pathogenesis of staphylococcal pneumonia. Due to the fact that Protein A captures immunoglobulin and displays toxic attributes, the possibility that this surface molecule may function as a vaccine in humans has not been rigorously pursued. Here the inventors demonstrate that Protein A variants no longer able to bind to immunoglobulins, which are thereby removed of their toxigenic potential, *i.e.*, are non-toxigenic, stimulate humoral immune responses that protect against staphylococcal disease.

[0015] In certain ~~embodiments~~examples the SpA variant is a full length SpA variant comprising a variant A, B, C, D, and E domain. In certain ~~aspects~~examples, the SpA variant comprises or consists of the amino acid sequence that is 80, 90, 95, 98, 99, or 100% identical to the amino acid sequence of SEQ ID NO:34 In other ~~embodiments~~examples the SpA variant comprises a segment of SpA. The SpA segment can comprise at least or at most 1, 2, 3, 4, 5 or more IgG binding domains. The IgG domains can be at least or at most 1, 2, 3, 4, 5 or more variant A, B, C, D, or E domains. In certain ~~aspects~~examples the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant A domains. In a further ~~aspect~~example the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant B domains. In still a further ~~aspect~~example the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant C domains. In yet a further ~~aspect~~example the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant D domains. In certain ~~aspects~~examples the SpA variant comprises at least or at most 1, 2, 3, 4, 5, or more variant E domains. In a further ~~aspect~~example the SpA variant comprises a combination of A, B, C, D, and E domains in various combinations and permutations. The combinations can include all or part of a SpA signal peptide segment, a SpA region X segment, and/or a SpA sorting signal segment. In other ~~aspects~~examples the SpA variant does not include a SpA signal peptide segment, a SpA region X segment, and/or a SpA sorting signal segment. In certain ~~aspects~~examples a variant A domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:4. In another ~~aspect~~example a variant B domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:6. In still anther ~~aspect~~example a variant C domain comprises a substitution at position(s) 7, 8, 34, and/or 35 of SEQ ID NO:5. In certain ~~aspects~~examples a variant D domain comprises a substitution at position(s) 9, 10, 37, and/or 38 of SEQ ID NO:2. In a further ~~aspect~~example a variant E domain comprises a substitution at position(s) 6, 7, 33, and/or 34 of SEQ ID NO:3.

[0015a] In one aspect, there is provided an isolated polypeptide comprising a Protein A (SpA) variant domain D, wherein the variant domain has at least one non-conservative amino acid substitution at positions corresponding to amino acids 9 and 10 of SEQ ID NO: 2, at least one non-conservative amino acid substitution at positions corresponding to amino acids 36 and 37 of SEQ ID NO:2, and an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:2.

[0016] In certain ~~aspects~~examples the SpA variant includes a substitution of (a) one or more amino acid substitution in an IgG Fc binding sub-domain of SpA domain A, B, C, D, and/or E that disrupts or decreases binding to IgG Fc, and (b) one or more amino acid substitution in a V_H3 binding sub-domain of SpA domain A, B, C, D, and/or E that disrupts or decreases binding to V_H3. In still further ~~aspects~~examples the amino acid sequence of a SpA variant comprises an amino acid sequence that is at least 50%, 60%, 70%, 80%, 90%, 95%, or 100% identical, including all values and ranges there between, to the amino acid sequence of SEQ ID NOs:2-6.

[0017] In a further ~~aspect~~example the SpA variant includes (a) one or more amino acid substitution in an IgG Fc binding sub-domain of SpA domain D, or at a corresponding amino acid position in other IgG domains, that disrupts or decreases binding to IgG Fc, and (b) one or more amino acid substitution in a V_H3 binding sub-domain of SpA domain D, or at a corresponding amino acid position in other IgG domains, that disrupts or decreases binding to V_H3. In certain ~~aspects~~examples amino acid residue F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 (SEQ ID NO:2, QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVLGEAKKLNES) of the IgG Fc binding sub-domain of domain D are modified or substituted. In certain ~~aspects~~examples amino acid residue Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 (SEQ ID NO:2) of the V_H3 binding sub-domain of domain D are modified or substituted such that binding to Fc or V_H3 is attenuated. In further ~~aspects~~examples corresponding modifications or substitutions can be engineered in corresponding positions of the domain A, B, C, and/or E. Corresponding positions are defined by alignment of the domain D amino acid sequence with one or more of the amino acid sequences from other IgG binding domains of SpA, for example see FIG. 1. In certain ~~aspects~~examples the amino acid substitution can be any of the other 20 amino acids. In a further ~~aspect~~example conservative amino acid substitutions can be specifically excluded from possible amino acid substitutions. In other ~~aspects~~examples only non-conservative substitutions are included. In any event, any substitution or combination of substitutions that reduces the binding of the domain such that SpA toxicity is significantly reduced is contemplated. The significance of the reduction in binding refers to a variant that produces minimal to no toxicity when introduced into a subject and can be assessed using in vitro methods described herein.

