platform provides various advantages including, *e.g.*, high affinity (dissociation constant [KD] ≈ 10^{-15}M), non-covalent binding between biotin and rhizavidin, a biotin-binding protein that has no significant predicted homology with human proteins. Among other things, such a MAPS multivalent pneumococcal vaccine could induce a T- and B-cell immune response against a broad range of S. pneumoniae serotypes; among other things, such a MAPS multivalent pneumococcal vaccine could induce immune response against *S. pneumoniae* serotypes that not included in the vaccine.

[0010] Among other things, the present disclosure describes compositions and methods for prevention and/or treatment of pneumococcal infections in patient populations in need thereof. In some examples, a set of pneumococcal polysaccharide serotypes to be included in compositions described herein are curated and selected based on a variety of considerations including, *e.g.*, characteristics of diseases (*e.g.*, epidemiology, prevalence, worldwide coverage, etc.), and/or compatibility and immunogenicity of various components in a single composition. In some example, the present disclosure provides a further surprising insight that certain pneumococcal polysaccharide serotypes can induce a stronger immune response when they are non-covalently associated with a certain polypeptide antigen. For example, in some examples, certain pneumococcal polysaccharide serotypes (*e.g.*, serotypes 1, 6B, 9V, 15B, 22F, 23A, 23B, 23F, and 33F) were demonstrated to induce a stronger immune response when they are non-covalently associated with a SPP2 fusion protein as described herein, as compared to the immune response observed when those serotypes were non-covalently associated with a CP1 fusion protein as described herein. In some embodiments, certain pneumococcal polysaccharide serotypes (*e.g.*, serotypes 2, 6A, 10A, 18C and 20B) were demonstrated to induce a stronger immune response when they are non-covalently associated with a CP1 fusion protein as described herein, as compared to the immune response observed when those serotypes were non-covalently associated with a SPP2 fusion protein as described herein.

[0011] In some examples, the present disclosure, among other things, describes compositions of SPP2 fusion polypeptides comprising a biotin-binding protein and *S. pneumoniae* polypeptide antigens that do not induce hemolysis and hemagglutination at a detectable level. In particular, the present disclosure describes asurprising insight that the relative position of *S. pneumoniae* polypeptide antigens SP0435 and PdT(G294P) in a fusion protein can

unexpectedly impact the activity of the fusion protein. Reversing the order of SP0435 and PdT(G294P) in a fusion protein, *i.e.*, positioning SP0435 to the C-terminus of PdT(G294P) in a fusion protein, unexpectedly abolished both hemolysis and hemagglutination.

[0012] In some examples, the present disclosure, among other things, provides an insight that immunization with SPP2 fusion protein and *S. pneumoniae* capsular polysaccharide (CPS) of a serotype can synergistically contribute to protection against invasive pneumococcal disease (IPD) by the same serotype. In some examples, the serotype is of serotype 3. In some examples, the SPP2 fusion protein is in a MAPS complex. In some examples, the CPS is in a MAPS complex or in a glycoconjugate (*e.g.*, PCV13). In some examples, immunization with a combination of SPP2 fusion protein and CPS of a serotype can increase protection (*e.g.*, as measured by percent survival) against IPD by the serotype relative to a reference without the combination of SPP2 fusion protein and CPS of the serotype. In some examples, the protection is increased by at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, or at least 10-fold relative to a reference. In some examples, such a reference is SPP2 fusion protein without CPS of the serotype. In some examples, such a reference is CPS of the serotype (*e.g.*, in a MAPS complex or glycoconjugate (*e.g.*, PCV13)) without SPP2 fusion protein.

[0013] One example of the present disclosure describes a multivalent pneumococcal immunogenic composition (*e.g.*, vaccine). In some aspects, the present disclosure refers toan immunogenic composition (*e.g.*, vaccine) comprising an immunogenic complex that comprises: (a) a biotinylated polysaccharide; and (b) a fusion protein comprising an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59, wherein the fusion protein comprises: (i) a biotin-binding moiety; and (ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof; and (iii) an SP0435 polypeptide or antigenic fragment thereof; wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form the immunogenic complex.

