~~34 to 40,~~ wherein the mean number of bile acid-peptide moieties conjugated per modified polypeptide antigen is at least ~~1~~about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50; or is between about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 and *n*, wherein *n* is the total number of accessible sites on the polypeptide antigen available for conjugation.

9.~~42.~~ The method of any one of claims 1 to 8,~~34 to 41,~~ wherein the bile acid is: cholic acid (CA), chenodeoxycholic acid (CDCA), deoxycholic acid (DCA), lithocholic acid (LCA), glycodeoxycholic acid (GDCA), glycocholic acid (GCA), taurocholic acid (TCA), ~~glycodeoxycholic acid (CDCA),~~ glycochenodeoxycholic acid (GCDCA), taurodeoxycholic acid (TDCA), glycolithocholic acid (GLCA), taurolithocholic acid (TLCA), taurohyodeoxycholic acid (THDCA), taurochenodeoxycholic acid (TCDCA), ursocholic acid (UCA), tauroursodeoxycholic acid (TUDCA), ursodeoxycholic acid (UDCA), or glycoursodeoxycholic acid (GUDCA).

10.~~43.~~ The method of any one of claims 1 to 9,~~34 to 42,~~ wherein the bile acid is an analog of CA, CDCA, DCA, LCA, GDCA, GCA, TCA, ~~CDCA,~~ GCDCA, TDCA, GLCA, TLCA, THDCA, TCDCA, UCA, TUDCA, UDCA, or GUDCA, wherein the analog: induces endocytosis; triggers ceramide accumulation on the inner leaflet of endosomes; or triggers increased acid sphingomyelinase (ASM)-mediated cleavage of sphingomyelin to form ceramide.

11.~~44.~~ The method of any one of claims 1 to 10,~~34 to 43,~~ wherein the nuclear localization signal is a/an: SV40 NLS (**SEQ ID NO: 1 or 7**), GWG-SV40NLS (**SEQ ID NO: 8**), hnRNPA1 M9 NLS (**SEQ ID NO: 9**), hnRNP D NLS (**SEQ ID NO: 10**), hnRNP M NLS (**SEQ ID NO: 11**), PQBP-1 NLS (**SEQ ID NO: 12**), NLS2-RG Domain RPS17 (**SEQ ID NO: 13**), NLS1 RPS17 (**SEQ ID NO: 14**), NLS2 RPS17 (**SEQ**

ID NO: 15), NLS3 RPS17 (**SEQ ID NO: 16**), cMyc NLS (**SEQ ID NO: 17**), HuR NLS (**SEQ ID NO: 18**), Tus NLS (**SEQ ID NO: 19**), or Nucleoplasmin NLS (**SEQ ID NO: 20**).

12.~~45.~~ The method of any one of claims 1 to 11,~~34 to 44,~~ wherein the nuclear localization signal is a variant of an NLS having nuclear localization activity, the NLS comprising or consisting of the amino acid sequence of any one of **SEQ ID NOs: 7 to 20**.

13.~~46.~~ The method of any one of claims 1 to 12,~~34 to 45,~~ wherein the polypeptide antigen is conjugated to the one or more bile acid-peptide moieties via a linker.

14.~~47.~~ The method of claim 13,~~46,~~ wherein the linker is a bifunctional linker, trifunctional linker, or multi-functional linker.

15.~~48.~~ The method of any one of claims 1 to 14,~~34 to 47,~~ wherein the modified polypeptide antigen molecule is conjugated to the one or more bile acid-peptide moieties via a solvent-accessible functional group of the polypeptide antigen.

16.~~49.~~ The method of any one of claims 1 to 15,~~34 to 48,~~ wherein the polypeptide antigen is or comprises a tumor-associated antigen (TAA), tumor-specific antigen (TSA), cell lysate derived from a tumor, tumor-derived exosomes, a neoantigen, a viral antigen, a bacterial antigen, a fungal antigen, or other antigen associated with a disease or disorder amenable to treatment by vaccination and/or immunotherapy.

17.~~50.~~ The method of any one of claims 1 to 16,~~34 to 49,~~ wherein the polypeptide antigen is or comprises a severe acute respiratory syndrome coronavirus (SARS-CoV) Spike protein or an antigenic fragment thereof.

18.~~51.~~ An immunogenic composition comprising:

a modified polypeptide antigen and a pharmaceutically acceptable excipient and/or adjuvant, wherein the modified polypeptide antigen ~~produced by the~~ comprises a polypeptide antigen covalently conjugated to a sufficient number of bile acid-peptide moieties to trigger an improved adaptive immune response to said polypeptide antigen upon administration to a subject as compared to a corresponding unmodified polypeptide antigen, wherein the peptide comprised in the bile acid-peptide moiety comprises a nuclear localization signal (NLS)~~method of any one of claims 34 to 50~~; or

a population of cells comprising ~~the~~ a modified polypeptide antigen and a pharmaceutically acceptable excipient and/or adjuvant, wherein the modified polypeptide antigen ~~produced by~~comprises a polypeptide antigen covalently conjugated to a sufficient number of bile acid-peptide moieties to trigger an improved adaptive immune response to said polypeptide antigen upon administration to a subject as compared to a corresponding unmodified polypeptide antigen, wherein the peptide comprised in the bile acid-peptide moiety comprises a nuclear localization signal (NLS) ~~the method of any one of claims 34 to 50, and a pharmaceutically acceptable excipient and/or adjuvant~~.

19.~~52.~~ The immunogenic composition of claim 18,~~51,~~ wherein the population of cells comprises dendritic cells, B cells, T cells, macrophages, engineered antigen-presenting cells, MHC class I-expressing cells, MHC class II-expressing cells, or any combination thereof.

~~53. A method for triggering an enhanced adaptive immune response in a subject against an unmodified polypeptide antigen of interest, the method comprising administering the immunogenic composition of claim 52 to the subject.~~

20. Use of the immunogenic composition of claim 18 or 19 in the manufacture of a medicament for triggering an enhanced adaptive immune response in a subject against an unmodified polypeptide antigen of interest.