[0018] In certain ~~embodiments~~examples, a variant SpA comprises at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more variant SpA domain D peptides. In certain ~~aspects~~examples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 or more amino acid residues of the variant SpA are substituted or modified - including but not limited to amino acids F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 (SEQ ID NO:2) of the IgG Fc binding sub-domain of domain D and amino acid residue Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 (SEQ ID NO:2) of the V_H3 binding sub-domain of domain D. In one ~~aspect~~example of the ~~invention~~disclosure glutamine residues at position 9 and/or 10 of SEQ ID NO:2 (or corresponding positions in other domains) are mutated. In another ~~aspect~~example, aspartic acid residues 36 and/or 37 of SEQ ID NO:2 (or corresponding positions in other domains) are mutated. In a further ~~aspect~~example, glutamine 9 and 10, and aspartic acid residues 36 and 37 are mutated. Purified non-toxigenic SpA or SpA-D mutants/variants described herein are no longer able to significantly bind (i.e., demonstrate attenuated or disrupted binding affinity) Fcγ or $F(ab)_2$ V_H3 and also do not stimulate B cell apoptosis. These non-toxigenic Protein A variants can be used as subunit vaccines and raise humoral immune responses and confer protective immunity against *S. aureus* challenge. Compared to wild-type full-length Protein A or the wild-type SpA-domain D, immunization with SpA-D variants resulted in an increase in Protein A specific antibody. Using a mouse model of staphylococcal challenge and abscess formation, it was observed that immunization with the non-toxigenic Protein A variants generated significant protection from staphylococcal infection and abscess formation. As virtually all *S. aureus* strains express Protein A, immunization of humans with the non-toxigenic Protein A variants can neutralize this virulence factor and thereby establish protective immunity. In certain ~~aspects~~examples the protective immunity protects or ameliorates infection by drug resistant strains of Staphylococcus, such as USA300 and other MRSA strains.

[0019] ~~Embodiments~~Examples include the use of Protein A variants in methods and compositions for the treatment of bacterial and/or staphylococcal infection. This application also provides an immunogenic composition comprising a Protein A variant or immunogenic fragment thereof. In certain ~~aspects~~examples, the immunogenic fragment is a Protein A domain D segment. In one aspect, there is provided an immunogenic composition comprising an isolated peptide comprising a Protein A

(SpA) variant domain D having non-conservative amino acid substitutions at positions corresponding to amino acid 9, 10, 36, and 37 of SEQ ID NO:2. Furthermore, the present ~~invention~~disclosure provides methods and compositions that can be used to treat (*e.g.*, limiting staphylococcal abscess formation and/or persistence in a subject) or prevent bacterial

infection. In some cases, methods for stimulating an immune response involve administering to the subject an effective amount of a composition including or encoding all or part of a Protein A variant polypeptide or antigen, and in certain ~~aspects~~examples other bacterial proteins. Other bacterial proteins include, but are not limited to (i) a secreted virulence factor, and/or a cell surface protein or peptide, or (ii) a recombinant nucleic acid molecule encoding a secreted virulence factor, and/or a cell surface protein or peptide.

[0020] In other ~~aspects~~examples, the subject can be administered all or part of a Protein A variant, such as a variant Protein A domain D segment. The polypeptide of the ~~invention~~disclosure can be formulated in a pharmaceutically acceptable composition. The composition can further comprise one or more of at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, or 19 additional staphylococcal antigen or immunogenic fragment thereof (*e.g.*, Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla (*e.g.*, H35 mutants), IsdC, SasF, vWbp, or vWh). Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa (GenBank CAC80837), Aap (GenBank accession AJ249487), Ant (GenBank accession NP_372518), autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (see PCT publications WO2007/113222, WO2007/113223, WO2006/032472, WO2006/032475, WO2006/032500, each of which is incorporated herein by reference in their entirety). The staphylococcal antigen or immunogenic fragment can be administered concurrently with the Protein A variant. The staphylococcal antigen or immunogenic fragment and the Protein A variant can be administered in the same composition. The Protein A variant can also be a recombinant nucleic acid

molecule encoding a Protein A variant. A recombinant nucleic acid molecule can encode the Protein A variant and at least one staphylococcal antigen or immunogenic fragment thereof. As used herein, the term "modulate" or "modulation" encompasses the meanings of the words "enhance," or "inhibit." "Modulation" of activity may be either an increase or a decrease in activity. As used herein, the term "modulator" refers to compounds that effect the function of a moiety, including up-regulation, induction, stimulation, potentiation, inhibition, down-regulation, or suppression of a protein, nucleic acid, gene, organism or the like.

[0021] In certain ~~embodiments~~examples the methods and compositions use or include or encode all or part of the Protein A variant or antigen. In other ~~aspects~~examples, the Protein A variant may be used in combination with secreted factors or surface antigens including, but not limited to one or more of an isolated Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh polypeptide or immunogenic segment thereof. Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In certain ~~embodiments~~examples, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP,

Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. can be specifically excluded from a formulation of the ~~invention~~disclosure.

