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 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. The method of claim 1, 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. The method of claim 1 or 2, 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. The method of any one of claims 1 to 3, 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. The method of claim 4, wherein the polypeptide antigen is reacted with between a 2-fold and 100-fold molar excess of the bile acid-peptide moiety.

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

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

8. The method of any one of claims 1 to 7, wherein the mean number of bile acid-peptide moieties conjugated per modified polypeptide antigen is at least 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. The method of any one of claims 1 to 8, 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), 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. The method of any one of claims 1 to 9, wherein the bile acid is an analog of CA, CDCA, DCA, LCA, GDCA, GCA, TCA, 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. The method of any one of claims 1 to 10, 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. The method of any one of claims 1 to 11, 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. The method of any one of claims 1 to 12, wherein the polypeptide antigen is conjugated to the one or more bile acid-peptide moieties via a linker.

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

15. The method of any one of claims 1 to 14, 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. The method of any one of claims 1 to 15, 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. The method of any one of claims 1 to 16, wherein the polypeptide antigen is or comprises a severe acute respiratory syndrome coronavirus (SARS-CoV) Spike protein or an antigenic fragment thereof.

18. An immunogenic composition comprising:

a modified polypeptide antigen and a pharmaceutically acceptable excipient and/or adjuvant, wherein the modified polypeptide antigen 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); or

a population of cells comprising a modified polypeptide antigen and a pharmaceutically acceptable excipient and/or adjuvant, wherein the modified polypeptide antigen 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).

19. The immunogenic composition of claim 18, 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.

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.