[0014] In some examples, where more than one species of an immunogenic complex is included in an immunogenic composition (*e.g.*, vaccine), one or more polypeptide antigens can

be utilized. In some examples, at least one polypeptide antigen in an immunogenic complex comprises: a pneumolysin (Ply) polypeptide or variant or antigenic fragment thereof; or an SP0435 polypeptide, or variant or antigenic fragment thereof. In some

examples, at least one polypeptide antigen in an immunogenic complex comprises: (a) a first polypeptide antigen comprising a Ply polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0435 polypeptide, or variant or antigenic fragment thereof. In some examples, at least one polypeptide antigen in an immunogenic complex comprises: an SP1500 polypeptide or variant or antigenic fragment thereof; or an SP0785 polypeptide or variant or antigenic fragment thereof. In some examples, at least one polypeptide antigen in an immunogenic complex comprises: (a) a first polypeptide antigen comprising an SP1500 polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0785 polypeptide or variant or antigenic fragment thereof.

[0015] In some examples where more than one species of an immunogenic complex is included in an immunogenic composition (*e.g.*, vaccine), one or more biotinylated polysaccharide antigens can be utilized. In some examples, at least one biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae.* In some examples, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

[0016] In some examples, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more (*e.g.*, 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, or 34) of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0017] In some examples, a biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more (*e.g.*, 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, or 33) of serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0018] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some examples, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100%

identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0019] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some examples, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0020] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some examples, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0021] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some examples, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0022] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: (a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of first fusion proteins, each first fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form an immunogenic complex. Additionally or alternatively, in some examples, immunogenic complexes in an immunogenic composition (*e.g.*, vaccine) described herein comprise (a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of each of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38; and (b) a plurality of second fusion proteins, each second fusion protein comprising (i) a biotin-binding moiety; (ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and (iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof; wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

[0023] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide that has or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide that has or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof. In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein that is or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 26 or SEQ ID NO: 61. In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein CP1 as described herein.

[0024] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide that has or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide that has or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof. In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein that is or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 19 or SEQ ID NO: 58. In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein SPP2 as described herein.

[0025] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising one or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty one or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein). In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty two or more species of immunogenic complexes, wherein a species refers to complexes comprising a polysaccharide antigen of a single *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0026] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty or more species of immunogenic complexes. In some examples, each species is a population of complexes comprising a polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0027] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty three or more species of immunogenic complexes. In some examples, each species is a population of complexes comprising a

polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0028] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising thirty four or more species of immunogenic complexes. In some examples, each species is a population of complexes comprising a polysaccharide antigen of a distinct *Streptococcus pneumoniae* serotype (*e.g.*, ones described herein).

[0029] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; a second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin binding moiety of the first fusion protein; a third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin binding moiety of the first fusion protein; a fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin binding moiety of the first fusion protein; a fifth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin binding moiety of the first fusion protein; a sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin binding moiety of the first fusion protein; a seventh species of immunogenic

second fusion protein; a twenty-ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirtieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin binding moiety of the second fusion protein; and a thirty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0030] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof; a second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin binding moiety of the first fusion protein; a third species of immunogenic complex comprising a biotinylated polysaccharide antigen of

biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-sixth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-seventh species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-eighth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin binding moiety of the second fusion protein; a twenty-ninth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirtieth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-second species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin binding moiety of the second fusion protein; a thirty-third species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin binding moiety of the second fusion protein; and a thirty-fourth species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0031] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising: a first species of immunogenic complex comprising a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with a biotin-binding moiety of a first fusion protein, wherein each first fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising

biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin binding moiety of the second fusion protein.

[0032] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein each species of immunogenic complexes contributes a stoichiometrically equal ratio, by weight, of the polysaccharide antigen.

[0033] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein at least one species of immunogenic complex contributes a stoichiometrically different ratio, by weight, of the polysaccharide antigen.

[0034] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a plurality of immunogenic complexes comprising two or more species of immunogenic complexes, wherein different species of immunogenic complexes contribute a stoichiometrically different ratio, by weight, of the polysaccharide antigen.