[0022] In still further ~~aspects~~examples, the isolated Protein A variant is multimerized, *e.g.*, dimerized or a linear fusion of two or more polypeptides or peptide segments. In certain ~~aspects~~examples of the ~~invention~~disclosure, a composition comprises multimers or concatamers of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more isolated cell surface proteins or segments thereof. Concatamers are linear polypeptides having one or more repeating peptide units. SpA polypeptides or fragments can be consecutive or separated by a spacer or other peptide sequences, *e.g.*, one or more additional bacterial peptide. In a further ~~aspect~~example, the other polypeptides or peptides contained in the multimer or concatamer can include, but are not limited to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 of Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh or immunogenic fragments thereof. Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

[0023] The term "Protein A variant" or "SpA variant" refers to polypeptides that include a SpA IgG domain having two or more amino acid substitutions that disrupt

binding to Fc and V_H3. In certain ~~aspect~~example, a SpA variant includes a variant domain D peptide, as well as variants of SpA polypeptides and segments thereof that are non-toxigenic and stimulate an immune response against staphylococcus bacteria Protein A and/or bacteria expressing such. In one aspect, there is provided a peptide composition comprising, in a pharmaceutically acceptable composition, a peptide comprising a non-toxigenic Protein A (SpA) variant domain D having two or more mutations that attenuate the binding of the Protein A domain D to IgG, Fcγ, VH3 $F(ab)_2$, von Willebrand factor (vWF), and tumor necrosis factor α receptor 1 (TNFR1), wherein the peptide comprises a variant domain D with at least one non-conservative amino acid substitution at positions corresponding to amino acid 9 and 10 of SEQ ID NO: 2 and at least one non-conservative amino acid substitution at positions corresponding to amino acids 36 and 37 of SEQ ID NO:2, wherein the composition is capable of stimulating an immune response in a subject in need thereof, and optionally wherein the peptide composition further comprises one or more of SpA variant domain E, variant domain A, variant domain B, or variant domain C.

[0024] In one aspect, there is provided the use of a Protein A (SpA) variant comprising at least one variant domain D having non-conservative amino acid substitution at positions corresponding to amino acid 9, 10, 36 and 37 of SEQ ID NO:2 and at least 85% sequence identity to SEQ ID NO: 2 in the manufacture of a composition for eliciting an immune response against a staphylococcus bacterium in a subject, and optionally wherein SpA variant further comprises one or more of SpA variant domain E, variant domain A, variant domain B, or variant domain C. ~~Embodiments~~Examples of the present ~~invention~~disclosure include methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of a Protein A variant or a segment thereof. In certain ~~aspects~~examples, the methods for eliciting an immune response against a staphylococcus bacterium or staphylococci in a subject comprising providing to the subject an effective amount of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted proteins and/or cell surface proteins or segments/fragments thereof. A secreted protein or cell surface protein includes, but is not limited to Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and/or vWh proteins and

immunogenic fragments thereof. Additional staphylococcal antigens that can be used in combination with a Protein A variant include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein.

[0025] In one aspect, there is provided the use of an isolated peptide comprising a Protein A (SpA) variant domain D having non-conservative amino acid substitutions at positions corresponding to amino acid 9, 10, 36 and 37 of SEQ ID NO:2 and at least 85% sequence identity to SEQ ID NO: 2, in the manufacture of a medicament for treating a staphylococcal infection in a subject having or suspected of having a staphylococcal infection or for preventing a staphylococcal infection in a subject at risk of developing a staphylococcal infection, and optionally wherein the polypeptide further comprises one or more of SpA variant domain E, variant domain A, variant domain B, or variant domain C. ~~Embodiments~~Examples of the ~~invention~~disclosure include compositions that include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to Protein A, or a second protein or peptide that is a secreted bacterial protein or a bacterial cell surface protein. In a further ~~embodiment~~example of the ~~invention~~disclosure a composition may include a polypeptide,

peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Protein A domain D polypeptide (SEQ ID NO:2), domain E (SEQ ID NO:3), domain A (SEQ ID NO:4), domain C (SEQ ID NO:5), domain B (SEQ ID NO:6), or a nucleic acid sequence encoding a Protein A domain D, domain E, domain A, domain C, or domain B polypeptide. In certain ~~aspects~~examples a Protein A polypeptide segment will have an amino acid sequence of SEQ ID NO:8. Similarity or identity, with identity being preferred, is known in the art and a number of different programs can be used to identify whether a protein (or nucleic acid) has sequence identity or similarity to a known sequence. Sequence identity and/or similarity is determined using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith & Waterman (1981), by the sequence identity alignment algorithm of Needleman & Wunsch (1970), by the search for similarity method of Pearson & Lipman (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux *et al.* (1984), preferably using the default settings, or by inspection. Preferably, percent identity is calculated by using alignment tools known to and readily ascertainable to those of skill in the art. Percent identity is essentially the number of identical amino acids divided by the total number of amino acids compared times one hundred.

[0026] Still further ~~embodiments~~examples include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition including (i) a SpA variant, *e.g.*, a variant SpA domain D polypeptide or peptide thereof; or, (ii) a nucleic acid molecule encoding such a SpA variant polypeptide or peptide thereof, or (iii) administering a SpA variant domain D polypeptide with any combination or permutation of bacterial proteins described herein. In a preferred ~~embodiment~~example the composition is not a staphylococcus bacterium. In certain ~~aspects~~examples the subject is a human or a cow. In a further ~~aspect~~example the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci may be *Staphylococcus aureus*.

[0027] In one aspect, there is provided a vaccine comprising the composition of the present invention or the polypeptide of the present invention and a pharmaceutically acceptable excipient. In another aspect, there is provided a method of making a vaccine comprising the steps of mixing antigens to make the composition of the present invention and adding a pharmaceutically acceptable excipient. In a further aspect, there is provided a use of the composition of the present invention or the polypeptide of the present invention in the manufacture of a vaccine for treatment or prevention of staphylococcal infection. Yet still further ~~embodiments~~examples include vaccines comprising a pharmaceutically acceptable composition having an isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacterium. The vaccine may comprise an isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described. In certain ~~aspects~~examples of the ~~invention~~disclosure the isolated SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described are multimerized, *e.g.*, dimerized or concatamerized. In a further ~~aspect~~example, the vaccine composition is contaminated by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.05% (or any range derivable therein) of other Staphylococcal proteins. A composition may further comprise an isolated non-SpA polypeptide. Typically the vaccine comprises an adjuvant. In certain ~~aspects~~examples a protein or peptide of the ~~invention~~disclosure is linked (covalently or non-covalently) to the adjuvant, preferably the adjuvant is chemically conjugated to the protein.

[0028] In still yet further ~~embodiments~~examples, a vaccine composition is a pharmaceutically acceptable composition having a recombinant nucleic acid encoding all or part of a SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacteria. The vaccine composition may comprise a recombinant nucleic acid encoding all or part of a SpA variant polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein. In certain ~~embodiments~~examples the recombinant nucleic acid contains a heterologous promoter. Preferably the recombinant nucleic acid is a vector. More preferably the vector is a plasmid or a viral vector. In some ~~aspects~~examples the

vaccine includes a recombinant, non-staphylococcus bacterium containing the nucleic acid. The recombinant non-staphylococci may be Salmonella or another gram-positive bacteria. The vaccine may comprise a pharmaceutically acceptable excipient, more preferably an adjuvant.

[0029] Still further ~~embodiments~~examples include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition of a

SpA variant polypeptide or segment/fragment thereof and further comprising one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh protein or peptide thereof. In a preferred ~~embodiment~~example the composition comprises a non-staphylococcus bacterium. In a further ~~aspect~~example the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci for which a subject is being treated may be *Staphylococcus aureus*. Methods of the ~~invention~~disclosure also include SpA variant compositions that contain 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted virulence factors and/or cell surface proteins, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh in various combinations. In certain ~~aspects~~examples a vaccine formulation includes Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and vWh. In certain ~~aspects~~examples an antigen combination can include (1) a SpA variant and IsdA; (2) SpA variant and ClfB; (3) SpA variant and SdrD; (4) SpA variant and Hla or Hla variant; (5) SpA variant and ClfB, SdrD, and Hla or Hla variant; (6) SpA variant, IsdA, SdrD, and Hla or Hla variant; (7) SpA variant, IsdA, ClfB, and Hla or Hla variant; (8) SpA variant, IsdA, ClfB, and SdrD; (9) SpA variant, IsdA, ClfB, SdrD and Hla or Hla variant; (10) SpA variant, IsdA, ClfB, and SdrD; (11) SpA variant, IsdA, SdrD, and Hla or Hla variant; (12) SpA variant, IsdA, and Hla or Hla variant; (13) SpA variant, IsdA, ClfB, and Hla or Hla variant; (14) SpA variant, ClfB, and SdrD; (15) SpA variant, ClfB, and Hla or Hla variant; or (16) SpA variant, SdrD, and Hla or Hla variant.

[0030] In certain ~~aspects~~examples, a bacterium delivering a composition of the ~~invention~~disclosure will be limited or attenuated with respect to prolonged or persistent growth or abscess formation. In yet a further ~~aspect~~example, SpA variant(s) can be overexpressed in an attenuated bacterium to further enhance or supplement an immune response or vaccine formulation.

[0031] Certain ~~embodiments~~examples are directed to methods for eliciting an immune response against a staphylococcus bacterium in a subject comprising providing to the subject an effective amount of a peptide comprising a coagulase polypeptide or an immunogenic segment thereof having an amino acid sequence that is at least 80, 85, 90, 95, 98, to 100% identical to SEQ ID NO:27 or a segment thereof or at least 80, 85,

90, 95, 98, to 100% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof.

[0032] In certain ~~aspects~~examples, the subject is provided with an effective amount of an coagulase polypeptide by administering to the subject a composition comprising: (i) an isolated coagulase polypeptide or segment thereof having an amino acid sequence that is at least 90% identical to SEQ ID NO:27 or a segment thereof or is at least 90% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof; or (ii) at least one isolated recombinant nucleic acid molecule encoding a coagulase polypeptide or a segment thereof having an amino acid sequence that is at least 90% identical to SEQ ID NO:27 or a segment thereof or is at least 90% identical to amino acids 27-508 of SEQ ID NO:32 or a segment thereof. In a further ~~aspect~~example, the composition comprises an isolated coagulase polypeptide having the amino acid sequence of SEQ ID NO:27 or the amino acid sequence of amino acids 27-508 of SEQ ID NO:32.