[0035] In some examples, an immunogenic composition (*e.g.*, vaccine) comprises a fusion protein comprising a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0036] Another example of the present disclosure provides an immunogenic complex comprising a fusion protein comprising a biotin-binding moiety of rhizavidin and one or more *Streptococcus pneumoniae* polypeptide antigens. In some examples, a biotin-binding moiety is a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a

polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0037] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* non-covalently associated with a fusion protein described herein. In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

[0038] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0039] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0040] In some examples, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises a pneumolysin (Ply) polypeptide or variant or antigenic fragment thereof; an SP0435 polypeptide or variant or antigenic fragment thereof; or a combination thereof. In some examples, an immunogenic complex comprises at least one *Streptococcus pneumoniae* polypeptide antigen, wherein the at

least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising a pneumolysin (Ply) polypeptide, or varaiant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0435 polypeptide or variant or antigenic fragment thereof. In some examples, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and (b) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof.

[0041] In some examples, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises an SP1500 polypeptide or variant of antigenic fragment thereof; an SP0785 polypeptide or variant or antigenic fragment thereof; or a combination thereof. In some examples, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an SP1500 polypeptide or variant or antigenic fragment thereof; and (b) a second polypeptide antigen comprising an SP0785 polypeptide or variant or antigenic fragment thereof. In some examples, an immunogenic complex comprises at least one polypeptide antigen, wherein the at least one polypeptide antigen comprises: (a) a first polypeptide antigen comprising an amino acid sequence 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%, or at least 99.5% identical to SEQ ID NO: 12 or variant or an antigenic fragment thereof; and (b) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or variant or an antigenic fragment thereof.

[0042] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0043] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0044] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0045] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0046] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0047] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0048] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first

polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0049] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0050] In some examples, an immunogenic complex comprises a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently associated with a fusion protein, wherein the fusion protein comprises (a) a biotin-binding moiety; (b) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or a variant or an antigenic fragment thereof; and (c) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or a variant or an antigenic fragment thereof.

[0051] In some examples, an immunogenic complex comprises a biotin-binding moiety, wherein the biotin-binding moiety is a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

[0052] In some examples, an immunogenic complex comprises a fusion protein (*e.g.*, ones described herein) and a polysaccharide antigen (*e.g.*, ones described herein), wherein the ratio of fusion protein to polysaccharide antigen in the complex is about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 6:1, about 7:1, about 8:1, about 9:1, or about 10:1 by weight. In some examples, an immunogenic composition (*e.g.*, vaccine) comprises one or more immunogenic complexes disclosed herein.

[0053] In some examples, pharmaceutical compositions comprising immunogenic composition (*e.g.*, vaccine) compositions are disclosed herein. In one aspect, the present disclosure refers to a pharmaceutical composition comprising the vaccine disclosed herein, and a pharmaceutically acceptable carrier. In some examples, a pharmaceutical composition comprises an immunogenic complex disclosed herein, and a pharmaceutically acceptable carrier. In some examples, a pharmaceutical composition disclosed herein comprises one or more adjuvants. In some examples, the one or more adjuvants is or comprises a co-stimulation factor.

[0054] In some examples, the one or more adjuvants are selected from the group consisting of aluminum phosphate, aluminum hydroxide, and phosphated aluminum hydroxide. In some examples, wherein the one or more adjuvants is or comprises aluminum phosphate. In some examples, a pharmaceutical composition disclosed herein is formulated for injection.

[0055] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein induces an immune response. In some examples, the immune response comprises an innate immune response. In some examples, the immune response comprises an antibody or B cell response. In some examples, the immune response comprises a T cell response. In some examples, the immune response comprises a CD4+ T

cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response. In some examples, the immune response comprises (i) an antibody or B cell response, and (ii) a T cell response. In some examples, the immune response comprises (i) an antibody or B cell response, (ii) a T cell response, and (iii) an innate immune response. In some examples, the immune response is to at least one polysaccharide antigen or at least one polypeptide antigen of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a T cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response and a T cell response, to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a CD4+ T cell response, including a T_H1, T_H2, or T_H17 response, a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response, to at least one polypeptide of a fusion protein.

[0056] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae.* In some examples, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include

polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such an opsonic/bactericidal response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, an opsonic/bactericidal response can include production of antibodies that opsonize one or more serotypes of *Streptococcus pneumoniae*, which leads to killing of such serotype(s) by one or more immune pathways. In some examples, such an opsonic/bactericidal response can be determined by an opsonophagocytic assay (OPA) or a concentrated opsonophagocytic assay (COPA) known in the art and/or as described herein.

[0057] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject. In some examples, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of transmission may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0058] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits replication, or reduces bacterial load, of one or

more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of replication, or reduction of bacterial load, may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0059] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of Invasive Pneumonoccal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of IPD or reduction in the rate of occurrence of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5,

6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of Invasive Pneumonoccal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0060] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some

examples, such inhibition, or reduction in the rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0061] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In

some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0062] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one

or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0063] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least

one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0064] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces the rate of occurrence of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such

reduction in the severity of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0065] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0066] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization of mucosal surface(s) by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical

composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0067] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein reduces or inhibits colonization of the nasopharynx by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0068] In some examples, upon administration to a subject, a pharmaceutical composition disclosed herein inhibits or reduces asymptomatic infection by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition or reduction of asymptomatic infection may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described

herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition or reduction of asymptomatic infection may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition or reduction of asymptomatic infection may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein a pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0069] Some aspects of the present disclosure provide a method of making an immunogenic composition (*e.g.*, vaccine), comprising non-covalently complexing a biotinylated polysaccharide antigen with a fusion protein comprising an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59, wherein the fusion protein comprises: (a) a biotin-binding moiety; (b) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3)or antigenic fragment thereof; and (c) an SP0435 polypeptide or antigenic fragment thereof. In some examples, the method further comprises non-covalently complexing a plurality of second biotinylated polysaccharide antigens with a plurality of a second fusion protein comprising at least one polypeptide antigen selected from: an SP1500 polypeptide or antigenic fragment thereof; an SP0785 polypeptide or antigenic fragment thereof; or a combination thereof. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31,

32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A,

12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0070] In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected

from one or more of 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus*

pneumoniae having a serotype selected from each of 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20B, 22F, 23F, and 33F. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 16F, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 16F, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some examples, the plurality of first biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 1, 4, 6A, 6B, 9V, 15B, 18C, 19A, 23F, and 33F. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from one or more of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38. In some examples, the plurality of second biotinylated polysaccharide antigens comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from each of 2, 3, 5, 6C, 7C, 7F, 8, 9N, 10A, 11A, 12F, 14, 15A, 17F, 19F, 20B, 22F, 23A, 23B, 24F, 31, 35B, and 38.

[0071] Some examples provide for a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, the method comprising administering to the subject an immunologically effective amount of an immunogenic composition (*e.g.*, vaccine) disclosed herein. In some examples, a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, comprises administering to a subject an immunologically effective amount of an immunogenic complex disclosed herein. In some examples, a method of immunizing a subject against *Streptococcus pneumoniae* infection and/or colonization, comprises administering to a subject an immunologically effective amount of a pharmaceutical composition disclosed herein.

[0072] In some examples, administration of the immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition induces an immune response. In some examples, the immune response comprises an innate immune response. In some examples, the immune response comprises an antibody or B cell response. In some examples, the immune response comprises a T cell response. In some examples, the immune response comprises a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response. In some examples, the immune response comprises (i) an antibody or B cell response and (ii) a T cell response. In some examples, the immune response comprises (i) an antibody or B cell response, (ii) a T cell response, and (iii) an innate immune response. In some examples, the immune response is to at least one polysaccharide antigen or at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a T cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) a CD4+ T cell response (*e.g.*, T_H1, T_H2, or T_H17 response), a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a T

cell response, to at least one polypeptide of a fusion protein. In some examples, the immune response comprises (i) an antibody or B cell response to at least one polysaccharide antigen, and (ii) an antibody or B cell response, and a CD4+ T cell response, including T_H1, T_H2, or T_H17 response, or a CD8+ T cell response, a CD4+ and CD8+ T cell response, or a CD4-/CD8- T cell response, to at least one polypeptide of a fusion protein.

[0073] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) induces an opsonic/bactericidal response against one or more serotypes of *Streptococcus pneumoniae.* In some examples, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such an opsonic/bactericidal response may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such an opsonic/bactericidal response may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones as described and/or utilized herein) inhibits transmission of one or more serotypes of *Streptococcus pneumoniae* from the subject to another subject. In some examples, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described

herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of transmission may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition of transmission may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0074] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of IPD may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (e.g.,