[0033] In one aspect, there is provided a pharmaceutical composition comprising the immunoglobulin or composition of immunoglobulins of the present invention and a pharmaceutically acceptable excipient. In another aspect, there is provided a use of the pharmaceutical composition of the present invention in the manufacture of a medicament for the treatment or prevention of staphylococcal infection. In a further aspect, there is provided the use of a polypeptide comprising a Protein A (SpA) variant domain D having at least one non-conservative amino acid substitution at positions corresponding to amino acids 9 and 10 of SEQ ID NO: 2, at least one non-conservative amino acid substitution at positions corresponding to amino acids 36 and 37 of SEQ ID NO:2, and an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:2 in the manufacture of a composition for eliciting an immune response against a staphylococcus bacterium in a subject, and optionally wherein the polypeptide further comprises one or more of SpA variant domain E, variant domain A, variant domain B, or variant domain C. Certain ~~embodiments~~examples are directed to methods for treating a staphylococcal infection in a subject comprising providing to a subject having or suspected of having or at risk of developing a staphylococcal infection an effective amount of an isolated peptide comprising a coagulase polypeptide having an amino acid sequence that is at least 80, 85, 90, 95, 98, to 100% identical to SEQ ID NO:27 or is at least 80, 85, 90, 95, 98, to 100% identical to amino acids 27-508 of SEQ ID NO:32. In a particular

~~aspect~~example, the coagulase polypeptide has an amino acid sequence of SEQ ID NO:27 or has an amino acid identical to amino acids 27-508 of SEQ ID NO:32. In certain ~~aspects~~examples, the subject is diagnosed with a persistent staphylococcal infection. In a further ~~aspect~~example, the coagulase polypeptide elicits production of an antibody that binds Coa or vWbpvWh in the subject.

[0034] ~~Embodiments~~Examples include methods of preventing or treating staphylococcal infection comprising the step of administering an immunogenic composition comprising a Staphylococcal coagulase or an immunogenic segment thereof.

[0035] Certain ~~embodiments~~examples are directed to methods of preparing an immunoglobulin for use in prevention or treatment of staphylococcal infection comprising the steps of immunizing a recipient with a coagulase polypeptide and isolating immunoglobulin from the recipient. In one aspect, there is provided a method of preparing an immunoglobulin or a composition of immunoglobulins for use in prevention or treatment of staphylococcal infection comprising the step of isolating immunoglobulins from plasma or serum that has been obtained from a recipient that has been immunized with the vaccine of the present invention.

[0036] A further ~~embodiment~~example is directed to an immunoglobulin prepared by the method described herein. In one aspect, there is provided an immunoglobulin or a composition of immunoglobulins prepared by the method of present invention, wherein the immunoglobulin or composition of immunoglobulins comprises at least one immunoglobulin specific for polypeptide comprising a Protein A (SpA) variant domain D, wherein the variant domain has at least one non-conservative amino acid substitution at positions corresponding to amino acids 9 and 10 of SEQ ID NO: 2, at least one non-conservative amino acid substitution at positions corresponding to amino acids 36 and 37 of SEQ ID NO:2, and an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:2.

[0037] A further ~~embodiment~~example is directed to methods for treatment or prevention of staphylococcal infection comprising a step of administering to a patient an effective amount of pharmaceutical preparation of immunoglobulin that binds a coagulase.

[0038] Other ~~embodiments~~examples are directed to a use of the pharmaceutical preparation of coagulase immunoglobulins in the manufacture of a medicament for the treatment or prevention of staphylococcal infection.

[0038a] In one aspect, there is provided the use of an isolated peptide comprising a Protein A (SpA) variant domain D having at least one non-conservative amino acid substitution at positions corresponding to amino acid 9 and 10 of SEQ ID NO:2, at least one non-conservative amino acid substitution at positions corresponding to amino acids 36 and 37 of SEQ ID NO:2, and an amino acid sequence that is at least 85% identical to the amino acid sequence of SEQ ID NO:2 in the manufacture of a medicament for treating a staphylococcal infection in a subject having or suspected of having a staphylococcal infection or for preventing a staphylococcal infection in a subject at risk of developing a staphylococcal infection, and optionally wherein the peptide further comprises one or more of SpA variant domain E, variant domain A, variant domain B, or variant domain C.

[0039] Yet still further ~~embodiments~~examples include vaccines comprising a pharmaceutically acceptable composition having an isolated coagulase polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacterium. The vaccine may comprise an isolated coagulase

polypeptide, or any other combination or permutation of protein(s) or peptide(s) described. In certain ~~aspects~~examples of the ~~invention~~disclosure the isolated coagulase polypeptide, or any other combination or permutation of protein(s) or peptide(s) described are multimerized, *e.g.*, dimerized or concatamerized. In a further ~~aspect~~example, the vaccine composition is contaminated by less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.5, 0.25, 0.05% (or any range derivable therein) of other Staphylococcal proteins. A composition may further comprise an isolated non-coagulase polypeptide. Typically the vaccine comprises an adjuvant. In certain ~~aspects~~examples a protein or peptide of the ~~invention~~disclosure is linked (covalently or non-covalently) to the adjuvant, preferably the adjuvant is chemically conjugated to the protein.

[0040] In still yet further ~~embodiments~~examples, a vaccine composition is a pharmaceutically acceptable composition having a recombinant nucleic acid encoding all or part of a coagulase polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein, wherein the composition is capable of stimulating an immune response against a staphylococcus bacterium. The vaccine composition may comprise a recombinant nucleic acid encoding all or part of a coagulase polypeptide, or any other combination or permutation of protein(s) or peptide(s) described herein. In certain ~~embodiments~~examples the recombinant nucleic acid

contains a heterologous promoter. Preferably the recombinant nucleic acid is a vector. More preferably the vector is a plasmid or a viral vector. In some ~~aspects~~examples the vaccine includes a recombinant, non-staphylococcus bacterium containing the nucleic acid. The recombinant non-staphylococci may be Salmonella or another gram-positive bacteria. The vaccine may comprise a pharmaceutically acceptable excipient, more preferably an adjuvant.

[0041] Still further ~~embodiments~~examples include methods for stimulating in a subject a protective or therapeutic immune response against a staphylococcus bacterium comprising administering to the subject an effective amount of a composition of a coagulase polypeptide or segment/fragment thereof and further comprising one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, or vWh protein or peptide thereof. In a preferred ~~embodiment~~example the composition comprises a non-staphylococcus bacterium. In a further ~~aspect~~example the composition is formulated in a pharmaceutically acceptable formulation. The staphylococci for which a subject is being treated may be *Staphylococcus aureus*. Methods of the ~~invention~~disclosure also include coagulase compositions that contain one or more of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or more secreted virulence factors and/or cell surface proteins, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, SpA and variants thereof, vWbp, or vWh in various combinations. In certain ~~aspects~~examples a vaccine formulation includes Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, and vWh. In certain ~~aspects~~examples an antigen combination can include (1) a Coa and/or vWbp, and IsdA; (2) a Coa and/or vWbp, and ClfB; (3) a Coa and/or vWbp, and SdrD; (4) a Coa and/or vWbp, and Hla or Hla variant; (5) a Coa and/or vWbp, and ClfB, SdrD, and Hla or Hla variant; (6) a Coa and/or vWbp, and IsdA, SdrD, and Hla or Hla variant; (7) a Coa and/or vWbp, and IsdA, ClfB, and Hla or Hla variant; (8) a Coa and/or vWbp, and IsdA, ClfB, and SdrD; (9) a Coa and/or vWbp, and IsdA, ClfB, SdrD and Hla or Hla variant; (10) a Coa and/or vWbp, and IsdA, ClfB, and SdrD; (11) a Coa and/or vWbp, and IsdA, SdrD, and Hla or Hla variant; (12) a Coa and/or vWbp, and IsdA, and Hla or Hla variant; (13) a Coa and/or vWbp, and IsdA, ClfB, and Hla or Hla variant; (14) a Coa and/or vWbp, and ClfB, and SdrD; (15) a Coa and/or vWbp, and

or amount of antibodies that specifically recognize a protein or cell surface protein, assays measuring T-cell activation or proliferation, and/or assays that measure modulation in terms of activity or expression of one or more cytokines.

[0063] In still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsxA protein. In certain ~~aspects~~examples the EsxA protein will have all or part of the amino acid sequence of SEQ ID NO:11.

[0064] In still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsxB protein. In certain ~~aspects~~examples the EsxB protein will have all or part of the amino acid sequence of SEQ ID NO:12.

[0065] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an SdrD protein. In certain ~~aspects~~examples the SdrD protein will have all or part of the amino acid sequence of SEQ ID NO:13.

[0066] In further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an SdrE protein. In certain ~~aspects~~examples the SdrE protein will have all or part of the amino acid sequence of SEQ ID NO:14.

[0067] In still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdA protein. In certain ~~aspects~~examples the IsdA protein will have all or part of the amino acid sequence of SEQ ID NO:15.

[0068] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdB protein. In certain ~~aspects~~examples the IsdB protein will have all or part of the amino acid sequence of SEQ ID NO:16.

[0069] ~~Embodiments~~Examples of the ~~invention~~disclosure include compositions that include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a EsaB protein. In certain ~~aspects~~examples the EsaB protein will have all or part of the amino acid sequence of SEQ ID NO:17.

[0070] In a further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a ClfB protein. In certain ~~aspects~~examples the ClfB protein will have all or part of the amino acid sequence of SEQ ID NO:18.

[0071] In still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an IsdC protein. In certain ~~aspects~~examples the IsdC protein will have all or part of the amino acid sequence of SEQ ID NO:19.

[0072] In yet further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a SasF protein. In certain ~~aspects~~examples the SasF protein will have all or part of the amino acid sequence of SEQ ID NO:20.

[0073] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a SdrC protein. In certain ~~aspects~~examples the SdrC protein will have all or part of the amino acid sequence of SEQ ID NO:21.

[0074] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a ClfA protein. In certain ~~aspects~~examples the ClfA protein will have all or part of the amino acid sequence of SEQ ID NO:22.

[0075] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Eap protein. In certain

~~aspects~~examples the Eap protein will have all or part of the amino acid sequence of SEQ ID NO:23.