1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition (e.g., ones described and/or utilized herein) reduces the severity of Invasive Pneumococcal Disease (IPD) associated with or induced by one or more serotypes of Streptococcus pneumoniae. In some examples, such reduction in the severity of IPD may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of Streptococcus pneumoniae, wherein an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of IPD may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of Streptococcus pneumoniae, wherein an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of IPD may be directed against two or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of Streptococcus pneumoniae, wherein an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0075] In some examples, administration of an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition (e.g., ones described and/or utilized herein) inhibits or reduces the rate of occurrence of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of Streptococcus pneumoniae. In some examples, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of Streptococcus pneumoniae, wherein an immunogenic composition (e.g., vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s). In some examples, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in rate of occurrence, of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of bacteremia, sepsis, and/or meningitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of bacteremia, sepsis, and/or meningitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0076] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of organ damage associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s). In some examples, such reduction in the severity of organ damage may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of organ damage may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0077] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such

serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of pneumonia associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of pneumonia may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of pneumonia may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0078] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or

reduction in the rate of occurrence, of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of otitis media associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of otitis media may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of otitis media may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include

polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0079] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) inhibits or reduces the rate of occurrence of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such inhibition, or reduction in the rate of occurrence, of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces the severity of sinusitis associated with or induced by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction in the severity of sinusitis may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of sinusitis may be directed

against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction in the severity of sinusitis may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0080] In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an

immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization of mucosal surface(s) by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of mucosal surface(s) may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, administration of an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition (*e.g.*, ones described and/or utilized herein) reduces or inhibits colonization of nasopharynx by one or more serotypes of *Streptococcus pneumoniae*. In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic

complex, or pharmaceutical composition described herein does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s). In some examples, such reduction or inhibition of colonization of nasopharynx may be directed against two or more (*e.g.*, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) serotypes of *Streptococcus pneumoniae*, wherein an immunogenic composition (*e.g.*, vaccine), immunogenic complex, or pharmaceutical composition described herein (i) includes polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s); and (ii) does not include polysaccharide(s) present in at least one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more) of such serotype(s).

[0081] In some examples of the methods disclosed herein, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48. In some examples, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38. In some examples, the *Streptococcus pneumoniae* has a serotype selected from one or more of 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

[0082] In some examples, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with one dose of an immunogenic composition (*e.g.*, vaccine). In some examples, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with two doses of an immunogenic composition (*e.g.*, vaccine). In some examples, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with three doses of an immunogenic composition (*e.g.*, vaccine). In some examples, the subject is immunized against *Streptococcus pneumoniae* infection and/or colonization with four doses of an immunogenic composition (*e.g.*, vaccine).

[0082a] In one aspect, the present disclosure refers to a fusion protein comprising an

amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59, wherein the fusion protein comprises: (i) a biotin-binding moiety; (ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus peneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof; and (iii) an SP0435 polypeptide or antigenic fragment thereof.

Claims

We claim:

1. A vaccine comprising an immunogenic complex that comprises:

(a) a biotinylated polysaccharide; and

(b) a fusion protein comprising an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59, wherein the fusion protein comprises:

(i) a biotin-binding moiety;

(ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof; and

(iii) an SP0435 polypeptide or antigenic fragment thereof;

wherein the biotinylated polysaccharide antigen is non-covalently associated with the biotin-binding moiety of the fusion protein to form the immunogenic complex.

2. The vaccine of claim 1, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae.*

3. The vaccine of claim 1, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48.

4. The vaccine of claim 1, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 1, 2, 3, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15A, 15B, 16F, 17F, 18C, 19A, 19F, 20B, 22F, 23A, 23B, 23F, 24F, 31, 33F, 35B, and 38.

5. The vaccine of any one of claims 1-3, wherein the biotinylated polysaccharide antigen comprises a polysaccharide of *Streptococcus pneumoniae* having a serotype selected from 6C, 7C, 15A, 16F, 23A, 23B, 24F, 31, 35B, and 38.

6. The vaccine of claim 1, comprising:

a plurality of different species of immunogenic complexes comprising:

(a) a plurality of first biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and

(b) a plurality of first fusion proteins, wherein each first fusion protein comprises an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59, and wherein each first fusion protein comprises:

(i) a biotin-binding moiety;

(ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof, wherein the non-hemolytic pneumolysin (Ply) polypeptide comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 6 or an antigenic fragment thereof; and

(iii) an SP0435 polypeptide or antigenic fragment thereof, wherein the SP0435 polypeptide or antigenic fragment thereof comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 8 or an antigenic fragment thereof;

wherein each of the plurality of first biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of first fusion proteins to form one of the immunogenic complexes.