[0076] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Ebh protein. In certain ~~aspects~~examples the Ebh protein will have all or part of the amino acid sequence of SEQ ID NO:24.

[0077] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an Emp protein. In certain ~~aspects~~examples the Emp protein will have all or part of the amino acid sequence of SEQ ID NO:25.

[0078] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to an EsaC protein. In certain ~~aspects~~examples the EsaC protein will have all or part of the amino acid sequence of SEQ ID NO:26. Sequence of EsaC polypeptides can be found in the protein databases and include, but are not limited to accession numbers ZP_02760162 (GI:168727885), NP_645081.1 (GI:21281993), and NP_370813.1 (GI:15923279), each of which is incorporated herein by reference as of the priority date of this application.

[0079] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Coa protein. In certain ~~aspects~~examples the Coa protein will have all or part of the amino acid sequence of SEQ ID NO:27.

[0080] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a Hla protein. In certain ~~aspects~~examples the Hla protein will have all or part of the amino acid sequence of SEQ ID NO:28.

[0081] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a vWa protein. In certain ~~aspects~~examples the vWa protein will have all or part of the amino acid sequence of SEQ ID NO:29.

[0082] In yet still further ~~embodiments~~examples of the ~~invention~~disclosure a composition may include a polypeptide, peptide, or protein that is or is at least 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical or similar to a vWbp protein. In certain ~~aspects~~examples the vWbp protein will have all or part of the amino acid sequence of SEQ ID NO:32.

[0083] In certain ~~aspects~~examples, a polypeptide or segment/fragment can have a sequence that is at least 85%, at least 90%, at least 95%, at least 98%, or at least 99% or more identical to the amino acid sequence of the reference polypeptide. The term "similarity" refers to a polypeptide that has a sequence that has a certain percentage of amino acids that are either identical with the reference polypeptide or constitute conservative substitutions with the reference polypeptides.

[0084] The polypeptides described herein may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or more variant amino acids within at least, or at most 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400,

500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NO:2-30, or SEQ ID NO:32-34.

[0085] A polypeptide segment as described herein may include 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids, or any range derivable therein, of SEQ ID NO:2-30, or SEQ ID NO:33-34.

[0086] The compositions may be formulated in a pharmaceutically acceptable composition. In certain ~~aspects~~examples of the ~~invention~~disclosure the staphylococcus bacterium is an *S. aureus* bacterium.

[0087] In further ~~aspects~~examples, a composition may be administered more than one time to the subject, and may be administered 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 or more times. The administration of the compositions include, but is not limited to oral, parenteral, subcutaneous, intramuscular, intravenous, or various combinations thereof, including inhalation or aspiration.

[0088] In still further ~~embodiments~~examples, a composition comprises a recombinant nucleic acid molecule encoding a polypeptide described herein or segments/fragments thereof. Typically a recombinant nucleic acid molecule encoding a polypeptide described herein contains a heterologous promoter. In certain ~~aspects~~examples, a recombinant nucleic acid molecule of the ~~invention~~disclosure is a vector, in still other ~~aspects~~examples the vector is a

plasmid. In certain ~~embodiments~~examples the vector is a viral vector. In certain ~~aspects~~examples a composition includes a recombinant, non-staphylococcus bacterium containing or expressing a polypeptide described herein. In particular ~~aspects~~examples the recombinant non-staphylococcus bacteria is *Salmonella* or another gram-positive bacteria. A composition is typically administered to mammals, such as human subjects, but administration to other animals that are capable of eliciting an immune response is contemplated. In further ~~aspects~~examples the staphylococcus bacterium containing or expressing the polypeptide is *Staphylococcus aureus*. In further ~~embodiments~~examples the immune response is a protective immune response.

[0089] In further ~~embodiments~~examples a composition comprises a recombinant nucleic acid molecule encoding all or part of one or more of a Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, SpA, vWbp, or vWh protein or peptide or variant thereof. Additional staphylococcal antigens that can be used in combination with the polypeptides described herein include, but are not limited to 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein. In particular ~~aspects~~examples, a bacteria is a recombinant non-staphylococcus bacteria, such as a *Salmonella* or other gram-positive bacteria.

[0090] Compositions of the ~~invention~~disclosure are typically administered to human subjects, but administration to other animals that are capable of eliciting an immune response to a staphylococcus bacterium is contemplated, particularly cattle, horses, goats, sheep and other domestic animals, *i.e.*, mammals.

[0091] In certain ~~aspects~~examples the staphylococcus bacterium is a *Staphylococcus aureus*. In further ~~embodiments~~examples the immune response is a protective immune response. In still further ~~aspects~~examples, the methods and compositions of the ~~invention~~disclosure can be used to prevent, ameliorate, reduce, or treat infection of tissues or glands, *e.g.*, mammary glands, particularly mastitis and other infections. Other methods include, but are not limited to prophylactically reducing bacterial burden in a subject not exhibiting signs of infection, particularly those subjects suspected of or at risk of being colonized by a target bacteria, *e.g.*, patients that are or will be at risk or susceptible to infection during a hospital stay, treatment, and/or recovery.