7. The vaccine of claim 6, wherein the plurality of different species of immunogenic complexes further comprises:

(a) a plurality of second biotinylated polysaccharide antigens, wherein the plurality comprises polysaccharide antigens of one or more of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 6C, 6D, 6E, 6F, 6G, 6H, 7A, 7B, 7C, 7F, 8, 9A, 9L, 9N, 9V, 10A, 10B, 10C, 10F, 11A, 11B, 11C, 11D, 11E, 11F, 12A, 12B, 12F, 13, 14, 15A, 15B, 15C, 15F, 16A, 16F, 17A, 17F, 18A, 18B, 18C, 18F, 19A, 19B, 19C, 19F, 20A, 20B, 21, 22A, 22F, 23A, 23B, 23F, 24A, 24B, 24F, 25A, 25F, 27, 28A, 28F, 29, 31, 32A, 32F, 33A, 33B, 33C, 33D, 33E, 33F, 34, 35A, 35B, 35C, 35F, 36, 37, 38, 39, 40, 41A, 41F, 42, 43, 44, 45, 46, 47A, 47F, and 48; and

(b) a plurality of second fusion proteins, wherein each second fusion protein comprises:

(i) a biotin-binding moiety;

(ii) a first polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 12 or an antigenic fragment thereof; and

(iii) a second polypeptide antigen comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 10 or an antigenic fragment thereof;

wherein each of the plurality of second biotinylated polysaccharide antigens is non-covalently associated with the biotin-binding moiety of one or more of the plurality of second fusion proteins to form an immunogenic complex.

8. The vaccine of claim 7, wherein each of the plurality of second fusion proteins, is or comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 26 or SEQ ID NO: 61.

9. The vaccine of claim 7, wherein the plurality of different species of immunogenic complexes comprises:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins.

10. The vaccine of claim 7, wherein the plurality of different species of immunogenic complexes comprises:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins.

11. The vaccine of claim 7, wherein the plurality of different species of immunogenic complexes comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion protein;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 3 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins.

12. The vaccine of claim 7, wherein the plurality of different species of immunogenic complexes comprise:

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 1 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 4 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9V non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 18C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 33F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of first fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 2 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 5 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 6C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7C non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 7F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 8 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 9N non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 10A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 11A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 12F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 14 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 15A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 16F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 17F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 19F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 20B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 22F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23A non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 23B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 24F non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 31 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins;

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 35B non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins; and

a biotinylated polysaccharide antigen of *Streptococcus pneumoniae* serotype 38 non-covalently complexed with the biotin-binding moiety of one or more of the plurality of second fusion proteins.

13. The vaccine of claim 1, wherein the biotin-binding moiety is (i) a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 1 or a biotin-binding fragment thereof; or (ii) a polypeptide comprising an amino acid sequence 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%, or 100% identical to SEQ ID NO: 2 or SEQ ID NO: 57, or a biotin-binding fragment thereof.

14. A pharmaceutical composition comprising the vaccine of claim 1, and a pharmaceutically acceptable carrier.

15. A method of making a vaccine, comprising non-covalently complexing a biotinylated polysaccharide antigen with a fusion protein comprising an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59,

wherein the fusion protein comprises:

(a) a biotin-binding moiety;

(b) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof; and

(c) an SP0435 polypeptide or antigenic fragment thereof.

16. A fusion protein comprising an amino acid sequence at least 90% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59,

wherein the fusion protein comprises:

(i) a biotin-binding moiety;

(ii) a non-hemolytic pneumolysin (Ply) polypeptide comprising mutations at amino acid residues corresponding to amino acid residues 294, 385, 428, and 433 of wild-type *Streptococcus pneumoniae* pneumolysin (SEQ ID NO: 3) or antigenic fragment thereof; and

(iii) an SP0435 polypeptide or antigenic fragment thereof.

17. The vaccine of claim 1, wherein the fusion protein comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59.

18. The vaccine of claim 6, wherein each first fusion protein comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59.

19. The method of claim 15, wherein the fusion protein comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59.

20. The fusion protein of claim 16, wherein the fusion protein comprises an amino acid sequence 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%, or 100% identical to SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 58, or SEQ ID NO: 59.