[0092] Any ~~embodiment~~example discussed with respect to one ~~aspect~~example of the ~~invention~~disclosure applies to other ~~aspects~~examples of the ~~invention~~disclosure as well. In particular, any ~~embodiment~~example discussed in the context of a SpA variant polypeptide or peptide or nucleic acid may be implemented with respect to other antigens, such as Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein (or nucleic acids), and vice versa. It is also understood that any one or more of Eap, Ebh, Emp, EsaC, EsxA, EsxB, SdrC, SdrD, SdrE, IsdA, IsdB, ClfA, ClfB, Coa, Hla, IsdC, SasF, vWbp, vWh, 52kDa vitronectin binding protein (WO 01/60852), Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Cna, collagen binding protein (US6288214), EFB (FIB), Elastin binding protein (EbpS), EPB, FbpA, fibrinogen binding protein (US6008341), Fibronectin binding protein (US5840846), FnbA, FnbB, GehD (US 2002/0169288), HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg2+ transporter, MHC II analogue (US5648240), MRPII, Npase, RNA III activating

protein (RAP), SasA, SasB, SasC, SasD, SasK,SBI, SdrF(WO 00/12689), SdrG / Fig (WO 00/12689), SdrH (WO 00/12689), SEA exotoxins (WO 00/02523), SEB exotoxins (WO 00/02523), SitC and Ni ABC transporter, SitC/MntC/saliva binding protein (US5,801,234), SsaA, SSP-1, SSP-2, and/or Vitronectin binding protein can be specifically excluded from a claimed composition.

[0093] ~~Embodiments~~Examples of the ~~invention~~disclosure include compositions that contain or do not contain a bacterium. A composition may or may not include an attenuated or viable or intact staphylococcal bacterium. In certain ~~aspects~~examples, the composition comprises a bacterium that is not a staphylococcal bacterium or does not contain staphylococcal bacteria. In certain ~~embodiments~~examples a bacterial composition comprises an isolated or recombinantly expressed staphylococcal Protein A variant or a nucleotide encoding the same. The composition may be or include a recombinantly engineered staphylococcus bacterium that has been altered in a way that comprises specifically altering the bacterium with respect to a secreted virulence factor or cell surface protein. For example, the bacteria may be recombinantly modified to express more of the virulence factor or cell surface protein than it would express if unmodified.

[0094] The term "isolated" can refer to a nucleic acid or polypeptide that is substantially free of cellular material, bacterial material, viral material, or culture medium (when produced by recombinant DNA techniques) of their source of origin, or chemical precursors or other chemicals (when chemically synthesized). Moreover, an isolated compound refers to one that can be administered to a subject as an isolated compound; in other words, the compound may not simply be considered "isolated" if it is adhered to a column or embedded in an agarose gel. Moreover, an "isolated nucleic acid fragment" or "isolated peptide" is a nucleic acid or protein fragment that is not naturally occurring as a fragment and/or is not typically in the functional state.

[0095] Moieties of the ~~invention~~disclosure, such as polypeptides, peptides, antigens, or immunogens, may be conjugated or linked covalently or noncovalently to other moieties such as adjuvants, proteins, peptides, supports, fluorescence moieties, or labels. The term "conjugate" or "immunoconjugate" is broadly used to define the operative association of one moiety with another agent and is not intended to refer solely to any type of operative association, and is particularly not limited to chemical "conjugation." Recombinant fusion proteins are particularly contemplated.

Compositions of the ~~invention~~disclosure may further comprise an adjuvant or a pharmaceutically acceptable excipient. An adjuvant may be covalently or non-covalently coupled to a polypeptide or peptide of the ~~invention~~disclosure. In certain ~~aspects~~examples, the adjuvant is chemically conjugated to a protein, polypeptide, or peptide.

[0096] The term "providing" is used according to its ordinary meaning to indicate "to supply or furnish for use." In some ~~embodiments~~examples, the protein is provided directly by administering the protein, while in other ~~embodiments~~examples, the protein is effectively provided by administering a nucleic acid that encodes the protein. In certain ~~aspects~~examples the ~~invention~~disclosure contemplates compositions comprising various combinations of nucleic acid, antigens, peptides, and/or epitopes.

[0097] The subject will have (*e.g.*, are diagnosed with a staphylococcal infection), will be suspected of having, or will be at risk of developing a staphylococcal infection. Compositions of the present ~~invention~~disclosure include immunogenic compositions wherein the antigen(s) or epitope(s) are contained in an amount effective to achieve the intended purpose. More specifically, an effective amount means an amount of active ingredients necessary to stimulate or elicit an immune response, or provide resistance to, amelioration of, or mitigation of infection. In more specific ~~aspects~~examples, an effective amount prevents, alleviates or ameliorates symptoms of disease or infection, or prolongs the survival of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any preparation used in the methods of the ~~invention~~disclosure, an effective amount or dose can be estimated initially from *in vitro* studies, cell culture, and/or animal model assays. For example, a dose can be formulated in animal models to achieve a desired immune response or circulating antibody concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[0098] The embodiments in the Example section are understood to be embodiments of the invention that are applicable to all aspects of the invention.

[0099] The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives