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(54) **Titre : COMPOSITIONS DE VACCIN CONTRE STAPHYLOCOCCUS AUREUS**
(54) **Title: STAPHYLOCOCCUS AUREUS VACCINE COMPOSITIONS**

(57) **Abrégé/Abstract:**

The present disclosure relates to immunogenic compositions for inducing an immune response in a subject for the treatment and/or prevention of a Staphylococcus aureus infection. The immunogenic compositions disclosed herein comprise a S. aureus protein A (SpA) polypeptide and a S. aureus Leukocidin A (LukA) and/or Leukocidin B (LukB) variant polypeptide. The present disclosure further relates to methods of generating an immune response against S. aureus in a subject that involve administering the disclosed immunogenic compositions.



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(54) Title: STAPHYLOCOCCUS AUREUS VACCINE COMPOSITIONS

(57) Abstract: The present disclosure relates to immunogenic compositions for inducing an immune response in a subject for the treatment and/or prevention of a *Staphylococcus aureus* infection. The immunogenic compositions disclosed herein comprise a *S. aureus* protein A (SpA) polypeptide and a *S. aureus* Leukocidin A (LukA) and/or Leukocidin B (LukB) variant polypeptide. The present disclosure further relates to methods of generating an immune response against *S. aureus* in a subject that involve administering the disclosed immunogenic compositions.



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STAPHYLOCOCCUS AUREUS VACCINE COMPOSITIONS

[0001] This application claims the priority benefit of U.S. Provisional Patent Application Serial No. 63/170,089, filed April 2, 2021, and U.S. Provisional Patent Application Serial No. 63/249,452, filed September 28, 2021, which are hereby incorporated by reference in their entirety.

FIELD

[0002] The present disclosure relates to *Staphylococcus aureus* immunogenic compositions, and use of the described compositions for inducing an immune response in a subject for the treatment and/or prevention of *Staphylococcus* infection.

BACKGROUND

[0003] *Staphylococcus aureus* causes a broad range of invasive diseases, including sepsis, infective endocarditis, and toxic shock, along with less severe skin and soft tissue infections (Tong et al., “*Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management,” *Clin. Microbiol. Rev.* 28(3):603-661 (2015)). Currently, no vaccine is approved to combat *S. aureus* and therapeutic options are further limited by emerging antibiotic resistance (Sause et al., “Antibody-Based Biologics and Their Promise to Combat *Staphylococcus aureus* Infections,” *Trends Pharmacol. Sci.* 37(3):231-241 (2016)). The ability of *S. aureus* to cause diverse clinical syndromes is often linked to major changes in genome content (Copin et al., “After the Deluge: Mining *Staphylococcus aureus* Genomic Data for Clinical Associations and Host-Pathogen Interactions,” *Curr. Opin. Microbiol.* 41:43-50 (2018) and Recker et al., “Clonal Differences in *Staphylococcus aureus* Bacteraemia-Associated Mortality,” *Nat. Microbiol.* 2(10):1381-1388 (2017)). Notably, approximately 40% of the genome is not shared by all *S. aureus* isolates (Bosi et al., “Comparative Genome-Scale Modelling of *Staphylococcus aureus* Strains Identifies Strain-Specific Metabolic Capabilities Linked to Pathogenicity,” *Proc. Natl. Acad. Sci. USA* 113(26):E3801-3809 (2016)), thereby further complicating the identification of conserved targets for the generation of vaccines and biologics.

[0004] The present disclosure is directed to overcoming these and other limitations in the art.

SUMMARY

[0005] A first aspect of the present disclosure relates to an immunogenic composition comprising (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and (ii) a *Staphylococcus aureus* Leukocidin A (LukA) variant polypeptide. In an alternative aspect, the invention provides
5 a combination of two or more compositions, together comprising (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and (ii) a *Staphylococcus aureus* Leukocidin A (LukA) variant polypeptide.

[0006] In one aspect, the LukA variant polypeptide comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113,
10 and Val193 of SEQ ID NO: 25.

[0007] Additional aspects of the disclosure relate to immunogenic compositions or a combination of two or more immunogenic compositions comprising a LukA variant polypeptide comprising one or more additional amino acid substitutions, deletions, and/or additions to those described above.

[0008] Another aspect of the present disclosure relates to immunogenic compositions or a combination of two or more immunogenic compositions, together comprising (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, (ii) a *Staphylococcus aureus* Leukocidin A (LukA) variant polypeptide, and (iii) a *Staphylococcus aureus* Leukocidin B (LukB) polypeptide or variant thereof.

[0009] Additional aspects of the disclosure relate to immunogenic compositions or a combination of two or more immunogenic compositions comprising one or more nucleic acid molecules encoding the *S. aureus* protein A (SpA) polypeptide or variant thereof, the LukA variant polypeptide, and the LukB polypeptide or variant thereof of the immunogenic compositions as described herein.

[0010] Another aspect of the present disclosure is directed to an immunogenic composition or a combination of two or more immunogenic compositions comprising one or more vectors comprising the one or more nucleic acid molecules encoding the *S. aureus* protein A (SpA) polypeptide or variant thereof, the LukA variant polypeptide, and the LukB polypeptide or variant thereof of the immunogenic compositions as described herein.

[0011] Another aspect of the present disclosure is directed to an immunogenic composition comprising a host cell, where the host cell comprises the one or more nucleic acid molecules or vectors as described herein.

[0012] Another aspect of the present disclosure relates to a method of treating or preventing a staphylococcal infection a subject in need thereof. The method involves

administering an effective amount of the immunogenic composition or the combination of immunogenic compositions as described herein to a subject under conditions effective to treat or prevent a staphylococcal infection in said subject.

[0013] Another aspect of the present disclosure relates to a method of eliciting an immune response against *Staphylococcus aureus* in a subject in need thereof. The method involves administering an effective amount of the immunogenic composition or the combination of immunogenic compositions as described herein to a subject under conditions effective to elicit said immune response against *S. aureus* in said subject.

[0014] Another aspect of the present disclosure relates to a method of decolonizing or preventing colonization or recolonization of a *Staphylococcus* bacterium in a subject in need thereof. The method involves administering an effective amount of the immunogenic composition or the combination of immunogenic compositions as described herein to a subject under conditions effective to decolonize or prevent colonization or recolonization of a *Staphylococcus* bacterium in said subject.

[0015] Another aspect of the present disclosure relates to use of the immunogenic composition or the combination of immunogenic compositions as described herein in a method of generating an immune response against *S. aureus* in a subject.

[0016] *Staphylococcus aureus* (*S. aureus*) is responsible for a large number of hospital and community acquired infections. To escape clearance by the immune system, *S. aureus* employs a wide range of strategies. Staphylococcus protein A (SpA), a surface protein, is one key virulence factor of *S. aureus* that displays at least two functions associated with promoting infection. First, cell wall-anchored SpA on the bacterial surface binds to the Fc γ -domain of IgG and disables the effector functions of antibodies. Antibodies are bound unspecifically “upside down” thereby protecting staphylococci from opsonophagocytic killing (OPK) by host immune cells and preventing proper clearance. Second, SpA serves as a key immune evasion determinant that prevents the development of protective immunity during *S. aureus* colonization and infection. During colonization and invasive disease, released SpA crosslinks VH3 clonal B cell receptors and triggers the secretion of antibodies not specific to *S. aureus* that are unable to recognize staphylococcal determinants as antigens. This B cell superantigen activity (*i.e.*, the VH3-binding activity of released SpA) is responsible for preventing the development of protective immunity against *S. aureus* during colonization or invasive disease. The use of a SpA variant as vaccine antigen that has lost its immunoglobulin binding activity induces SpA specific antibodies that (1) neutralize its ability to bind IgG via Fc γ , (2) neutralize its ability to

bind IgG via VH3-idiotypic heavy chains and enables anti-staphylococcal immunity to develop, and (3) induce opsonophagocytic clearance via surface bound SpA.

[0017] Staphylococcal leukocidins A and B form a bi-component toxin (LukAB) having a different mode of action in promoting *S. aureus* infection. LukAB is a secreted toxin that, upon binding to phagocytic cells, assembles into a pore, inserts into the membrane, and lyses the host cell. This allows *S. aureus* to escape attack from neutrophils and escape clearance by the host. Antibodies induced by immunization with a LukA, LukB, or a LukAB toxoid will neutralize LukAB toxin activity resulting in surviving phagocytic cells that can clear *S. aureus*.

[0018] An immunogenic composition comprising a combination of these antigens, *i.e.*, SpA, LukA, LukB, and LukAB, will induce antibodies that neutralize two *S. aureus* virulence factors and prevent two independent key escape mechanisms of *S. aureus* to allow antibody mediated opsonophagocytosis to be effective.

BRIEF DESCRIPTION OF THE FIGURES

[0019] FIG.1 is an alignment of fifteen different *Staphylococcus aureus* LukA amino acid sequences including LukA of clonal complex (CC) 8 (SEQ ID NO: 1); CC45 (SEQ ID NO: 2); HMPREF0772_044(TCH60) of CC30 (SEQ ID NO: 27); SAR2108(MRSA252) of CC30 (SEQ ID NO: 36); SALG_02329(A9635) of CC45 (SEQ ID NO: 34); SAPIG2061(ST398) of CC398 (SEQ ID NO: 35); SATG_01930(D139) of CC10 (SEQ ID NO: 37); NEWMAN of CC8 (SEQ ID NO: 26); SAB1876C(RF122) of CC151 (SEQ ID NO: 32); SAV2005(Mu50) of CC5 (SEQ ID NO: 38); SA1813(N315) of CC5 (SEQ ID NO: 31); SACOL2006 of CC8 (SEQ ID NO: 33); HMPRE0776_0173 USA300(TCH959) of CC7 (SEQ ID NO: 29); HMPREF0774_2356 TCH130 of CC72 (SEQ ID NO: 28); and MW1942 (MW2) of CC1 (SEQ ID NO: 30). The amino acid sequence of a majority LukA sequence generated from a comparison of the aligned sequences is provided as SEQ ID NO: 25. The locations of amino acid substitutions described herein are also identified within each of the LukA sequences.

[0020] FIG. 2 is an alignment of fourteen different *Staphylococcus aureus* LukB amino acid sequences including LukB CC8 (SEQ ID NO: 15); CC45 (SEQ ID NO: 16); A9635 of CC45 (SEQ ID NO: 40); E1410 of CC30 (SEQ ID NO: 43); MRSA252 of CC30 (SEQ ID NO: 45); D139 of CC10 (SEQ ID NO: 42); Mu.50 of CC5 (SEQ ID NO: 46); JKD6008 of CC239 (SEQ ID NO: 44); COL of CC8 (SEQ ID NO: 41); USA300_FPR3757 of CC8 (SEQ ID NO: 115); NEWMAN of CC8 (SEQ ID NO: 116); RF122 of CC151 (SEQ ID NO: 98); MW2 of CC1 (SEQ ID NO: 47); and TCH130 of CC72 (SEQ ID NO: 99). The amino acid sequence of a majority LukB sequence generated from a comparison of the aligned sequences is provided as

SEQ ID NO: 39. The locations of amino acid substitutions described herein are also identified within each of the LukB sequences.

[0021] FIG. 3 shows the cytotoxicity of different LukAB variants used for immunization. Intoxication of primary human polymorphonuclear leukocytes (“PMNs” (n=4) with a titration of the different LukAB variants was carried out for 1 hr. Cell viability was evaluated with CellTiter. Data are the mean $\pm$ SEM from 4 donors, obtained in 2 separate experiments.

[0022] FIGs. 4A–4B show antibody titers against LukAB CC8 or CC45 in mice immunized with different LukAB variants. Envigo Hsd:ND4 (4 week old) mice (n=5/antigen) were subcutaneously administered 20 μ g of LukAB in 50 μ l of 10% glycerol 1X TBS mixed with 50 μ l of the adjuvant, TiterMax® Gold. A cohort of 5 mice also received a mock immunization consisting of an equal volume of 10% glycerol 1X TBS and TiterMax® Gold. Following two boosts (interval of 2 weeks between boosts) of the same antigen adjuvant cocktail, mice were bled via cardiac puncture and serum was obtained. Sera from immunized mice with indicated immunization antigens was pooled and serially diluted to determine antibody titers for CC8 LukAB (FIG. 4A) or CC45 LukAB (FIG. 4B). Plates were coated with 2 μ g/ml of CC8 or CC45 LukAB. Heatmap shows average absorbance value from duplicate measurements.

[0023] FIG. 5 provides the neutralization profile for sera from mice immunized with different LukAB variants against various LukAB toxins. Intoxication of human PMNs (n=4) for 1 hr with 0.156 μ g/ml (LD₉₀) of the indicated LukAB variants in the presence of 4-0.031% sera from mice immunized with the indicated antigens. Sera from immunized mice with indicated immunization antigens was pooled and heat inactivated before use. Cell viability was determined with CellTiter. Heatmap displays the average percentage of death cells of 4 donors, with black representing no cell death, and white 100% cell death.

[0024] FIGs. 6A–6C are tables showing the percentage of dead human polymorphonuclear leukocytes following intoxication with LD₉₀ of LukAB toxin sequence variants in the absence or presence of 2% (FIG. 6A), 1% (FIG. 6B), and 0.5% (FIG. 6C) mouse sera from mice immunized with the indicated antigen. Data are presented as the percent of dead cells. Cells with no shading represent lowest cell death and cells with darkest grey shading represent highest cell death.

[0025] FIGs. 7A–7D show intoxication of LukAB RARPR-33 at high concentrations is not cytotoxic. Freshly isolated human PMNs from healthy donors (n=4-6) were incubated for 1 hour with different concentrations of LukAB variants. Cell viability was determined by absorbance of CellTiter (FIGs. 7A and 7B). Percentage of dead cells was calculated by

subtracting background (healthy cells + PBS) and normalizing to Triton X100 treated cells which were set at 100% dead. Mean $\pm$ SEM is shown. Human PMNs isolated from healthy donors (n=4-6) were incubated for 2 hours with different concentrations of LukAB variants. Cell viability was determined by LDH release. (FIGs. 7C and 7D). Mean $\pm$ SEM is shown.

5 **[0026]** FIGs. 8A–8D shows intoxication of LukAB RARPR-33 and the D39A/R23E toxoid at high concentrations. Freshly isolated human PMNs from healthy donors (n=5) were incubated for 2 hours with different concentrations of LukAB variants. Cell viability was determined by absorbance of CellTiter (FIGs. 8A and 8B). Percentage of dead cells was calculated by subtracting background (healthy cells + PBS) and normalizing to Triton X100
10 treated cells which were set at 100% dead. Mean $\pm$ SEM is shown. Human PMNs isolated from healthy donors (n=5) were incubated for 2 hours with different concentrations of the LukAB variants. Cell viability was determined by LDH release (FIGs. 8C and 8D). Mean $\pm$ SEM is shown.

[0027] FIG. 9 shows the neutralization profile of sera from mice immunized with two
15 different LukAB toxoids against various LukAB toxins. Intoxication of human PMNs (n=4) for 1 hr with 0.156 μ g/ml (LD₉₀) of the indicated LukAB toxin variants in the presence of 0.125% sera from mice immunized with the two different antigens. Sera from mice immunized with both indicated immunization antigens was pooled and heat inactivated before use. Cell viability was determined with CellTiter. Bar graphs show mean + SEM of 4 different donors. Statistical
20 significance was determined using unpaired t test and $P < 0.05$ were considered significant. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$.

[0028] FIG. 10 is a schematic of the immunization schedule for male Göttingen Minipigs. Göttingen minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus*
25 in the SSI model. Eight days later the bacterial burden was determined. The table of FIG. 14B provides an overview of the experimental groups that were tested.

[0029] FIGs. 11A–11B show the efficacy of LukAB RARPR-33 and Spa* +/- GLA-SE in the SSI model in minipigs. Minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus* in the SSI model. Eight days later the bacterial burden was determined. The bacterial
30 load in the mid muscle (FIG. 15A) and deep muscle (FIG. 15B) is shown eight days post challenge with *S. aureus*. Each dot represents 1 minipig and geometric mean is indicated. Dotted line represents limit of detection. Statistical significance was determined using ANOVA with Dunnett post hoc test to correct for multiple comparisons, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

[0030] FIG. 12 is a schematic of the immunization schedule for male Göttingen Minipigs. The minipigs received three intramuscular immunizations, 3 weeks apart. Three weeks post the third immunization, animals were challenged with 10^6 CFU *S. aureus* in the surgical site infection model. Eight days post challenge (day 71) the bacterial burden was determined at the surgical site and in the spleen. At several timepoints blood was drawn and serum collected. The table shows the details of the three experimental groups.

[0031] FIGs. 13A–13C are graphs showing the immunogenicity of LukAB RARPR-33 and SpA*. Göttingen minipigs (n=3) were immunized with 100 µg LukAB RARPR-33 + 100 µg SpA* combined with AS01b (25 µg MPL and 25 µg QS-21) or 10 µg GLA-SE. The control group received antigen formulation buffer. Sera was collected before each immunization and three weeks post the third immunization. Specificity towards LukAB CC8 (FIG. 13A), LukAB CC45 (FIG. 13B) or SpA* (FIG. 13C) was determined by ELISA. EC_{50} titers are shown. Each point represents a single animal. The geometric mean $\pm$ geometric stdev of each group is shown. Dotted line indicates limit of detection and is set at 30. Samples below this value are censored to 30. Statistical significance was determined after three immunizations between the animals immunized with LukAB RARPR-33 + SpA* combined with AS01b or GLA-SE to the buffer control group, using one-way Tobit model with a Bonferroni correction to correct for multiple comparison, **P<0.01, ***P<0.001, ****P<0.0001.

[0032] FIGs. 14A–14D show cross-neutralization of LukAB by vaccine induced antibodies. Göttingen minipigs (n=3) were immunized with 100 µg LukAB RARPR-33 + 100 µg SpA* combined with AS01b (25 µg MPL and 25 µg QS-21) or 10 µg GLA-SE. The control group received antigen formulation buffer. Sera was collected before each immunization and three weeks post the third immunization. THP-1 cells were incubated with different sequence variants of LukAB toxins (CC8 (FIG. 14A), CC45 (FIG. 14B), CC22a (FIG. 14C), CC398 (FIG. 14D)) in the presence of serially diluted sera from minipigs before and after immunization. Relative potency titers representing the difference in IC_{50} titers (the serum dilution at which 50% of cytotoxicity was observed) between serum samples and a reference LukAB monoclonal antibody are shown. Graph shows geometric mean $\pm$ geometric Stdev. Each dot represents 1 animal. Statistical significance was determined for the samples derived from animals after three immunizations with LukAB RARPR-33 + SpA* combined with AS01b or GLA-SE and were compared to the buffer control group. One-way ANOVA with Dunnett post hoc test to correct for multiple comparison was used, *P<0.05, **P<0.01, ***P<0.001.

[0033] FIGs. 15A–15C show the efficacy of the immune response at the surgical site and the spleen in animals immunized with LukAB RARPR-33 + SpA* combined with different

adjuvants and challenged with *S. aureus*. Göttingen minipigs (n=3) were immunized with 100 µg LukAB RARPR-33 and 100 µg SpA*, adjuvanted with AS01b (25 µg MPL and 25 µg QS-21) or 10 µg of GLA-SE. The control group received only buffer. Three weeks post the third immunization, animals were challenged with 10⁶ CFU *S. aureus* CC398 in the SSI model. Eight days post challenge the bacterial burden (Log₁₀ CFU/gram of tissue) was determined at the surgical site in the mid muscle (FIG. 15A), deep muscle (FIG. 15B), and in the spleen (FIG. 15C). Each point represents a single animal. The geometric mean of each group is indicated. Statistical significance was determined using ANOVA with Dunnett post hoc test to correct for multiple comparisons, *P < 0.05, **P < 0.01.

[0034] FIGs. 16A is a schematic of the immunization schedule for male Göttingen Minipigs. Göttingen minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus* in the SSI model. Eight days later the bacterial burden was determined. The table of FIG. 16B provides an overview of the experimental groups that were tested.

[0035] FIGs. 16C–16D show the efficacy of LukAB RARPR-33 and SpA* in the SSI model in minipigs. Minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus* in the SSI model. Eight days later the bacterial burden was determined. The bacterial load in the mid muscle (FIG. 16C) and deep muscle (FIG. 16D) is shown eight days post challenge with *S. aureus*. Each dot represents 1 minipig and geometric mean is indicated. Dotted line represents limit of detection. Statistical significance was determined using ANOVA with Dunnett post hoc test to correct for multiple comparisons, *P < 0.05.

[0036] FIGs. 17A is a schematic of the immunization schedule for male Göttingen Minipigs. Göttingen minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus* in the SSI model. Eight days later the bacterial burden was determined. The table of FIG. 17B provides an overview of the experimental groups that were tested.

[0037] FIGs. 17C–17D show the efficacy of LukAB RARPR-33 and SpA* in the SSI model in minipigs. Minipigs were intramuscularly immunized on 3 separate occasions with 3 weeks apart. Three weeks post the final immunization, minipigs were challenged with *S. aureus* in the SSI model. Eight days later the bacterial burden was determined. The bacterial load in the mid muscle (FIG. 17C) and deep muscle (FIG. 17D) is shown eight days post challenge with *S. aureus*. Each dot represents 1 minipig and geometric mean is indicated. Dotted line represents

limit of detection. Statistical significance was determined using ANOVA with Dunnett post hoc test to correct for multiple comparisons, $**P<0.01$, $***P<0.0001$.

[0038] FIGs. 18A-E show the immunogenicity of LukAB RARPR-33 and SpA* in combination with different adjuvants. Experimental setup is shown in FIG 18A, where Swiss Webster mice were subcutaneously immunized on 3 separate occasions with 2 weeks apart, and then blood was collected at the indicated timepoints. FIG. 18B is an overview of the groups that were included. Antibody specificity towards LukAB CC8 (FIG. 18C), LukAB CC45 (FIG. 18D) or SpA* (FIG. 18E) in sera was determined by ELISA. EC_{50} titers are shown. Each point represents a single animal. The geometric mean $\pm$ geometric stdev of each group is shown. Dotted line indicates limit of detection and is set at 30. Samples below this value are censored to 30.

[0039] FIGs. 19A and 19B show the results of LukAB CC8 and CC45 toxin neutralization assays, respectively, which were performed with sera samples from 5 mice, from groups 1-5 (as listed in Fig. 18B), isolated two weeks post the final immunization. Relative potency titers representing the difference in IC_{50} titers (the serum dilution at which 50% of cytotoxicity was observed) between serum samples and a reference LukAB monoclonal antibody are shown. Graph shows geometric mean $\pm$ geometric Stdev. Each dot represents 1 animal.

DETAILED DESCRIPTION

Definitions

[0040] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular compositions or methodologies described, as these may vary. It is also to be understood that the terminology used in the description is for the purpose of describing the particular versions or embodiments only, and is not intended to limit the scope of embodiments herein which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of embodiments herein, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated by reference in their entirety. Nothing herein is to be construed as an admission that embodiments herein are not entitled to antedate such disclosure by virtue of prior invention.

[0041] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise.

[0042] Unless otherwise stated, any numerical values, such as a concentration or a concentration range described herein, are to be understood as being modified in all instances by the term “about.” Thus, a numerical value typically includes $\pm 10\%$ of the recited value. For example, a concentration of 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v). As used herein, the use of a numerical range expressly includes all possible subranges, all individual numerical values within that range, including integers within such ranges and fractions of the values unless the context clearly indicates otherwise.

[0043] Unless otherwise indicated, the term “at least” preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the invention.

[0044] As used herein, the terms “comprises,” “comprising,” “includes,” “including,” “has,” “having,” “contains” or “containing,” or any other variation thereof, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers and are intended to be non-exclusive or open-ended. For example, a composition, a mixture, a process, a method, an article, or an apparatus that comprises a list of elements is not necessarily limited to only those elements but can include other elements not expressly listed or inherent to such composition, mixture, process, method, article, or apparatus. Further, unless expressly stated to the contrary, “or” refers to an inclusive or and not to an exclusive or. For example, a condition A or B is satisfied by any one of the following: A is true (or present) and B is false (or not present), A is false (or not present) and B is true (or present), and both A and B are true (or present). As used herein, the conjunctive term “and/or” between multiple recited elements is understood as encompassing both individual and combined options. For instance, where two elements are conjoined by “and/or,” a first option refers to the applicability of the first element without the second. A second option refers to the applicability of the second element without the first. A third option refers to the applicability of the first and second elements together. Any one of these options is understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or” as used herein. Concurrent applicability of more than one of the options is also understood to fall within the meaning, and therefore satisfy the requirement of the term “and/or.”

[0045] As used herein, the term “consists of,” or variations such as “consist of” or “consisting of,” as used throughout the specification and claims, indicate the inclusion of any

recited integer or group of integers, but that no additional integer or group of integers can be added to the specified method, structure, or composition.

[0046] As used herein, the term “consists essentially of,” or variations such as “consist essentially of” or “consisting essentially of,” as used throughout the specification and claims, indicate the inclusion of any recited integer or group of integers, and the optional inclusion of any recited integer or group of integers that do not materially change the basic or novel properties of the specified method, structure or composition.

[0047] As used herein, “subject” means any animal, preferably a mammal, most preferably a human. The term “mammal” as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys, humans, etc., more preferably a human.

[0048] It should also be understood that the terms “about,” “approximately,” “generally,” “substantially,” and like terms, used herein when referring to a dimension or characteristic of a component of the preferred invention, indicate that the described dimension/characteristic is not a strict boundary or parameter and does not exclude minor variations therefrom that are functionally the same or similar, as would be understood by one having ordinary skill in the art. At a minimum, such references that include a numerical parameter would include variations that, using mathematical and industrial principles accepted in the art (*e.g.*, rounding, measurement or other systematic errors, manufacturing tolerances, etc.), would not vary the least significant digit.

[0049] The terms “identical” or percent “identity,” in the context of two or more nucleic acids or polypeptide sequences (*e.g.*, Staphylococcus LukA, LukB, SpA polypeptides and the polynucleotides that encode them), refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

[0050] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0051] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the

search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (see generally, Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, (1995 Supplement)).

[0052] Examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al. (1990) *J. Mol. Biol.* 215: 403-410 and Altschul et al. (1997) *Nucleic Acids Res.* 25: 3389- 3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

[0053] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0054] A further indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions.

[0055] As used herein, the term "polynucleotide," synonymously referred to as "nucleic acid molecule," "nucleotides" or "nucleic acids," refers to any polyribonucleotide or polydeoxyribonucleotide, which can be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that can be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions

comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short nucleic acid chains, often referred to as oligonucleotides.

[0056] As used herein, the term "vector," refers to e.g. any number of nucleic acids into which a desired sequence can be inserted, e.g., by restriction and ligation, for transport between genetic environments or for expression in a host cell. Nucleic acid vectors can be DNA or RNA. Vectors include, but are not limited to, plasmids, phage, phagemids, bacterial genomes, virus genomes, self-amplifying RNA, replicons.

[0057] As used herein, the term "host cell" refers to a cell comprising a nucleic acid molecule of the invention. The "host cell" can be any type of cell, e.g., a primary cell, a cell in culture, or a cell from a cell line. In one embodiment, a "host cell" is a cell transfected or transduced with a nucleic acid molecule of the invention. In another embodiment, a "host cell" is a progeny or potential progeny of such a transfected or transduced cell. A progeny of a cell may or may not be identical to the parent cell, e.g., due to mutations or environmental influences that can occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome.

[0058] The term "expression" as used herein, refers to the biosynthesis of a gene product. The term encompasses the transcription of a gene into RNA. The term also encompasses translation of RNA into one or more polypeptides, and further encompasses all naturally occurring post-transcriptional and post-translational modifications. The expressed polypeptide can be within the cytoplasm of a host cell, into the extracellular milieu such as the growth medium of a cell culture or anchored to the cell membrane.

[0059] As used herein, the terms "peptide," "polypeptide," or "protein" can refer to a molecule comprised of amino acids and can be recognized as a protein by those of skill in the art. The conventional one-letter or three-letter code for amino acid residues is used herein. The terms "peptide," "polypeptide," and "protein" can be used interchangeably herein to refer to polymers of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example,

disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as conjugation with a labeling component. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), as well as other modifications known in the art.

[0060] The polypeptide sequences described herein are written according to the usual convention whereby the N-terminal region of the peptide is on the left and the C-terminal region is on the right. Although isomeric forms of the amino acids are known, it is the L-form of the amino acid that is represented unless otherwise expressly indicated.

[0061] 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 polypeptide refers to one that can be administered to a subject as an isolated polypeptide; in other words, the polypeptide may not simply be considered “isolated” if it is adhered to a column or embedded in a 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.

[0062] As used herein the phrase “immune response” or its equivalent “immunological response” refers to the development of a humoral (antibody mediated), cellular (mediated by antigen-specific T cells or their secretion products) or both humoral and cellular response directed against a protein, peptide, carbohydrate, or polypeptide of the disclosure in a recipient subject. Such a response can be an active response induced by administration of immunogen or a passive response induced by administration of antibody, antibody containing material, or primed T-cells. A cellular immune response is elicited by the presentation of polypeptide epitopes in association with Class I or Class II MHC molecules, to activate antigen-specific CD4 (+) T helper cells and/or CD8 (+) cytotoxic T cells. The response can also involve activation of monocytes, macrophages, NK cells, basophils, dendritic cells, astrocytes, microglia cells, eosinophils, or other components of innate immunity. As used herein “active immunity” refers to any immunity conferred upon a subject by administration of an antigen.

[0063] The present disclosure is directed to immunogenic compositions suitable for eliciting an immune response to *Staphylococcus aureus*. As described herein, in some embodiments, the immunogenic composition comprises a *Staphylococcus aureus* protein A (SpA) polypeptide and a *S. aureus* Leukocidin A (LukA) variant polypeptide. In some embodiments, the immunogenic composition further comprises a *S. aureus* Leukocidin B (LukB)

polypeptide or variant polypeptide thereof. In some embodiments, the immunogenic composition comprises a *S. aureus* SpA protein and a *S. aureus* LukB variant polypeptide. The disclosure is further directed to uses and methods of using the immunogenic compositions in the treatment and/or prevention of *S. aureus* infection.

5 [0064] In a general aspect the invention thus provides for a composition comprising:

- (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
- (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptide comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.

10 In certain embodiments, the composition further comprises (iii) a *S. aureus* Leukocidin B (LukB) polypeptide or variant thereof. In certain embodiments, the composition further comprises (iv) an adjuvant.

[0065] The components (i), (ii), (iii) and (iv) of the composition can be formulated as a single product i.e. as a single composition. Alternatively, the components (i), (ii), (iii) and (iv)

15 can each be formulated in a single composition or in compositions comprising a combination of two or more of the components together. Accordingly, in a further aspect, the invention provides for a combination of two or more compositions, together comprising:

- (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
 - (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptide comprising
- 20 an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.

In certain embodiments, the combination of two or more compositions, further comprises (iii) a *S. aureus* Leukocidin B (LukB) polypeptide or variant thereof. In certain embodiments, the combination of two or more compositions, further comprises (iv) an adjuvant.

25 [0066] In certain embodiments, the combination of compositions can be combined to a single composition prior to use. In other embodiments, the combination of compositions is used as separate compositions that are to be administered in combination with each other.

***S. aureus* Leukocidin A (LukA) Polypeptides of the Immunogenic Composition**

[0067] In one aspect, the immunogenic composition of the present disclosure comprises a

30 *S. aureus* LukA variant polypeptide. Suitable LukA variant polypeptides comprise one or more amino acid residue insertions, substitutions, and/or deletions that render a LukAB bi-component complex containing such LukA variant non-cytotoxic. The LukA variant polypeptide also stabilizes the LukAB heterodimer, increases the melting temperature, and/or increases solubility of the heterodimer.

[0068] In all embodiments, the LukA variant polypeptide of the immunogenic composition can be a variant of the full-length LukA protein comprising all of the amino acid residues corresponding to a full-length mature LukA protein sequence. As referred to herein, a “mature” leukocidin protein sequence, is a sequence of the leukocidin protein lacking the amino-terminal secretion signal, which typically comprises the first 27-28 amino acid residues on the amino terminus.

[0069] In any embodiment, the LukA variant polypeptides of the immunogenic composition can be a variant of a less than the full-length mature LukA protein. In any embodiment, the variant LukA polypeptide is at least 100 amino acid residues in length. In any embodiment, the variant LukA polypeptide is at least 110, at least 120, at least 130, at least 140, at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, at least 240, at least 250, at least 260, at least 270, at least 280, at least 290, at least 300 amino acid residues in length.

[0070] While exemplary LukA variant proteins and polypeptides of the immunogenic composition as described herein are variant LukA proteins of clonal complexes CC8 (SEQ ID NO: 1) and CC45 (SEQ ID NO: 2) (see Table 1 below), one of skill in the art will readily appreciate that the amino acid substitutions and/or deletions of LukA identified in the context of SEQ ID NO: 1 and SEQ ID NO: 2 are amino acid residues that are conserved across various clonal complexes or within regions of LukA that are highly conserved across the various clonal complexes. Indeed, an alignment of LukA protein sequences from fifteen different strains of *S. aureus* (see FIG. 1) shows that the amino acid residues identified herein as residues subject to variation are residues that are conserved across all 15 of the aligned LukA amino acid sequences. While the position of the identified residue of variation may differ between individual LukA sequences, the sequence alignment shows the correspondence between these positions. For clarity, a LukA consensus sequence, having the amino acid sequence of SEQ ID NO: 25, was generated from the sequence alignment and utilized for the purpose of assigning the location of particular amino acid variations. For example, an amino acid substitution at lysine residue 83 in SEQ ID NO: 25 corresponds to the lysine residue at position 80 in the LukA sequence of SEQ ID NO: 1, the lysine residue at position 81 in the LukA sequence of SEQ ID NO: 2, and the lysine residue at position 83 in the LukA sequences of SEQ ID NOs: 26–38. Thus, the identified amino acid variations described herein can be universally applied to the corresponding amino acid residues of any LukA amino acid sequence known now or in the future.

[0071] In accordance with this aspect of the disclosure, in any embodiment, the LukA variant polypeptide of the immunogenic composition comprises an amino acid residue insertion,

substitution, and/or deletion at one or more amino acid residues corresponding to residues Lys83, Ser141, Val113, Val193 of SEQ ID NO: 25. In any embodiment, the LukA variant polypeptide further comprises an amino acid substitution or deletion at the amino acid residue corresponding to Glu323 of SEQ ID NO: 25 in addition to the one or more amino acid residue insertions, substitutions, and/or deletions described above. In any embodiment, the amino acid substitution or deletion at Glu323 comprises a glutamic acid to alanine substitution at position 323 (Glu323Ala) of SEQ ID NO: 25.

[0072] In any embodiment, the amino acid substitution at the one or more identified positions of LukA (and other *S. aureus* proteins as described herein) is a conservative substitution. Such conservative substitutions involve substituting one amino acid residue for another that is a member of the same class, which acts as a functional equivalent, resulting in a silent alteration. That is to say, the change relative to the native sequence would not appreciably diminish the basic properties of LukA. These classes of amino acid residues include, nonpolar (hydrophobic) amino acids (*e.g.*, alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine); polar neutral amino acids (*e.g.*, glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine); positively charged (basic) amino acids (*e.g.*, arginine, lysine and histidine); and negatively charged (acidic) amino acids (*e.g.*, aspartic acid and glutamic acid).

[0073] In other embodiments, an amino acid substitution at the one or more identified positions of the variant leukocidin or SpA polypeptide as described herein is a non-conservative alteration (*i.e.*, a substitution that disrupts the sequence, structure, function, or activity of the identified region). Such substitution may be desirable for purposes of reducing or alleviating cytotoxicity of the protein. A non-conservative substitution involves the substitution of an amino acid residue of one particular class with an amino acid residue of a different class. For example, a substitution of a nonpolar (hydrophobic) amino acid residue with a polar neutral amino acid or *vice versa*. In another embodiment, the non-conservative substitution involves the substitution of a positively charged (basic) amino acid residue, with a negatively charged (acidic) amino acid residue, such as aspartic acid and glutamic acid or *vice versa*. Such Molecular alterations can be accomplished by methods well known in the art, including primer extension on a plasmid template using single stranded templates (Kunkel et al., *Proc. Acad. Sci., USA* 82:488-492 (1985), which is hereby incorporated by reference in its entirety), double stranded DNA templates (Papworth, et al., *Strategies* 9(3):3-4 (1996), which is hereby incorporated by reference in its entirety), and by PCR cloning (Braman, J. (ed.), *IN VITRO MUTAGENESIS*

PROTOCOLS, 2nd ed. Humana Press, Totowa, N.J. (2002), which is hereby incorporated by reference in its entirety).

[0074] In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a lysine to methionine substitution at the residue corresponding to the lysine at position 83 (Lys83Met) of SEQ ID NO: 25. In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a serine to alanine substitution at the residue corresponding to the serine at position 141 (Ser141Ala) of SEQ ID NO: 25. In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a valine to isoleucine substitution at the residue corresponding to the valine at position 113 (Val113Ile) of SEQ ID NO: 25. In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a valine to isoleucine substitution at the residue corresponding to the valine at position 193 (Val193Ile) of SEQ ID NO: 25.

[0075] In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a glutamic acid to alanine substitution at the residue corresponding to the glutamic acid residue position 323 (Glu323Ala) of SEQ ID NO: 25 in addition to any one or more of the substitutions at the residues corresponding to Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25

[0076] In any embodiment, the LukA variant polypeptide of the immunogenic composition comprises a protein or polypeptide thereof having an amino acid residue insertion, substitution, and/or deletion at two of the aforementioned amino acid residues corresponding to Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25. In any embodiment, the LukA variant polypeptide comprises an amino acid residue insertion, substitution, and/or deletion at three of the aforementioned amino acid residues. In any embodiment, the LukA variant polypeptide comprises an amino acid residue insertion, substitution, and/or deletion at all four of the aforementioned amino acid residues. In any embodiment, the LukA variant polypeptide comprises the amino acid substitutions of lysine to methionine, serine to alanine, and valine to isoleucine at the aforementioned amino acid residues corresponding to Lys83Met, Ser141Ala, Val113Ile, and Val193Ile of SEQ ID NO: 25. In any embodiment, the variant LukA protein or polypeptide thereof further comprises the amino acid substitution corresponding to Glu323Ala of SEQ ID NO: 25, *i.e.*, the variant LukA comprises substitutions corresponding to Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala of SEQ ID NO: 25.

[0077] An exemplary LukA variant polypeptide of the immunogenic composition described herein possesses the amino acid substitutions corresponding to Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala in SEQ ID NO: 25. In any embodiment, the LukA variant

polypeptide of the immunogenic composition is CC8 LukA variant comprising any one or more amino acid substitutions selected from Lys80Met, Ser138Ala, Val110Ile, Val190Ile, and Glu320Ala in SEQ ID NO: 1. In any embodiment, the LukA variant polypeptide of the immunogenic composition is CC8 LukA variant comprising amino acid substitutions corresponding to each of Lys80Met, Ser138Ala, Val110Ile, Val190Ile, and Glu320Ala in SEQ ID NO: 1. In any embodiment, this LukA variant polypeptide has the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3.

[0078] In any embodiment, the LukA variant polypeptide of the immunogenic composition is a CC45 LukA variant polypeptide comprising any one or more amino acid substitutions corresponding to Lys81Met, Ser139Ala, Val111Ile, Val191Ile, and Glu321Ala in SEQ ID NO: 2. In any embodiment, the LukA variant polypeptide of the immunogenic composition is a CC45 LukA variant polypeptide comprising amino acid substitutions corresponding to each of Lys81Met, Ser139Ala, Val111Ile, Val191Ile, and Glu321Ala in SEQ ID NO: 2. In some embodiments, this LukA variant polypeptide has the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 4. Other exemplary variant LukA proteins include any one of the LukA proteins of SEQ ID NOs: 26–38 comprising the amino acid substitutions corresponding to the substitutions of Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala in SEQ ID NO: 25.

[0079] In any embodiment, the LukA variant polypeptide of the immunogenic composition as described herein comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In one embodiment, the amino acid substitutions at the one or more aforementioned residues introduces cysteine residues capable of forming disulfide bonds to stabilize conformation of the LukAB heterodimer structure. For example, in one embodiment, the LukA variant polypeptide described herein comprises a tyrosine to cysteine substitution at the amino acid residue corresponding to Tyr74 (Tyr74Cys) of SEQ ID NO: 25, and comprises an asparagine to cysteine substitution at the amino acid residue corresponding to Asp140 (Asp140Cys) of SEQ ID NO: 25. These cysteine residues at positions 74 and 140 form a disulfide bond thereby increasing the thermostability of the variant LukA relative to wild-type LukA or relative to other variant LukA proteins and polypeptides described herein not containing paired cysteine residues capable of forming a disulfide bond.

[0080] In another embodiment, the LukA variant polypeptide of the immunogenic composition described herein comprises a glycine to cysteine substitution at the amino acid residue corresponding to Gly149 (Gly149Cys) of SEQ ID NO: 25, and comprises a glycine to cysteine substitution at the amino acid residue corresponding to Gly156 (Gly156Cys) of SEQ ID NO: 25. These cysteine residues introduced at positions 149 and 156 form a disulfide bond thereby increasing the thermostability of the variant LukA relative to wild-type LukA or relative to other variant LukA polypeptides described herein not containing paired cysteine residues capable of forming a disulfide bond.

[0081] In any embodiment, the variant LukA polypeptide of the immunogenic composition comprises amino acid substitutions at each amino acid residue corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above. In any embodiment, the variant LukA polypeptide of the immunogenic composition comprises amino acid substitutions at each amino acid residue corresponding to amino acid residues Tyr71, Asp137, Gly146, and Gly153 of SEQ ID NO:1. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above. In any embodiment, the variant LukA polypeptide of the immunogenic composition comprises amino acid substitutions at each amino acid residue corresponding to amino acid residues Tyr72, Asp138, Gly147, and Gly154 of SEQ ID NO: 2. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above.

[0082] In any embodiment, the variant LukA protein or polypeptide of the immunogenic composition comprises an amino acid substitution at one or more amino acid residues corresponding to Lys83, Ser141, Val113, Val193, and Glu323 in combination with an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the variant LukA polypeptide comprises amino acid substitutions at amino acid residues corresponding to residues Lys83, Ser141, Val113, Val193, and Glu323 and residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25.

[0083] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys80, Ser138, Val110, Val190, Glu320, Tyr71, Asp137, Gly146, and Gly153 of SEQ ID NO: 1. In any embodiment, an exemplary LukA variant polypeptide is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of

Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Glu320Ala, Tyr71Cys, Asp137Cys, Gly146Cys, and Gly153Cys of SEQ ID NO: 1. In any embodiment, this CC8 LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 5, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 5.

[0084] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC45 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys81, Ser139, Val111, Val191, Glu321, Tyr72, Asp138, Gly147, and Gly154 of SEQ ID NO: 2. In any embodiment, an exemplary LukA variant polypeptide is a CC45 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Glu321Ala, Tyr72Cys, Asp138Cys, Gly147Cys, and Gly154Cys of SEQ ID NO: 2. In some embodiments, this CC45 LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 6, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 6.

[0085] Other exemplary LukA variant polypeptides of the immunogenic composition include any one of the LukA proteins of SEQ ID NOs: 26–38 comprising the amino acid substitutions corresponding to Lys83Met, Ser141Ala, Val113Ile, Val193Ile, Glu323Ala, Tyr74Cys, Asp140Cys, Gly149Cys, and Gly156Cys of SEQ ID NO: 25.

[0086] In any embodiment, the LukA variant polypeptide of the immunogenic composition as described herein comprises an amino acid substitution or deletion at the amino acid residue corresponding to amino acid residue Thr249 of SEQ ID NO: 25. In any embodiment, the LukA variant comprises a substitution at the residue corresponding to Thr249, where the substitution is a threonine to valine substitution at this residue (Thr249Val).

[0087] In any embodiment, the LukA variant protein or polypeptide of the immunogenic composition as described herein comprises the amino acid substitution at amino acid residue corresponding to Thr249 of SEQ ID NO: 25 in combination with any one of the other amino acid residue substitutions described herein, *i.e.*, substitutions at residues corresponding to Lys83, Ser141, Val113, Val193, Glu323 Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the LukA variant protein or polypeptide described herein comprises an amino acid substitution at the amino acid residue corresponding to Thr249 of SEQ ID NO: 25 in combination with at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or all nine of the other amino acid residue substitutions described herein. In any embodiment, the variant LukA protein or polypeptide comprises amino acid substitutions at each

residue corresponding to Lys83, Ser141, Val113, Val193, Glu323, and Thr249 of SEQ ID NO: 25.

[0088] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC8 LukA variant polypeptide having an amino acid substitution at residue Thr246 alone or in combination with any one or more amino acid substitutions corresponding to each of Lys80, Ser138, Val110, Val190, and Glu320 of SEQ ID NO: 1. In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys80, Ser138, Val110, Val190, Glu320, and Thr246 of SEQ ID NO: 1. In any embodiment, an exemplary LukA variant polypeptide is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Glu320Ala, and Thr246Val of SEQ ID NO: 1. In one embodiment, an exemplary LukA variant polypeptide has amino acid substitutions at residues corresponding to each of the aforementioned positions has an amino acid sequence of SEQ ID NO: 7, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 7.

[0089] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC45 LukA variant polypeptide having an amino acid substitution at residue Thr247 alone or in combination with any one or more amino acid substitutions corresponding to each of Lys81, Ser139, Val111, Val191, and Glu321 of SEQ ID NO: 2. In any embodiment, an exemplary LukA variant polypeptide of the immunogenic composition is a CC45 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys81, Ser139, Val111, Val191, Glu321, and Thr247 of SEQ ID NO: 2. In any embodiment, an exemplary LukA variant polypeptide is a CC45 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Glu321Ala, and Thr247Val of SEQ ID NO: 2. In one embodiment, an exemplary LukA variant polypeptide having amino acid substitutions at residues corresponding to each of the aforementioned positions has an amino acid sequence of SEQ ID NO: 8, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 8.

[0090] Other exemplary variant LukA proteins of the immunogenic composition include any one of the LukA proteins of SEQ ID NOs: 26–38 comprising the described amino acid substitutions at the amino acid residues corresponding to Lys83, Ser141, Val113, Val193, Glu323, and Thr249 of SEQ ID NO:25.

[0091] In any embodiment, the variant LukA protein or polypeptide of the immunogenic composition comprises amino acid substitutions at each residue corresponding to Lys83, Ser141, Val113, Val193, Glu323, Thr249, Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25.

[0092] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic

composition is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys80, Ser138, Val110, Val190, Glu320, Tyr71, Asp137, Gly146, Gly153, and Thr246 of SEQ ID NO: 1. In any embodiment, an exemplary LukA variant

polypeptide is a CC8 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Glu320Ala, Tyr71Cys,

Asp137Cys, Gly146Cys, Gly153Cys, and Thr246Val of SEQ ID NO: 1. In one embodiment, an exemplary LukA variant polypeptide has amino acid substitutions at residues corresponding to each of the aforementioned positions has an amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 9.

[0093] In any embodiment, an exemplary LukA variant polypeptide of the immunogenic

composition is a CC45 LukA variant polypeptide having amino acid substitutions at residues corresponding to each of Lys81, Ser139, Val111, Val191, Glu321, Tyr72, Asp138, Gly147, Gly154 and Thr247 of SEQ ID NO: 2. In any embodiment, an exemplary LukA variant

polypeptide is a CC45 LukA variant polypeptide having amino acid substitutions at residues

corresponding to each of Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Glu321Ala, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys and Thr247Ala of SEQ ID NO: 2. In one embodiment, an

exemplary LukA variant polypeptide having amino acid substitutions at residues corresponding to each of the aforementioned positions has an amino acid sequence of SEQ ID NO: 10, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least

99% sequence similarity to the amino acid sequence of SEQ ID NO: 10.

[0094] Other exemplary variant LukA proteins of the immunogenic composition include

any one of the LukA proteins of SEQ ID NOs: 26–38 comprising the described amino acid substitutions of residues corresponding to Lys83, Ser141, Val113, Val193, Glu323, Thr249, Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25.

[0095] Table 1 below provides exemplary variant LukA amino acid sequences of the immunogenic composition as disclosed herein.

Table 1. Exemplary LukA Polypeptide Amino Acid Sequences

SEQ ID NO	Name	Description
1	LukA CC8 WT	HKDSQDQNKKEHVDKSQQKDKRNVTNKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQDFDIDPTYDKNVLLVKK QGSIHSLNLFESHKEEKNSNWLKYPSEYHVDQVQRNRKT EILDQLPKNKISTAKVDSTFSYSSGGKFDSTKGIGRTSSNSY SKTISYNQQNYDTIASGKNNNWHVHWSVIANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFLTYLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYPKEG
2	LukA CC45 WT	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQDFDIDPTYDKNVLLV KKQGSIHSLNLFESHNETNASWLKYPSEYHVDQVQRNP KTEILDQLPKNKISTAKVDSTFSYSLGGKFDSTKGIGRTSSN SYSKSISYNQQNYDTIASGKNNNRHVHWSVANDLKYGN EIKNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGF NPEFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPI LEQNKGQRFIVVYEVDWKNKTVKVVEKYSDDQNKPYKEG
3	LukA CC8 W95 E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile	HKDSQDQNKKEHVDKSQQKDKRNVTNKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQDFDIDPTYDKNVLLV K <u>M</u> QGSIHSLNLFESHKEEKNSNWLKYPSEYH <u>I</u> DFQVQRNRK TEILDQLPKNKISTAKVD <u>A</u> TFSYSSGGKFDSTKGIGRTSSNS YSKTISYNQQNYDTIASGKNNNWHVHWS <u>I</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFLTYLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYPK <u>A</u> G
4	LukA CC45 W95 E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQDFDIDPTYDKNVLLV K <u>M</u> QGSIHSLNLFESHNETNASWLKYPSEYH <u>I</u> DFQVQRNP KTEILDQLPKNKISTAKVD <u>A</u> TFSYSLGGKFDSTKGIGRTSSN SYSKSISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNE IKNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGFNP EFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSDDQNKPYK <u>A</u> G
5	LukA CC8 W95W72 E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	HKDSQDQNKKEHVDKSQQKDKRNVTNKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQDFDIDPT <u>C</u> DKNVLLV K <u>M</u> QGSIHSLNLFESHKEEKNSNWLKYPSEYH <u>I</u> DFQVQRNRK TEILDQLPKNKISTAKV <u>C</u> A <u>T</u> FSYSSG <u>C</u> KFDSTK <u>C</u> I <u>G</u> RTSSNS YSKTISYNQQNYDTIASGKNNNWHVHWS <u>I</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFLTYLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYPK <u>A</u> G

SEQ ID NO	Name	Description
6	LukA CC45 W95W72 E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDP <u>T</u> CDKNVLLV K <u>M</u> QGSIHSLNLFESHNETNASWLKYPSEYH <u>I</u> DFQVQRNP KTEILDQLPKNKISTAKV <u>C</u> A <u>T</u> FSYSLG <u>C</u> KFDSTK <u>C</u> IGRTSSN SYSKISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNE IKNRNDEFLFYRNTLSTVENPELSFASKYRYPALVRSGFNP EFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSQDNKPYK <u>A</u> G
7	LukA CC8 W97 E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val	HKDSQDQNKKEHVDKSQQKDKRNVTNKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQFDFIDDP <u>T</u> YDKNVLLV <u>M</u> QGSIHSLNLFESHKEEKNSNWLKYPSEYH <u>I</u> DFQVKRNRK TEILDQLPKNKISTAKV <u>N</u> A <u>T</u> FSYSSGGKFDSTKGIGRTSSNS YSKTISYNQQNYDTIASGKNNNWHVHWS <u>I</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFL <u>V</u> YLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYK <u>A</u> G
8	LukA CC45 W97 E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDP <u>T</u> YDKNVLLV K <u>M</u> QGSIHSLNLFESHNETNASWLKYPSEYH <u>I</u> DFQVQRNP KTEILDQLPKNKISTAKV <u>D</u> A <u>T</u> FSYSLGGKFDSTKGIGRTSSN SYSKISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNE IKNRNDEFLFYRNTLSTVENPELSFASKYRYPALVRSGFNP EFL <u>V</u> YISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSQDNKPYK <u>A</u> G
9	LukA CC8 W97 W72 E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	HKDSQDQNKKEHVDKSQQKDKRNVTNKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQFDFIDDP <u>T</u> CDKNVLLV <u>M</u> QGSIHSLNLFESHKEEKNSNWLKYPSEYH <u>I</u> DFQVKRNRK TEILDQLPKNKISTAKV <u>C</u> A <u>T</u> FSYSSG <u>C</u> KFDSTK <u>C</u> IGRTSSNS YSKTISYNQQNYDTIASGKNNNWHVHWS <u>I</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFL <u>V</u> YLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYK <u>A</u> G
10	LukA CC45 W97 W72 E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDP <u>T</u> CDKNVLLV K <u>M</u> QGSIHSLNLFESHNETNASWLKYPSEYH <u>I</u> DFQVQRNP KTEILDQLPKNKISTAKV <u>C</u> A <u>T</u> FSYSLG <u>C</u> KFDSTK <u>C</u> IGRTSSN SYSKISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNE IKNRNDEFLFYRNTLSTVENPELSFASKYRYPALVRSGFNP EFL <u>V</u> YISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSQDNKPYK <u>A</u> G

SEQ ID NO	Name	Description
11	LukA CC45 W94 E321A, Lys81Leu, Ser139Ala, Val111Ile, Val191Ile	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDPYDKNVLLV K <u>L</u> QGSIHSLKFEHRNETNASWLKYPSEYH <u>I</u> DFQVQRNPK TEILDQLPKNKISTAKVD <u>A</u> TFSYSLGGKFDSTKGIGRTSSNS YSKSISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNEI KNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGFNP EFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSQNKPYK <u>A</u> G
12	LukA CC45 W96 E321A, Lys81Leu, Ser139Ala, Val111Ile, Val191Ile, Thr247Val	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDPYDKNVLLV K <u>L</u> QGSIHSLKFEHRNETNASWLKYPSEYH <u>I</u> DFQVQRNPK TEILDQLPKNKISTAKVD <u>A</u> TFSYSLGGKFDSTKGIGRTSSNS YSKSISYNQQNYDTIASGKNNNRHVHWS <u>I</u> VANDLKYGNEI KNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGFNP EFL <u>Y</u> YISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPILE QNKDGQRFIVVYEVDWKNKTVKVVEKYSQNKPYK <u>A</u> G
13	LukA CC8 Glu320Ala	HKDSQDQNKKEHVDKSQQKDKRNVNVDKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQFDFIDDPYDKNVLLV <u>K</u> QGSIHSLKFEHKEEKNSNWLKYPSEYH <u>V</u> DFQVQRNRKT EILDQLPKNKISTAKVDSTFSYSSGGKFDSTKGIGRTSSNSY SKTISYNQQNYDTIASGKNNNWHVHWS <u>V</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFLTYLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKYSDDNKPYK <u>A</u> G
14	LukA CC45 Glu321Ala	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDPYDKNVLLV K <u>K</u> QGSIHSLKFEHRNETNASWLKYPSEYH <u>V</u> DFQVQRNP KTEILDQLPKNKISTAKVDSTFSYSLGGKFDSTKGIGRTSSN SYSKSISYNQQNYDTIASGKNNNRHVHWS <u>V</u> VANDLKYGN EIKNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGF NPEFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPI LEQNKGQRFIVVYEVDWKNKTVKVVEKYSQNKPYK <u>A</u> G
113	LukA CC8 delta 10	HKDSQDQNKKEHVDKSQQKDKRNVNVDKDKNSTAPDDIGK NGKITKRTETVYDEKTNILQNLQFDFIDDPYDKNVLLV <u>K</u> QGSIHSLKFEHKEEKNSNWLKYPSEYH <u>V</u> DFQVQRNRKT EILDQLPKNKISTAKVDSTFSYSSGGKFDSTKGIGRTSSNSY SKTISYNQQNYDTIASGKNNNWHVHWS <u>V</u> IANDLKYGGEV KNRNDELLFYRNTRIATVENPELSFASKYRYPALVRSGFNP EFLTYLSNEKSNEKTQFEVITYTRNQDILKNRPGIHYAPPILE KNKDGQRLIVTYEVDWKNKTVKVVDKY

SEQ ID NO	Name	Description
114	LukA CC45 delta 10	ANKDSQDQTKKEHVDKAQQKEKRNVDKDKNTPGPDDIG KNGKVTKRTVSEYDKETNILQNLQFDFIDDPYDKNVLLV KKQGSIHSLKFEHRNETNASWLKYPSEYHVDFQVQRNP KTEILDQLPKNKISTAKVDSTFSYSLGGKFDSTKGIGRTSSN SYSKSISYNQQNYDTIASGKNNNRHVHWSVVANDLKYGN EIKNRNDEFLFYRNTRLSTVENPELSFASKYRYPALVRSGF NPEFLTYISNEKTNDKTRFEVITYTRNQDILKNKPGIHYGQPI LEQNKDQGQRFIVVYEVDWKNKTVKVVEKY

***S. aureus* Leukocidin B (LukB) Polypeptides of the Immunogenic Composition**

[0096] In some aspects, the immunogenic composition of the present disclosure comprises a *S. aureus* Leukocidin B (LukB) proteins or polypeptides. In any embodiment, the *S. aureus* LukB protein or polypeptide is a wildtype protein or polypeptide. Suitable LukB polypeptides include any one of LukB polypeptides disclosed herein, *e.g.*, polypeptide having any amino acid sequence selected from SEQ ID NO: 15, 16, and 39-51. In any embodiment, the LukB polypeptide is a CC8 LukB polypeptide. A suitable CC8 LukB polypeptide comprises the amino acid sequence of SEQ ID NO: 15. In any embodiment, the LukB polypeptide is a CC45 LukB polypeptide. A suitable CC45 LukB polypeptide comprises the amino acid sequence of SEQ ID NO: 16.

[0097] In any embodiment, the LukB polypeptide of the immunogenic composition disclosed here comprises a LukB variant polypeptide. Suitable LukB variant polypeptides comprise one or more amino acid residue insertions, substitutions, and/or deletions that improve LukB stability thereby contributing to LukAB toxoid stability. As described herein, these variant LukB proteins and polypeptides are ideal vaccine antigen candidates which can be included in the immunogenic composition with a SpA polypeptide alone or in combination with a Leukocidin A (LukA) variant protein or polypeptide. When the immunogenic composition comprises the combination of LukB and LukA polypeptides, the resulting toxoid mimics the structure of *S. aureus* LukAB toxin, thereby facilitating the generation of a robust immune response against one of the most potent toxins of *S. aureus*.

[0098] In any embodiment, the LukB variant polypeptide of the immunogenic composition is a variant of the full-length LukB protein comprising all of the amino acid residues corresponding to a full-length mature LukB protein sequence. In any embodiment, the LukB variant polypeptide is a variant of a less than the full-length mature LukB protein. In any embodiment, the variant LukB polypeptide is at least 100 amino acid residues in length. In any embodiment, the variant LukB polypeptide is at least 110, at least 120, at least 130, at least 140,

at least 150, at least 160, at least 170, at least 180, at least 190, at least 200, at least 210, at least 220, at least 230, at least 240, at least 250, at least 260, at least 270, at least 280, at least 290, at least 300 amino acid residues in length.

[0099] While exemplary LukB variant proteins and polypeptides described herein are variant LukB proteins of clonal complexes CC8 (SEQ ID NO: 15) and CC45 (SEQ ID NO: 16) (see Table 2 below), one of skill in the art will readily appreciate that the amino acid substitutions and/or deletions of LukB identified in the context of SEQ ID NO: 15 and SEQ ID NO: 16 are amino acid residues that are conserved across various clonal complexes or within regions of LukB that are highly conserved across the various clonal complexes. An alignment of LukB protein sequences from fourteen different strains of *S. aureus* (see FIG. 2) shows that the amino acid residues identified herein as residues subject to variation are residues that are conserved across all 14 of the aligned LukB amino acid sequences. While the position of the identified residue of variation may differ between individual LukB sequences, the sequence alignment shows the correspondence between these positions. For clarity, a LukB consensus sequence, having the amino acid sequence of SEQ ID NO: 39, was generated from the sequence alignment and utilized for the purpose of assigning the location of particular amino acid variations. For example, an amino acid substitution at glutamic acid residue 109 in SEQ ID NO: 39 corresponds to the glutamic acid residue at position 109 in the LukB sequences of SEQ ID NOs: 15, 42, 44, and 46–51, the glutamic acid residue at position 110 in the LukB sequences of SEQ ID NOs: 16, 40, 43, and 45, and the glutamic acid residue at position 60 in the LukB sequence of SEQ ID NO: 41. Thus, the identified amino acid variations described herein can be universally applied to corresponding amino acid residues in any LukB amino acid sequences known now or in the future.

[0100] In any embodiment, a suitable LukB variant polypeptide of the immunogenic compositions as disclosed herein comprises an amino acid substitution or deletion at the amino acid residue corresponding to amino acid residue Val53 of SEQ ID NO: 39. In any embodiment, the amino acid substitution at Val53 comprises a valine to leucine (Val53Leu) substitution. In any embodiment, an exemplary LukB variant polypeptide comprising a substitution corresponding to the Val53Leu substitution in SEQ ID NO: 39.

[0101] In any embodiment, an exemplary LukB variant polypeptide of the immunogenic composition is a CC8 LukB variant polypeptide having an amino acid substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 15. In any embodiment, an exemplary LukB variant polypeptide is a CC8 LukB variant polypeptide having a valine to leucine amino acid substitution at the position corresponding to position 53

of SEQ ID NO: 15. In any embodiment, an exemplary CC8 LukB sequence having a valine to leucine substitution at position 53 comprises the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 17.

5 [0102] In any embodiment, an exemplary LukB variant polypeptide of the immunogenic composition is a CC45 LukB variant polypeptide having an amino acid substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 16. In any embodiment, an exemplary LukB variant polypeptide is a CC45 LukB variant polypeptide having a valine to leucine amino acid substitution at the position corresponding to position 53
10 of SEQ ID NO: 16. An exemplary LukB variant polypeptide comprising a valine to leucine substitution comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 18.

[0103] Other exemplary variant LukB proteins include any one of the LukB proteins of
15 SEQ ID NOs: 40–51 comprising an amino acid substitution corresponding to Val53Leu.

[0104] In any embodiment, the LukB variant polypeptide of the immunogenic composition as described herein comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39. In any embodiment, the amino acid substitution at the one or more
20 aforementioned residues introduces cysteine residues capable of forming a disulfide bond to stabilize conformation of the LukAB heterodimer structure. For example, in one embodiment, the LukB variant protein or polypeptide described herein comprises a glutamic acid to cysteine substitution at the amino acid residue corresponding to Glu45 (Glu45Cys) of SEQ ID NO: 39, and comprises an threonine to cysteine substitution at the amino acid residue corresponding to
25 Thr121 (Thr121Cys) of SEQ ID NO: 39. These cysteine residues at positions 45 and 121 form a disulfide bond thereby increasing the thermostability of the variant LukB relative to wild-type LukB or relative to other variant LukB proteins and polypeptides described herein not containing paired cysteine residues capable of forming a disulfide bond.

[0105] In any embodiment, the LukB variant protein or polypeptide of the
30 immunogenic composition described herein comprises a glutamic acid to cysteine substitution at the amino acid residue corresponding to Glu109 (Glu109Cys) of SEQ ID NO: 39, and comprises an arginine to cysteine substitution at the amino acid residue corresponding to Arg154 (Arg154Cys) of SEQ ID NO: 39. These cysteine residues introduced at positions 109 and 154 form a disulfide bond thereby increasing the thermostability of the variant LukB

relative to wild-type LukB or relative to other variant LukB proteins and polypeptides described herein not containing paired cysteine residues capable of forming disulfide bonds.

[0106] In any embodiment, the LukB variant polypeptide of the immunogenic composition is a CC8 LukB variant polypeptide comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 15. In any embodiment, the LukB variant polypeptide of the immunogenic composition is a CC8 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 15. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above. In any embodiment, an exemplary LukB variant polypeptide comprising cysteine amino acid substitutions at residues corresponding to Glu45, Glu109, Thr121, and Arg154 comprises the amino acid sequence of SEQ ID NO: 21, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 21.

[0107] In any embodiment, the LukB variant polypeptide of the immunogenic composition as described herein comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu110, Thr122, and Arg155 of SEQ ID NO: 16. In any embodiment, the LukB variant polypeptide of the immunogenic composition is a CC45 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue corresponding to amino acid residues Glu45, Glu110, Thr122, and Arg155 of SEQ ID NO: 16. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above. In any embodiment, an exemplary LukB variant polypeptide comprising cysteine amino acid substitutions at residues corresponding to Glu45, Glu110, Thr122, and Arg155 comprises the amino acid sequence of SEQ ID NO: 22, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 22.

[0108] In any embodiment, the LukB variant polypeptide of the immunogenic composition as disclosed herein comprises an amino acid substitution at the amino acid residue corresponding to Val53 of SEQ ID NO: 39 in combination with an amino acid residue substitution at one or more amino acid residues corresponding to Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39. In any embodiment, the LukB variant polypeptide is a CC8 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue

corresponding to amino acid residues Val53, Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 15. In any embodiment, the LukB variant polypeptide is a CC8 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue corresponding to amino acid residues Val53Leu, Glu45Cys, Glu109Cys, Thr121Cys, and Arg154Cys of SEQ ID NO: 15. In any embodiment, an exemplary CC8 LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 19, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 19.

[0109] In any embodiment, the LukB variant polypeptide of the immunogenic composition is a CC45 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue corresponding to amino acid residues Val53, Glu45, Glu110, Thr122, and Arg155 of SEQ ID NO: 16. In any embodiment, the LukB variant polypeptide is a CC45 LukB variant polypeptide comprising an amino acid substitution at each amino acid residue corresponding to amino acid residues Val53Leu, Glu45Cys, Glu110Cys, Thr123Cys, and Arg155Cys of SEQ ID NO: 16. In any embodiment, an exemplary CC45 LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 20, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 20.

[0110] Other exemplary LukB variant polypeptides of the immunogenic composition include any one of the LukB proteins of SEQ ID NOs: 40–51 comprising the described amino acid substitutions of residues corresponding to Val53, Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39 of SEQ ID NO: 39.

[0111] Table 2 below provides exemplary variant LukB amino acid sequences of the immunogenic composition as disclosed herein.

Table 2. Exemplary LukB Polypeptide Amino Acid Sequences

SEQ ID NO	Name	Description
15	LukB CC8 WT	KINSEIKQVSEKNLDGDTKMYTRTATTSDSQKNITQSLQFN FLTEPNYDKETVFIKAKGTIGSGLRILDPNGYWNSTLRWPG SYSVSIQNVDNNTNVTDFAPKNQDESREVKYTYGYKT GGDFSINRGGLTGNITKESNYSETISYQQPSYRTLDDQSTSH KGVGWKVEAHLINMGHDHTRQLTNDSDNRTKSEIFSLTR NGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDK GKSQFVVHYKRSMDEFKIDWNRHGFWGYWSGENHVDKK EEKLSALYEVDWKTHNVKFVKVLNDNEKK

SEQ ID NO	Name	Description
16	LukB CC45 WT	EIKSKITTVSEKNLDGDTKMYTRTATTSDTEKKISQSLQFNFLTEPNYDKETVFIKAKGTIGSGLKILNPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDESREVKYTYGYKTGGDFSINRGGLTG NITKEKNYSETISYQQPSYRTLIDQPTTNKGVAWKVEAHSINNMGHDHTRQLTNDSDDRVKSEIFSLTRNGNLWAKDNFTPKNKMPVTVSEGFNPEFLAVMSHDKNKDGKSRFIVHYKRSMDDFKLDWNKHGFWGYWSGENHVDQKEEKLSALYEVDWKTHDVKLIKTFNDKEKK
17	LukB CC8 Val53Leu	KINSEIKQVSEKNLDGDTKMYTRTATTSDSQKNITQSLQFNFLTEPNYDKETLFIKAKGTIGSGLRILDPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDESREVKYTYGYKTGGDFSINRGGLTG NITKESNYSETISYQQPSYRTLLDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSDNRTKSEIFSLTRNGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDKGKSQFVVHYKRSMDEFKIDWNRHGFWGYWSGENHVDKKEEKLSALYEVDWKTHNVKFKVLNDNEKK
18	LukB CC45 Val53Leu	EIKSKITTVSEKNLDGDTKMYTRTATTSDTEKKISQSLQFNFLTEPNYDKETLFIKAKGTIGSGLKILNPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDESREVKYTYGYKTGGDFSINRGGLTG NITKEKNYSETISYQQPSYRTLIDQPTTNKGVAWKVEAHSINNMGHDHTRQLTNDSDDRVKSEIFSLTRNGNLWAKDNFTPKNKMPVTVSEGFNPEFLAVMSHDKNKDGKSRFIVHYKRSMDDFKLDWNKHGFWGYWSGENHVDQKEEKLSALYEVDWKTHDVKLIKTFNDKEKK
19	LukB CC8 Val53Leu, Glu45Cys, Glu109Cys, Thr121Cys, and Arg154Cys	KINSEIKQVSEKNLDGDTKMYTRTATTSDSQKNITQSLQFNFLTCPNYDKETLFIKAKGTIGSGLRILDPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDCSREVKYTYGYKC GGDFSINRGGLTG NITKESNYSETISYQQPSYCTLLDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSDNRTKSEIFSLTRNGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDKGKSQFVVHYKRSMDEFKIDWNRHGFWGYWSGENHVDKKEEKLSALYEVDWKTHNVKFKVLNDNEKK
20	LukB CC45 Val53Leu, Glu45Cys, Thr122Cys, Glu110Cys, Arg155Cys	EIKSKITTVSEKNLDGDTKMYTRTATTSDTEKKISQSLQFNFLTCPNYDKETLFIKAKGTIGSGLKILNPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDCSREVKYTYGYKC GGDFSINRGGLTG NITKEKNYSETISYQQPSYCTLIDQPTTNKGVAWKVEAHSINNMGHDHTRQLTNDSDDRVKSEIFSLTRNGNLWAKDNFTPKNKMPVTVSEGFNPEFLAVMSHDKNKDGKSRFIVHYKRSMDDFKLDWNKHGFWGYWSGENHVDQKEEKLSALYEVDWKTHDVKLIKTFNDKEKK
21	LukB CC8 Glu45Cys, Glu109Cys, Thr121Cys, and Arg154Cys	KINSEIKQVSEKNLDGDTKMYTRTATTSDSQKNITQSLQFNFLTCPNYDKETVFIKAKGTIGSGLRILDPNGYWNSTLRWPGSYSVSIQNVDDNNNSTNVTDFAPKNQDCSREVKYTYGYKC GGDFSINRGGLTG NITKESNYSETISYQQPSYCTLLDQSTSHKGVGWKVEAHLINNMGHDHTRQLTNDSDNRTKSEIFSLTRNGNLWAKDNFTPKDKMPVTVSEGFNPEFLAVMSHDKKDKGKSQFVVHYKRSMDEFKIDWNRHGFWGYWSGENHVDKKEEKLSALYEVDWKTHNVKFKVLNDNEKK

SEQ ID NO	Name	Description
22	LukB CC45 Glu45Cys, Thr122Cys, Glu110Cys, Arg155Cys	EIKSKITTVSEKNLDGDTKMYTRTATTSDTEKKISQSLQFNF LTCPNYDKETVFIKAKGTIGSGLKILNPNGYWNSTLRWPGS YSVSIQNVDDNNNSTNVTDFAFKNQDCSREVKYTYGYKC GGDFSINRGGTLGNITKEKNYSETISYQQPSYCTLIDQPTTN KGVAVKVEAHSINNMGHDHTRQLTNDSDDRVKSEIFSLTR NGNLWAKDNFTPKNKMPVTVSEGFNPEFLAVMSHDKNDK GKSRFIVHYKRSMDDFKLDWNKHGFWGYWSGENHVDQK EEKLSALYEVDWKTHDVKLIKTFNDKEKK

Staphylococcal Protein A (SpA) Polypeptides of the Immunogenic Composition

[0112] The immunogenic composition as described herein contains a *S. aureus* Protein A polypeptide. “Protein A” or “SpA,” are used interchangeably herein and refer to the cell wall anchored surface protein of *S. aureus*, which functions to provide for bacterial evasion from the innate and adaptive immune responses of the host to be infected. Protein A can bind immunoglobulins at their Fc portion, can interact with the V_H3 domain of B cell receptors in appropriately stimulating B cell proliferation and apoptosis, can bind von Willebrand factor A1 domains to activate intracellular clotting, and can also bind to the TNF Receptor-1 to contribute to the pathogenesis of staphylococcal pneumonia.

[0113] The majority of *S. aureus* strains express the structural gene for Protein A (SpA), a well characterized virulence factor whose cell wall anchored surface protein product (SpA) encompasses five highly homologous immunoglobulin binding domains designated E, D, A, B, and C. The immunoglobulin domains, which display ~80% identity at the amino acid level, are 56 to 61 residues in length, and are organized as tandem repeats. Each of the immunoglobulin binding domains is composed of anti-parallel α -helices that assemble into a three helix bundle and bind the Fc domain of immunoglobulin G (IgG), the V_H3 heavy chain (Fab) of IgM, the von Willebrand factor at its A1 domain, and the tumor necrosis factor α (TNF- α) receptor 1 (TNFR1).

[0114] SpA impedes neutrophil phagocytosis of staphylococci through binding the Fc component of IgG. Additionally, SpA is able to activate intravascular clotting via binding to von Willebrand factor A1 domains. Plasma proteins, such as fibrinogen and fibronectin act as bridges between staphylococci (ClfA and ClfB) and the platelet integrin GPIIb/IIIa, an activity that is supplemented through SpA association with vWF A1, which allows staphylococci to capture platelets via the GPIb- α platelet receptor. SpA also binds TNFR1, and this interaction contributes to the pathogenesis of staphylococcal pneumonia. SpA activates proinflammatory signaling through TNFR1 mediated activation of TRAF2, the p38/c-Jun kinase, mitogen

activated protein kinase (MAPK), and the Rel-transcription factor NF- κ B. SpA binding further induces TNFR1 shedding, an activity that appears to require the TNF-converting enzyme (TACE). Each of the disclosed activities are mediated through the five IgG binding domains and can be perturbed by the same amino acid substitutions, initially defined by their

requirement for the interaction between Protein A and human IgG1 (Cedergren et al., (1993)).

[0115] SpA also functions as a B cell superantigen by capturing the Fab region of V_H3 bearing IgM, the B cell receptor. Following intravenous challenge, staphylococcal SpA mutations show a reduction in staphylococcal load in organ tissues and dramatically diminished ability to form abscesses.

[0116] In any embodiment, the SpA polypeptide of the immunogenic composition is a wildtype (non-variant) SpA polypeptide. In any embodiment, the SpA polypeptide comprises at least one SpA A, B, C, D, or E IgG domain. In any embodiment, the SpA polypeptide comprises at least a SpA A domain. In any embodiment, the SpA A domain comprises an amino acid sequence of SEQ ID NO: 55 or 48. In any embodiment, the SpA polypeptide comprises at least a SpA B domain. In any embodiment, the SpA B domain comprises an amino acid sequence of SEQ ID NO: 56 or 49. In any embodiment, the SpA polypeptide comprises at least a SpA C domain. In any embodiment, the SpA C domain comprises an amino acid sequence of SEQ ID NO: 57 or 50. In any embodiment, the SpA polypeptide comprises at least a SpA D domain. In any embodiment, the SpA D domain comprises an amino acid sequence of SEQ ID NO: 58 or 51. In any embodiment, the SpA polypeptide comprises at least a SpA E domain. In any embodiment, the SpA E domain comprises an amino acid sequence of SEQ ID NO: 59 or 52. In any embodiment, the SpA polypeptide comprises at least two of the SpA IgG domains, at least three of the SpA IgG domains, at least four of the SpA IgG domains, or all five of the SpA IgG domains. In any embodiment, the SpA polypeptide comprises an amino acid sequence of SEQ ID NO: 53 or a sequence having 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% sequence identity to SEQ ID NO: 53. Exemplary SpA domains and full-length sequences are provided in Table 3 below.

[0117] In any embodiment, the SpA polypeptide of the immunogenic composition is a SpA variant polypeptide. As referred to herein, the terms “Protein A variant,” “SpA variant,” “Protein A variant polypeptide,” and “SpA variant polypeptide” refer to a polypeptide including a SpA IgG domain having at least one amino acid substitution that disrupts the binding to Fc and V_H3. In certain embodiments, the SpA variant polypeptide includes a variant A domain, a variant B domain, a variant C domain, a variant D domain, and/or a variant E

domain. Suitable SpA variant polypeptides include those variants and fragments thereof that are non-toxic and stimulate an immune response against staphylococcus bacteria Protein A and Protein A-like proteins and/or bacteria expressing the same.

[0118] Described herein are SpA variant polypeptides that do not bind to

immunoglobulins and, therefore, are non-cytotoxic variants of the wildtype SpA polypeptide. The SpA variant polypeptides are non-toxic and stimulate humoral immune responses to protect against staphylococcal infection and disease.

[0119] In any embodiment, the SpA variant polypeptide of the immunogenic composition is a full-length SpA variant comprising at least one variant E, D, A, B, or C

domain. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:60 or 61. In any embodiment, the SpA variant polypeptide comprises an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:54.

[0120] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a fragment of the full-length SpA polypeptide. The SpA variant polypeptide fragment can comprise 1, 2, 3, 4, 5, or more IgG binding domains. The IgG binding domains can, for example, be 1, 2, 3, 4, 5, or more variant A, B, C, D, and/or E domains.

[0121] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises 1, 2, 3, 4, 5, or more variant A domains. In any embodiment, the SpA variant polypeptide comprises 1, 2, 3, 4, 5, or more variant B domains. In any embodiment, the SpA variant polypeptide comprises 1, 2, 3, 4, 5, or more variant C domains. In any embodiment, the SpA variant polypeptide comprises 1, 2, 3, 4, 5, or more variant D domains. In any embodiment, the SpA variant polypeptide comprises 1, 2, 3, 4, 5, or more variant E domains.

[0122] In any embodiment, the variant A domain of the SpA variant polypeptide, for example, comprises one or more amino acid substitutions within the amino acid sequence of SEQ ID NO: 55 or 48. The variant B domain, for example, comprises one or more amino acid substitutions within the amino acid sequence of SEQ ID NO: 56 or 49. The variant C domain, for example, comprises one or more amino acid substitutions within the amino acid sequence of

SEQ ID NO: 57 or 50. The variant D domain, for example, comprises one or more amino acid substitutions within the amino acid sequence of SEQ ID NO: 58 or 51. The variant E domain, for example, comprises one or more amino acid substitutions within the amino acid sequence of SEQ ID NO: 59 or 52.

5 **[0123]** In certain embodiments, the SpA variant polypeptide of the immunogenic composition comprises variant E, D, A, B, and/or C domains, which comprise an amino acid sequence having 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:59 or 52, SEQ ID NO:58 or 51, SEQ ID NO:55 or 48, SEQ ID NO:56 or 49, and SEQ ID NO:57 or 50, respectively.

10 **[0124]** In any embodiment, the SpA variant polypeptide comprises a variant E domain comprising a substitution at amino acid position 6, 7, 33, and/or 34 of SEQ ID NO: 59. In any embodiment, the SpA variant polypeptide comprises a variant D domain comprising a substitution at amino acid position 9, 10, 36, and/or 37 of SEQ ID NO:58. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant A domain
15 comprising a substitution at amino acid position 7, 8, 34, and/or 35 of SEQ ID NO:55. In any embodiment, the SpA variant polypeptide comprises a variant B domain comprising a substitution at amino acid position 7, 8, 34, and/or 35 of SEQ ID NO:56. In any embodiment, the SpA variant polypeptide comprises a variant C domain comprising a substitution at amino acid position 7, 8, 34, and/or 35 of SEQ ID NO:57. Amino acid substitutions in variant E, D,
20 A, B, and/or C domains are described in WO2011/005341 and WO2020232471, which are hereby incorporated by reference in their entirety.

[0125] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises one or more amino acid substitutions in an IgG Fc binding sub-domain of the SpA domain D, and/or at corresponding amino acid positions in the other IgG domains.
25 The one or more amino acid substitutions can disrupt or decrease the binding of the SpA variant polypeptide to the IgG Fc. In any embodiment, the SpA variant polypeptide further comprises one or more amino acid substitutions in a V_{H3} binding sub-domain of the SpA domain D, and/or at corresponding amino acid positions in the other IgG domains. The one or more amino acid substitutions can disrupt or decrease binding to V_{H3} .

30 **[0126]** The aforementioned amino acid substitutions in SpA domain D (i.e., substitutions in the IgG Fc sub-domain binding region or V_{H3} binding sub-domain region) can be incorporated into the SpA A, B, C, and/or E domains at corresponding positions of each domain. Corresponding positions are defined by an alignment of the SpA domain D with SpA domains A, B, C, and/or E to determine which residues of SpA domains A, B, C, and/or E

correspond to the variant SpA D residues. For example, an amino acid substitution at the glutamine residue at position 9 in SEQ ID NO: 58 of SpA domain D corresponds to the glutamine residue at position 7 in SEQ ID NO: 55 of SpA domain A, the glutamine residue at position 7 in SEQ ID NO: 56 of SpA domain B, the glutamine residue at position 7 in SEQ ID NO: 57 of SpA domain C, and the glutamine residue at position 6 in SEQ ID NO: 59 of SpA domain E. Thus, the identified amino acid variations described herein can be universally applied to the corresponding amino acid residues of any SpA domain amino acid sequence known now or in the future.

[0127] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises (a) one or more amino acid substitutions in an IgG Fc binding sub-domain of the SpA domain D, and/or at corresponding amino acid positions in the other IgG domains; and (b) one or more amino acid substitutions in a V_H3 binding sub-domain of the SpA domain D, and/or at corresponding amino acid positions in the other IgG domains. The one or more amino acid substitutions reduce the binding of the SpA variant polypeptide to an IgG Fc and V_H3 such that the SpA variant polypeptide has reduced or eliminated toxicity in a host organism.

[0128] In any embodiment, the amino acid residues F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 of the IgG Fc binding sub-domain of SpA D domain of SEQ ID NO: 58 are modified or substituted such that binding to IgG Fc is reduced or eliminated. In any embodiment, corresponding modifications are incorporated in SpA A, B, C, and/or E domains. Corresponding positions are defined by an alignment of the SpA domain D with SpA domains A, B, C, and/or E to determine the residues in SpA domains A, B, C, and/or E that correspond to the residues of interest in SpA domain D.

[0129] In any embodiment, the amino acid residues Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 of the V_H3 binding sub-domain of SpA D domain of SEQ ID NO: 58 are modified or substituted such that binding to V_H3 is reduced or eliminated. Corresponding modifications can be incorporated in SpA A, B, C, and/or E domains.

[0130] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more variant D domains. The variant D domains can comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acid residue substitutions or modifications. The amino acid residue substitutions or modifications can, for example, occur at amino acid residue F5, Q9, Q10, S11, F13, Y14, L17, N28, I31, and/or K35 of the IgG Fc binding sub-domain of the SpA domain D (SEQ ID NO: 58) and/or at amino acid residue Q26, G29, F30, S33, D36, D37, Q40, N43, and/or E47 of the V_H3 binding sub-domain of the SpA

domain D (SEQ ID NO: 58). In any embodiment, the amino acid residue substitution or modification is at amino acid residues Q9 and Q10 of SEQ ID NO: 58. In any embodiment, the amino acid residue substitution or modification is at amino acid residues D36 and D37 of SEQ ID NO: 58. Amino acid substitutions in variant A, B, C, D, and/or E domains are described in

5 WO2011/005341, which is incorporated by reference herein in its entirety.

[0131] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90% (but not 100%) sequence identity to SEQ ID NO: 53 or 72. In any embodiment, the SpA variant polypeptide comprises an amino acid sequence having 91%, 92%, 93%, 94%,

10 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO: 53 or 72 or a fragment of at least n consecutive amino acids of SEQ ID NO: 53 or 72, wherein n is at least 7, at least 8, at least 10, at least 20, at least 30, at least 40, at least 50, at least 75, at least 100, at least 125, at least 150, at least 175, at least 200, at least 225, at least 250, at least 275, at least 300, at least 325, at least 350, at least 375, at least 400, or at least 425 amino acids. In any embodiment, the SpA variant

15 polypeptide can comprise a deletion of one or more amino acids from the carboxy (C)-terminus (e.g., at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acids) and/or a deletion of one or more amino acids from the amino (N)-terminus (e.g., at least 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, or 35 amino acids) of SEQ ID NO: 72. In any embodiment, the final 35 C-terminal amino acids are deleted. In certain embodiments, the first 36 N-terminal amino acids are deleted. In any

20 embodiment, the SpA variant polypeptide comprises amino acids residues 37 to 327 of SEQ ID NO: 72.

[0132] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises all five SpA IgG binding domains, which arranged from the N- to C-terminus comprise in order the E domain, D domain, A domain, B domain, and C domain. In

25 any embodiment, the SpA variant polypeptide comprises consecutively the E, D, A, B, and C domains of SpA. In any embodiment, the SpA variant polypeptide comprises 1, 2, 3, 4, or 5 of the natural E, D, A, B, and/or C domains. In embodiments in which 1, 2, 3, 4, or 5 of the natural domains are deleted, the SpA variant polypeptide can prevent the excessive B cell expansion and apoptosis which can occur if SpA functions as a B cell superantigen. In any

30 embodiment, the SpA variant polypeptide comprises only the SpA E domain. In any embodiment, the SpA variant polypeptide comprises only the SpA D domain. In any embodiment, the SpA variant polypeptide comprises only the SpA A domain. In any embodiment, the SpA variant polypeptide comprises only the SpA B domain. In any embodiment, the SpA variant polypeptide comprises only the SpA C domain.

[0133] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises mutations of at least one of eleven (11) dipeptide sequence repeats relative to SEQ ID NO: 72 (e.g., a QQ dipeptide repeat and/or a DD dipeptide repeat). By way of an example, the SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO:73, wherein the XX dipeptide repeats at amino acid positions 7 and 8, 34 and 35, 60 and 61, 68 and 69, 95 and 96, 126 and 127, 153 and 154, 184 and 185, 211 and 212, 242 and 243, and 269 and 270 are substituted to reduce the affinity of the SpA variant polypeptide for immunoglobulins. Useful dipeptide substitutions for a Gln-Gln (QQ) dipeptide can include, but are not limited to, a Lys-Lys (KK), an Arg-Arg (RR), an Arg-Lys (RK), a Lys-Arg (KR), an Ala-Ala (AA), a Ser-Ser (SS), a Ser-Thr (ST), and a Thr-Thr (TT) dipeptide. Preferably, a QQ dipeptide is substituted with a KR dipeptide. Useful dipeptide substitutions for an Asp-Asp (DD) dipeptide can include, but are not limited to, an Ala-Ala (AA), a Lys-Lys (KK), an Arg-Arg (RR), a Lys-Arg (KR), a His-His (HH), and a Val-Val (VV) dipeptide. The dipeptide substitutions can, for example, decrease the affinity of the SpA variant polypeptide for the Fc portion of the human IgG and the Fab portion of VH3-containing human B cell receptors.

[0134] Thus, in any embodiment, the SpA variant polypeptide of the immunogenic composition can comprise SEQ ID NO:78, wherein one or more, preferably all 11 of the XX dipeptide repeats are substituted with amino acids that differ from the corresponding dipeptides of SEQ ID NO:72. In any embodiment, the SpA variant polypeptide comprises SEQ ID NO:79, wherein the amino acid doublet at positions 60 and 61 are Lys and Arg (K and R), respectively. In any embodiment, the SpA variant polypeptide comprises SEQ ID NO: 80 or SEQ ID NO: 81. In certain embodiments, the SpA variant polypeptide comprises SEQ ID NO: 75, wherein a preferred example of SEQ ID NO: 75 is SEQ ID NO: 76 or SEQ ID NO: 77 (SEQ ID NO: 77 is SEQ ID NO: 76 with an N-terminal methionine).

[0135] In any embodiment, the SpA variant polypeptide N-terminus comprises a deletion of the first 36 amino acids of SEQ ID NO:72, and the C-terminus comprises a deletion of the last 35 amino acids of SEQ ID NO:72. The SpA variant polypeptide comprising an N-terminal deletion of 36 amino acids of SEQ ID NO:72 and a C-terminal deletion of 35 amino acids of SEQ ID NO:72 can further comprise a deletion of the fifth Ig-binding domain (i.e., downstream of Lys-327 of SEQ ID NO:72). This SpA variant comprises the amino acid sequence of SEQ ID NO:73, wherein the XX dipeptides can be substituted with amino acids, such that the amino acids differ from the corresponding dipeptide sequences in SEQ ID NO:72. In any embodiment, the SpA variant polypeptide comprises SEQ ID NO:74.

[0136] In any embodiment, as noted above, a SpA variant polypeptide of the immunogenic composition comprises 1, 2, 3, or 4 of the natural A, B, C, D, and/or E domains. For example, a SpA variant polypeptide may comprise only the SpA E domain but not the D, A, B, or C domains. Thus, the SpA variant polypeptide can comprise a variant SpA E domain, wherein the SpA E domain comprises a substitution in at least one amino acid residue of SEQ ID NO: 83. The substitution can, for example, be at amino acid positions 60 and 61 of SEQ ID NO: 83. In any embodiment, the SpA variant polypeptide can comprise SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO:81, or SEQ ID NO:82. In any embodiment, the SpA variant polypeptide can comprise SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, or SEQ ID NO:82 with at least one amino acid substitution. SpA variant polypeptides are described in WO2015/144653, which is incorporated by reference herein in its entirety.

[0137] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid substitution at amino acids 43Q, 44Q, 96Q, 97Q, 162Q, 163Q, 220Q, 221Q, 278Q, and 279Q of SEQ ID NO:84. The amino acid substitution at amino acids 43Q, 44Q, 96Q, 97Q, 162Q, 163Q, 220Q, 221Q, 278Q, and 279Q of SEQ ID NO:84 can, for example, be a lysine (K) or an arginine (R) substitution. In certain embodiments, the SpA variant polypeptide comprises an amino acid substitution at amino acids 70D, 71D, 131D, 132D, 189D, 190D, 247D, 248D, 305D, and 306D of SEQ ID NO:84. The amino acid substitution at amino acids 70D, 71D, 131D, 132D, 189D, 190D, 247D, 248D, 305D, and 306D of SEQ ID NO:84 can, for example, be an alanine (A) or a valine (V) substitution. In certain embodiments, the SpA variant polypeptide can be selected from SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87 and SEQ ID NO: 100. SpA variant polypeptides are described in US2016/0304566, which is incorporated by referenced herein in its entirety.

[0138] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant A domain, for example, a variant A domain comprising an amino acid sequence of SEQ ID NO:62, 67, 88 or 93, or an amino acid sequence having at least 90% identity to any one of the amino acid sequences of SEQ ID NO:62, 67, 88 or 93. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant B domain, for example, a variant B domain comprising an amino acid sequence of SEQ ID NO:63, 68, 89, or 94, or an amino acid sequence having at least 90% identity to any one of the amino acid sequences of SEQ ID NO:63, 68, 89, or 94. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant C domain, for example, a variant C domain comprising an amino acid sequence of SEQ ID NO:64, 69, 90, or 95, or an amino acid sequence having at least 90% identity to any one of the amino acid sequences of

SEQ ID NO:64, 69, 90, or 95. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant D domain, for example, a variant D domain comprising an amino acid sequence of SEQ ID NO:66, 71, 91, or 96, or an amino acid sequence having at least 90% identity to any one of the amino acid sequences of SEQ ID

NO:66, 71, 91, or 96. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant E domain, for example, a variant E domain comprising an amino acid sequence of SEQ ID NO:65, 70, 92, or 97, or an amino acid sequence having at least 90% identity to any one of the amino acid sequences of SEQ ID NO:65, 70, 92, or 97.

[0139] In any embodiment, the variant A domain of the SpA variant polypeptide of the immunogenic composition can, for example, comprise an amino acid sequence of SEQ ID NO:62. The variant B domain can, for example, comprise an amino acid sequence of SEQ ID NO:63. The variant C domain can, for example, comprise an amino acid sequence of SEQ ID NO:64. The variant D domain can, for example, comprise an amino acid sequence of SEQ ID NO:66. The variant E domain can, for example, comprise an amino acid sequence of SEQ ID NO:65.

[0140] In any embodiment, the SpA variant polypeptide of the immunogenic composition can comprise a variant A, B, C, D, and E domain, which can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:62 or 67, SEQ ID NO:63 or 68, SEQ ID NO:64 or 69, SEQ ID NO:66 or 71, and SEQ ID NO:65 or 70, respectively.

[0141] In any embodiment, the SpA variant polypeptide of the immunogenic composition can comprise a variant A, B, C, D, and E domain, which can comprise an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to SEQ ID NO:88 or 93, SEQ ID NO:89 or 94, SEQ ID NO:90 or 95, SEQ ID NO:91 or 96 and SEQ ID NO:92 or 97, respectively.

[0142] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a variant D domain, where the variant D domain comprises a substitution at amino acid positions corresponding to positions 9, 10, and/or 33 of SEQ ID NO:58.

[0143] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises (i) lysine substitutions for glutamine amino acid residues in each of

SpA A-E domains at the amino acid positions corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58); and (ii) a glutamate substitution for a serine amino acid residue in each of SpA A-E domains at the amino acid position corresponding to position 33 of SpA D domain (SEQ ID NO:58). The SpA variant polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood and/or activate basophils. By not detectably crosslinking IgG and IgE in blood and/or activating basophils, the SpA variant polypeptide does not pose a significant safety or toxicity issue to human patients and/or does not pose a significant risk of anaphylactic shock in a human patient.

[0144] In any embodiment, the K_A binding affinity of the SpA variant polypeptide described herein for V_{H3} from human IgG is reduced as compared to a SpA variant polypeptide (SpA_{KKAA}) consisting of lysine substitutions for glutamine residues in each of SpA A-E domains corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58) and alanine substitutions for aspartic acid in SpA A-E domains corresponding to positions 36 and 37 of SpA D domain (SEQ ID NO:58). The SpA variant polypeptide consisting of glutamine to lysine substitutions in each of domains A-E at amino acid positions corresponding to positions 9 and 10 of domain D (SEQ ID NO: 58), and aspartic acid to alanine substitutions in each of domains A-E at amino acid positions corresponding to positions 36 and 37 of domain D for each is used as a comparator and is named SpA_{KKAA}. The SpA_{KKAA} variant polypeptide has an amino acid sequence of SEQ ID NO:54. In certain embodiments, the SpA variant polypeptide has a K_A binding affinity for V_{H3} from human IgG that is reduced by at least two-fold (2-fold) as compared to SpA_{KKAA}. In certain embodiments, the SpA variant polypeptide of the immunogenic composition has a K_A binding affinity for V_{H3} from human IgG that is reduced at least 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3-fold or more or any value in between as compared to SpA_{KKAA}. In certain embodiments, the SpA variant polypeptide of the immunogenic composition has a K_A binding affinity for V_{H3} from human IgG that is reduced at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300% or more or any value in between as compared to SpA_{KKAA}. In certain embodiments, the SpA variant polypeptide of the immunogenic composition has a K_A binding affinity for V_{H3} from human IgG that is less than about $1 \times 10^5 \text{ M}^{-1}$. In certain embodiments, the SpA variant polypeptide of the immunogenic composition has a K_A binding affinity for V_{H3} from human IgG that is less than about 3, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2.0, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1.0, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, or $0.1 \times 10^5 \text{ M}^{-1}$ or any value in between. In any embodiment, the SpA variant polypeptide of the immunogenic

composition does not have substitutions in any of the SpA A-E domains corresponding to positions 36 and 37 of SpA D domain (SEQ ID NO: 58).

[0145] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises (i) lysine substitutions for glutamine amino acid residues in each of SpA A-E domains at positions corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58); and (ii) a glutamate substitution for a serine amino acid residue in each of SpA A-E domains at positions corresponding to position 33 of SpA D domain (SEQ ID NO:58). In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA E domain having an amino acid sequence of SEQ ID NO: 65 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 65. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA D domain having an amino acid sequence of SEQ ID NO: 66 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 66. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA A domain having an amino acid sequence SEQ ID NO: 62 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 62. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA B domain having an amino acid sequence SEQ ID NO: 63 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 63. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA C domain having an amino acid sequence SEQ ID NO: 64 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 64. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence of SEQ ID NO: 60 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 60. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence of SEQ ID NO: 60.

[0146] In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises (i) lysine substitutions for glutamine amino acid residues in each of SpA A-E domains at positions corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58); and (ii) a threonine substitution for a serine amino acid residue in each of SpA A-E domains at positions corresponding to position 33 of SpA D domain (SEQ ID NO:58). In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA E domain having an amino acid sequence SEQ ID NO: 70 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 70. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA D domain having an amino acid sequence SEQ ID NO: 71 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 71. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA A domain having an amino acid sequence SEQ ID NO: 67 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 67. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA B domain having an amino acid sequence SEQ ID NO: 68 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 68. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises a SpA C domain having an amino acid sequence SEQ ID NO: 69 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 69. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence of SEQ ID NO: 61 or an amino acid sequence having at least 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%, or at least 99% identical to SEQ ID NO: 61. In any embodiment, the SpA variant polypeptide of the immunogenic composition comprises an amino acid sequence of SEQ ID NO: 61.

[0147] The SpA variant polypeptide does not, relative to a negative control, detectably crosslink IgG and IgE in blood and/or activate basophils. By not detectably crosslinking IgG and IgE in blood and/or activating basophils, the SpA variant polypeptide does not pose a

significant safety or toxicity issue to human patients or does not pose a significant risk of anaphylactic shock in a human patient. SpA variant polypeptides suitable for use in the compositions and methods disclosed herein are described in WO2020232471, which is hereby incorporated by referenced herein in its entirety.

- 5 [0148] Table 3 below provides exemplary SpA polypeptide amino acid sequences of the immunogenic composition as disclosed herein.

Table 3. Exemplary SpA Polypeptide Amino Acid Sequences

SEQ ID NO	Name	Description
48	WT SpA A Domain long	ADNNFNKEQQ NAFYEILNMPNLNEEQRNGF IQSLKDDPSQ SANLLSEAKKLNESQAPK
49	WT SpA B Domain long	ADNKFNKEQQ NAFYEILHLPNLNEEQRNGF IQSLKDDPSQ SANLLAEAKKLNDQAQPK
50	WT SpA C Domain long	ADNKFNKEQQ NAFYEILHLPNLTEEQRNGF IQSLKDDPSV SKEILAEAKKLNDQAQPK
51	WT SpA D Domain long	ADAQQNNFNK DQQSAFYEILNMPNLNEAQR NGFIQSLKDD PSQSTNVLGEAKKLNESQAPK
52	WT SpA E Domain long	AQHDEAQQNA FYQVLNMPNLNADQRNGFIQ SLKDDPSQSA NVLGEAQKLNDQAQPK
53	Full length WT SpA	AQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVL GEAQKLNDQAQPKADAQQNNFNKDQQSAFYEILNMPNLNEAQR NGFIQSLKDDPSQSTNVLGEAQQKLNESQAPKADNNFNKEKKNAF YEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSEAQQKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLL AEAQQKLNDQAQPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKDDPSVSKEILAEAKKLNDQAQPK
54	Full length SpA _{KKAA}	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANVL GEAQKLNDQAQPKADAQQNNFNKDKKSAFYEILNMPNLNEAQR NGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNAF YEILNMPNLNEEQRNGFIQSLKAAPSQSANLLSEAKKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSANLL AEAKKKLNDQAQPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKAAPSVSKEILAEAKKLNDQAQPK
55	WT SpA A Domain	NNFNKEQQNAFYEILNMPNLNEEQRNGFIQSLKDDPSQSANLLSE AKKLNES
56	WT SpA B Domain	NKFNKEQQNAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAE AKKLND
57	WT SpA C Domain	NKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSKEILAEA KKLND
58	WT SpA D Domain	QQNNFNKDQQSAFYEILNMPNLNEAQRNGFIQSLKDDPSQSTNVL GEAKKLNES
59	WT SpA E Domain	QHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLG EAQKLND

SEQ ID NO	Name	Description
60	SpAS33E	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQELKDDPSQSANVL GEAQKLNDSSQAPKADAQQNNFNKDKKSAFYEILNMPNLNEAQR NGFIQELKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNAF YEILNMPNLNEEQRNGFIQELKDDPSQSANLLSEAKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQELKDDPSQSANLL AEAKKLNDASQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QELKDDPSVSKEILAEAKKLNDASQAPK
61	SpAS33T	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQTLKDDPSQSANVL GEAQKLNDSSQAPKADAQQNNFNKDKKSAFYEILNMPNLNEAQR NGFIQTLKDDPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNAF YEILNMPNLNEEQRNGFIQTLKDDPSQSANLLSEAKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQTLKDDPSQSANLL AEAKKLNDASQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QTLKDDPSVSKEILAEAKKLNDASQAPK
62	SpAS _{33E} A domain	NNFNKEKKNAFYEILNMPNLNEEQRNGFIQELKDDPSQSANLLSE AKKLNES
63	SpAS _{33E} B Domain	NKFNKEKKNAFYEILHLPNLNEEQRNGFIQELKDDPSQSANLLAE AKKLNDAS
64	SpAS _{33E} C Domain	NKFNKEKKNAFYEILHLPNLTEEQRNGFIQELKDDPSVSKEILAEA KKLNDAS
65	SpAS _{33E} E Domain	QHDEAKKNAFYQVLNMPNLNADQRNGFIQELKDDPSQSANVLG EAQKLNDSS
66	SpAS _{33E} D Domain	QQNNFNKDKKSAFYEILNMPNLNEAQRNGFIQELKDDPSQSTNVL GEAKKLNES
67	SpAS _{33T} A domain	NNFNKEKKNAFYEILNMPNLNEEQRNGFIQTLKDDPSQSANLLSE AKKLNES
68	SpAS _{33T} B domain	NKFNKEKKNAFYEILHLPNLNEEQRNGFIQTLKDDPSQSANLLAE AKKLNDAS
69	SpAS _{33T} C domain	NKFNKEKKNAFYEILHLPNLTEEQRNGFIQTLKDDPSVSKEILAEA KKLNDAS
70	SpAS _{33T} E domain	QHDEAKKNAFYQVLNMPNLNADQRNGFIQTLKDDPSQSANVLG EAQKLNDSS
71	SpAS _{33T} D domain	QQNNFNKDKKSAFYEILNMPNLNEAQRNGFIQTLKDDPSQSTNVL GEAKKLNES
72	SpA long	MKKKNIYSIRKLGVGIVSLGTLISGGVTPAANAAQHDEAQQN AFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLNDSSQ APKADAQQNNFNKDKKSAFYEILNMPNLNEAQRNGFIQSLKDDP SQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNE EQRNGFIQSLKDDPSQSANLLSEAKKLNESQAPKADNKFNKEQQ NAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLNDASQ APKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSK EILAEAKKLNDASQAPKEEDNNKPGKEDNNKPGKEDNNKPGKED NNKPGKEDNNKPGKEDGNNKPGKEDNNKPGKEDNNKPGKEDNNK KPGKEDGNNKPGKEDGNNKPGKEDGNNKPGKEDGNNKPGKEDNNK TTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQAL PETGEENPFIGTTVFGLSLALGAALLAGRRREL

SEQ ID NO	Name	Description
73	SpAXX	AQHDEAXXNAFYQVLNMPNLNADQRNGFIQSLKXXPSQSANVL GEAQKLNDSDQAPKADAQQNNFNKDXXSIFYEILNMPNLNEAQR NGFIQSLKXXPSQSTNVLGEAKKLNESQAPKADNNFNKEXXNAF YEILNMPNLNEEQRNGFIQSLKXXPSQSANLLSEAKKLNESQAPK ADNKFNKEXXNAFYEILHLPNLNEEQRNGFIQSLKXXPSQSANLL AEAKKLNDADQAPKADNKFNKEXXNAFYEILHLPNLTEEQRNGFI QSLKXXPSVSKEILAEAKKLNDADQAPK
74	SpAkkAA	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANVL GEAQKLNDSDQAPKADAQQNNFNKDKKSIFYEILNMPNLNEAQR NGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNF YEILNMPNLNEEQRNGFIQSLKAAPSQSANLLSEAKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSANLL AEAKKLNDADQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKAAPSVSKEILAEAKKLNDADQAPK
75	SpAkR	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANVL GEAQKLNDSDQAPKADAXNNFNKDKKSIFYEILNMPNLNEAQR NGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNF YEILNMPNLNEEQRNGFIQSLKAAPSQSANLLSEAKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSANLL AEAKKLNDADQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKAAPSVSKEILAEAKKLNDADQAPK
76	SpAkR	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANVL GEAQKLNDSDQAPKADAKRNNFNKDKKSIFYEILNMPNLNEAQR NGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNF YEILNMPNLNEEQRNGFIQSLKAAPSQSANLLSEAKKLNESQAPK ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSANLL AEAKKLNDADQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKAAPSVSKEILAEAKKLNDADQAPK
77	SpAkR	MAQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANV LGEAQKLNDSDQAPKADAKRNNFNKDKKSIFYEILNMPNLNEAQ RNGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEKKNF FYEILNMPNLNEEQRNGFIQSLKAAPSQSANLLSEAKKLNESQAP KADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSANL LAEAKKLNDADQAPKADNKFNKEKKNAFYEILHLPNLTEEQRNGFI QSLKAAPSVSKEILAEAKKLNDADQAPK
78	SpAkR	AQHDEAXXNAFYQVLNMPNLNADQRNGFIQSLKXXPSQSANVL GEAQKLNDSDQAPKADAXNNFNKDXXSIFYEILNMPNLNEAQR NGFIQSLKXXPSQSTNVLGEAKKLNESQAPKADNNFNKEXXNAF YEILNMPNLNEEQRNGFIQSLKXXPSQSANLLSEAKKLNESQAPK ADNKFNKEXXNAFYEILHLPNLNEEQRNGFIQSLKXXPSQSANLL AEAKKLNDADQAPKADNKFNKEXXNAFYEILHLPNLTEEQRNGFI QSLKXXPSVSKEILAEAKKLNDADQAPK
79	SpAkR E domain	AQHDEAXXNAFYQVLNMPNLNADQRNGFIQSLKXXPSQSANVL GEAQKLNDSDQAPKADAXNNFNKD
80	SpAkR E domain	AQHDEAXXNAFYQVLNMPNLNADQRNGFIQSLKXXPSQSANVL GEAQKLNDSDQAPKADAKRNNFNKD
81	SpAkR E domain	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANVL GEAQKLNDSDQAPKADAKRNNFNKD

SEQ ID NO	Name	Description
82	SpA E domain	MAQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQSANV LGEAQKLND SQAPKADAKRNNFNKD
83	SpA E domain	AQHDEAQQNAFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVL GEAQKLND SQAPKADAQQNNFNKD
84	SpA 252	MKKKNIYSIRKLGVGIA SVTLGTLLISGGVTPAANAAQHDEAQQN AFYQVLNMPNLNADQRNGFIQSLKDDPSQSANVLGEAQKLND SQ APKADAQQNKFNKDQQSAFYEILNMPNLNEEQRNGFIQSLKDDP SQSTNVLGEAKKLNESQAPKADNNFNKEQQNAFYEILNMPNLNE EQRNGFIQSLKDDPSQSANLLAEAKKLNESQAPKADNKFNKEQQ NAFYEILHLPNLNEEQRNGFIQSLKDDPSQSANLLAEAKKLND AQ APKADNKFNKEQQNAFYEILHLPNLTEEQRNGFIQSLKDDPSVSK EILAEAKKLND AQAPKEEDNNKPGKEDNNKPGKEDGNKPGKED NKKPGKEDGNKPGKEDNKKPGKEDGNKPGKEDGNKPGKEDGN KPGKEDGNKPGKEDGNKPGKEDGNVHVVKPGDTVNDIAKANG TTADKIAADNKLADKNMIKPGQELVVDKKQPANHADANKAQA L PETGEENPFIGTTVFGLSLALGAALLAGRRREL
85	SpA5 (KKA A)	GPLGSAQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKAAPSQS ANVLGEAQKLND SQAPKADAKKNKFNKDQQSAFYEILNMPNLN EEQRNGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKEK KNAFYEILNMPNLNEEQRNGFIQSLKAAPSQSANLLAEAKKLNES QAPKADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKAAPSQS ANLLAEAKKLND AQAPKADNKFNKEKKNAFYEILHLPNLTEEQR NGFIQSLKAAPSVSKEILAEAKKLND AQAPK
100	SpA5 (RRA A)	GPLGSAQHDEARRNAFYQVLNMPNLNADQRNGFIQSLKAAPSQS ANVLGEAQKLND SQAPKADARRNKFNKDQQSAFYEILNMPNLN EEQRNGFIQSLKAAPSQSTNVLGEAKKLNESQAPKADNNFNKERR NAFYEILNMPNLNEEQRNGFIQSLKAAPSQSANLLAEAKKLNESQ APKADNKFNKEKRRNAFYEILHLPNLNEEQRNGFIQSLKAAPSQSA NLLAEAKKLND AQAPKADNKFNKEKRRNAFYEILHLPNLTEEQRN GFIQSLKAAPSVSKEILAEAKKLND AQAPK
86	SpA5 (KKVV)	GPLGSAQHDEAKKNAFYQVLNMPNLNADQRNGFIQSLKVVP SQS ANVLGEAQKLND SQAPKADAKKNKFNKDQQSAFYEILNMPNLN EEQRNGFIQSLKVVP SQSTNVLGEAKKLNESQAPKADNNFNKEK KNAFYEILNMPNLNEEQRNGFIQSLKVVP SQSANLLAEAKKLNES QAPKADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQSLKVVP SQS ANLLAEAKKLND AQAPKADNKFNKEKKNAFYEILHLPNLTEEQR NGFIQSLKVVP SVSKEILAEAKKLND AQAPK
87	SpA5 (RRVV)	GPLGSAQHDEARRNAFYQVLNMPNLNADQRNGFIQSLKVVP SQS ANVLGEAQKLND SQAPKADARRNKFNKDQQSAFYEILNMPNLN EEQRNGFIQSLKVVP SQSTNVLGEAKKLNESQAPKADNNFNKERR NAFYEILNMPNLNEEQRNGFIQSLKVVP SQSANLLAEAKKLNESQ APKADNKFNKEKRRNAFYEILHLPNLNEEQRNGFIQSLKVVP SQSA NLLAEAKKLND AQAPKADNKFNKEKRRNAFYEILHLPNLTEEQRN GFIQSLKVVP SVSKEILAEAKKLND AQAPK
88	SpA _{S33E} A domain long	ADNNFNKEKKNAFYEILNMPNLNEEQRNGFIQELKDDPSQSANLLSEAK KLNESQAPK
89	SpA _{S33E} B Domain long	ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQELKDDPSQSANLLAEAK KLND AQAPK

SEQ ID NO	Name	Description
90	SpA _{S33E} C Domain long	ADNKFNKEKKNAFYEILHLPNLTEEQRNGFIQELKDDPSVSKEILAEAKK LNDAAQAPK
91	SpA _{S33E} D Domain long	ADAQQNNFNKDKKSAFYEILNMPNLNEAQRNGFIQELKDDPSQSTNVLG E AKKLNESQAPK
92	SpA _{S33E} E Domain long	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQELKDDPSQSANVLGEAQ KLNSQAPK
93	SpA _{S33T} A domain long	ADNNFNKEKKNAFYEILNMPNLNEEQRNGFIQTLKDDPSQSANLLSEAK KLNESQAPK
94	SpA _{S33T} B Domain long	ADNKFNKEKKNAFYEILHLPNLNEEQRNGFIQTLKDDPSQSANLLAEAK KLNDAAQAPK
95	SpA _{S33T} C Domain long	ADNKFNKEKKNAFYEILHLPNLTEEQRNGFIQTLKDDPSVSKEILAEAKK LNDAAQAPK
96	SpA _{S33T} D Domain long	ADAQQNNFNKDKKSAFYEILNMPNLNEAQRNGFIQTLKDDPSQSTNVLG E AKKLNESQAPK
97	SpA _{S33T} E Domain long	AQHDEAKKNAFYQVLNMPNLNADQRNGFIQTLKDDPSQSANVLGEAQ KLNSQAPK

[0149] In accordance with all aspects of the disclosure herein, the LukA variant polypeptide, the LukB polypeptide, and the SpA polypeptide of the immunogenic composition as disclosed herein may further comprise one or more heterologous amino acid sequences.

- 5 Suitable heterologous amino acid sequences include, without limitation, a tag sequences, immunogens, signal sequences, etc. Suitable tag sequences include, without limitation, a polyhistidine-tag, a polyarginine tag, FLAG tag, Step-tag II, ubiquitin tag, a NusA tag, a chitin binding domain, a calmodulin-binding peptide, cellulose-binding domain, Hat-tag, S-tag, SBP, maltose-binding protein, glutathione S-transferase (*see* Terpe K., “Overview of Tag Protein
- 10 Fusions: From Molecular and Biochemical Fundamentals to Commercial Systems,” *Appl. Microbiol. Biotechnol.* 60:523-33 (2003), which is hereby incorporated by reference). Suitable immunogens include, without limitation, a T-cell epitope, a B-cell epitope. Suitable signal sequences include, without limitation, a PelB signal sequence, a Sec signal sequence, a Tat signal sequence, an AmyE signal sequence (*see* Freudl R., “Signal Peptides for Recombinant
- 15 Protein Secretion in Bacterial Expression Systems,” *Microbial Cell Factories* 17:52 (2018), which is hereby incorporated by reference. In some embodiments, the LukA, LukB, and SpA polypeptides as described herein comprise a PelB sequence (MKYLLPTAAAGLLLLAAQPAMA; SEQ ID NO: 23). In some embodiments, the LukA, LukB, and SpA polypeptides as described herein comprise His-tag (e.g., NSAHHHHHHGS;

SEQ ID NO: 24). In some embodiments the SpA, LukA and/or LukB polypeptides therefore as described herein comprise both the aforementioned PelB sequence and His-tag.

***S. aureus* LukA, LukB, and SpA Polynucleotides and Constructs**

[0150] Another aspect of the present disclosure is directed to nucleic acid molecules

5 encoding the LukA variant polypeptides, LukB polypeptides, and SpA polypeptides as described herein, and immunogenic compositions comprising one or more of these nucleic acid molecules. The nucleic acid molecules described herein include isolated polynucleotides, recombinant polynucleotide sequences, portions of expression vectors or portions of linear DNA sequences, including linear DNA sequences used for *in vitro* or *in vivo*
10 transcription/translation, and vectors compatible with prokaryotic and eukaryotic cell expression and secretion of the variant LukA, LukB, and SpA polypeptides as described herein. The polynucleotides of the disclosure may be produced by chemical synthesis such as solid phase polynucleotide synthesis on an automated polynucleotide synthesizer and assembled into complete single or double stranded molecules. Alternatively, the polynucleotides of the
15 disclosure may be produced by other techniques such as PCR followed by routine cloning. Techniques for producing or obtaining polynucleotides of a given sequence are well known in the art.

[0151] In any embodiment, the immunogenic composition disclosed herein comprises a polynucleotide encoding a LukA variant polypeptide. In any embodiment, the polynucleotide
20 encodes the LukA variant comprising a lysine to methionine substitution at the residue corresponding to the lysine at position 83 (Lys83Met) of SEQ ID NO: 25. In any embodiment, a polynucleotide of the present disclosure encodes the variant LukA polypeptide comprising a serine to alanine substitution at the residue corresponding to the serine at position 141 (Ser141Ala) of SEQ ID NO: 25. In any embodiment, a polynucleotide of the present disclosure
25 encodes a variant LukA polypeptide comprising a valine to isoleucine substitution at the residue corresponding to the valine at position 113 (Val113Ile) of SEQ ID NO: 25. In any embodiment, a polynucleotide of the present disclosure encodes a LukA polypeptide comprising a valine to isoleucine substitution at the residue corresponding to the valine at position 193 (Val193Ile) of SEQ ID NO: 25. In any embodiment, a polynucleotide of the
30 present disclosure encodes a variant LukA polypeptide thereof comprising the amino acid substitutions of lysine to methionine, serine to alanine, and valine to isoleucine at residues corresponding to the aforementioned amino acid residues, *i.e.*, Lys803Met, Ser141Ala, Val113Ile, and Val193Ile of SEQ ID NO: 25. In any embodiment, the polynucleotide of the present disclosure encodes a variant LukA polypeptide thereof further comprising the amino

acid substitution corresponding to Glu323Ala, *i.e.*, the polynucleotide encodes a variant LukA comprising substitutions corresponding to the Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala substitutions of SEQ ID NO: 25.

[0152] In one embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding a CC8 LukA variant sequence, *e.g.*, encoding a variant of SEQ ID NO: 1 comprising amino acid substitutions corresponding to Lys80Met, Ser138Ala, Val110Ile, Val190Ile, and Glu320Ala in SEQ ID NO: 1. An exemplary nucleic acid molecule encoding CC8 LukA is provided herein as SEQ ID NO: 101. Accordingly, in any embodiment, an exemplary nucleic acid molecule is a variant of SEQ ID NO: 101, wherein said variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 101.

[0153] In any embodiment, an exemplary nucleic acid molecule of the immunogenic composition is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 3 (LukA CC8 Glu320Ala, Lys80Met, Ser138Ala, Val110Ile, Val190Ile) or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3. An exemplary nucleic acid molecule encoding this LukA CC8 variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 103. In any embodiment, the nucleic acid molecule encoding this LukA CC8 variant comprises the nucleotide sequence of SEQ ID NO: 103.

[0154] In another embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding a CC45 LukA variant sequence, *e.g.*, encoding a variant of SEQ ID NO: 2 comprising amino acid substitutions corresponding to Lys81Met, Ser139Ala, Val111Ile, Val191Ile, and Glu321Ala in SEQ ID NO: 2. An exemplary nucleic acid molecule encoding CC45 LukA is provided herein as SEQ ID NO: 102. Accordingly, in any embodiment, an exemplary nucleic acid molecule is a variant of SEQ ID NO: 102, wherein said variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 102.

[0155] In another embodiment, an exemplary nucleic acid molecule of the immunogenic composition is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 4 (LukA CC45 Glu321Ala, Lys81Met, Ser139Ala, Val111Ile, Val191Ile), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 4. An exemplary nucleic acid molecule encoding this LukA CC45 variant comprises a nucleotide sequence having at

least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 104. In any embodiment, the nucleic acid molecule encoding this LukA CC8 variant comprises the nucleotide sequence of SEQ ID NO: 104.

[0156] In any embodiment, the one or more polynucleotides of the immunogenic composition encode a LukA variant protein or polypeptide comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the polynucleotide encodes a LukA variant protein or polypeptide comprising a tyrosine to cysteine substitution at the amino acid residue corresponding to Tyr74 (Tyr74Cys) of SEQ ID NO: 25, and comprises an asparagine to cysteine substitution at the amino acid residue corresponding to Asp140 (Asp140Cys) of SEQ ID NO: 25. In any embodiment, the polynucleotide encodes a LukA variant protein or polypeptide comprising a glycine to cysteine substitution at the amino acid residue corresponding to Gly149 (Gly149Cys) of SEQ ID NO: 25, and comprises a glycine to cysteine substitution at the amino acid residue corresponding to Gly156 (Gly156Cys) of SEQ ID NO: 25. In any embodiment, the polynucleotide encodes a variant LukA protein or polypeptide comprising amino acid substitutions at each amino acid residue corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the amino acid substitution at each of these amino acid residues is a cysteine residue as described above.

[0157] In any embodiment, the polynucleotide of the immunogenic composition encodes a variant LukA protein or polypeptide comprising amino acid substitution at one or more amino acid residues corresponding to Lys83, Ser141, Val113, Val193, and Glu323 in combination with an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, the polynucleotide encodes a variant LukA protein or polypeptide comprising amino acid substitutions at amino acid residues corresponding to residues Lys83, Ser141, Val113, Val193, and Glu323 and residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 5 (LukA CC8 Glu320Ala, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 5. In any embodiment, an exemplary nucleic acid molecule of the present disclosure is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 6 (LukA CC45 Glu321Ala, Lys81Met, Ser139Ala,

Val111Ile, Val191Ile, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 6.

[0158] In any embodiment, the one or more polynucleotides of the immunogenic composition encodes a LukA variant polypeptide comprising an amino acid substitution or deletion at the amino acid residue corresponding to amino acid residue Thr249 of SEQ ID NO: 25. In any embodiment, the polynucleotide encodes a LukA variant comprising a threonine to valine substitution at this residue corresponding to position 249 of SEQ ID NO: 25. In any embodiment, the polynucleotide of the present disclosure encodes a LukA variant polypeptide comprising the amino acid substitution at the position corresponding to Thr249 in combination with any one of or all of the amino acid substitutions at residues corresponding to Lys83, Ser141, Val113, Val193, Glu323, Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25. In any embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 7 (LukA CC8 Glu320Ala, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, and Thr246Val), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 7. In any embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 8 (LukA CC45 Glu321Ala, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 8.

[0159] In any embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 9 (LukA CC8 Glu320Ala, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val, Tyr71Cys, Asp137Cys, Gly146Cys, and Gly153Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 9. In any embodiment, an exemplary nucleic acid molecule encoding this LukA CC8 variant of SEQ ID NO: 9 comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 105. In any embodiment, the nucleic acid molecule encoding this LukA CC8 variant comprises the nucleotide sequence of SEQ ID NO: 105.

[0160] In any embodiment, an exemplary nucleic acid molecule is a nucleic acid molecule encoding the LukA variant sequence of SEQ ID NO: 10 (LukA CC45 Glu321Ala, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val, Tyr72Cys, Asp138Cys, Gly147Cys,

and Gly154Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 10.

In any embodiment, an exemplary nucleic acid molecule encoding this LukA CC45 variant of SEQ ID NO: 10 comprises a nucleotide sequence having at least 85%, at least 90%, at least

95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 106. In any embodiment, the nucleic acid molecule encoding this LukA CC8 variant comprises the nucleotide sequence of SEQ ID NO: 106.

[0161] In any embodiment, the one or more polynucleotides of the immunogenic composition disclosed herein further encodes a LukB polypeptide as disclosed herein. In any embodiment, the polynucleotide encodes a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 15. An exemplary nucleic acid molecule encoding CC8 LukB is provided herein as SEQ ID NO: 107. Accordingly, in any embodiment, an exemplary nucleic acid molecule is a variant of SEQ ID NO: 107, wherein said variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 107.

[0162] In any embodiment, the polynucleotide encodes a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 16. An exemplary nucleic acid molecule encoding CC45 LukB is provided herein as SEQ ID NO: 108. Accordingly, in any embodiment, an exemplary nucleic acid molecule is a variant of SEQ ID NO: 108, wherein said variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 108.

[0163] In any embodiment, the polynucleotide encodes a variant LukB polypeptide comprising an amino acid substitution or deletion at the amino acid residue corresponding to amino acid residue Val53 of SEQ ID NO: 39. In any embodiment, the amino acid substitution at Val53 comprises a valine to leucine (Val53Leu) substitution.

[0164] In any embodiment, an exemplary polynucleotide of the present disclosure encodes a variant LukB protein or polypeptide of SEQ ID NO: 17 (LukB CC8 V53L), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 17. An exemplary nucleic acid molecule encoding this LukB CC8 V53L variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 109. In any embodiment, the nucleic acid molecule encoding this LukA CC8 variant comprises the nucleotide sequence of SEQ ID NO: 109.

[0165] In any embodiment, an exemplary polynucleotide of the present disclosure encodes a variant LukB protein or polypeptide of SEQ ID NO: 18 (LukB CC45 V53L), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 18. An exemplary nucleic acid molecule encoding this LukB CC45 V53L variant comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to SEQ ID NO: 110. In any embodiment, the nucleic acid molecule encoding this LukA CC45 variant comprises the nucleotide sequence of SEQ ID NO: 110.

[0166] In any embodiment, the polynucleotide of the immunogenic composition encodes a variant LukB protein or polypeptide comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39. In any embodiment, the amino acid substitution at the one or more aforementioned residues introduces one or more cysteine residues capable of forming a disulfide bond to stabilize conformation of the LukAB heterodimer structure. In any embodiment, the polynucleotide encodes a LukB variant protein or polypeptide comprising a glutamic acid to cysteine substitution at the amino acid residue corresponding to Glu45 (Glu45Cys) of SEQ ID NO: 39, and threonine to cysteine substitution at the amino acid residue corresponding to Thr121 (Thr121Cys) of SEQ ID NO: 39. In any embodiment, the polynucleotide encodes a LukB variant protein or polypeptide comprising a glutamic acid to cysteine substitution at the amino acid residue corresponding to Glu109 (Glu109Cys) of SEQ ID NO: 39, and an arginine to cysteine substitution at the amino acid residue corresponding to Arg154 (Arg154Cys) of SEQ ID NO: 39.

[0167] In any embodiment, the polynucleotide of the immunogenic composition encodes a variant LukB protein or polypeptide comprising amino acid substitutions at each amino acid residue corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39. In any embodiment, the amino acid substitutions at each of these amino acid residues involves the introduction of a cysteine residue as described above. In any embodiment, the polynucleotide encodes a variant LukB protein or polypeptide comprising the amino acid sequence of SEQ ID NO: 21 (LukB CC8 Glu45Cys, Glu109Cys, Thr121Cys, and Arg154Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 21. In any embodiment, the polynucleotide encodes a variant LukB protein or polypeptide comprising the amino acid sequence of SEQ ID NO: 22 (LukB CC45 Glu45Cys, Thr122Cys, Glu110Cys,

Arg155Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 22.

[0168] In any embodiment, the polynucleotide of the present disclosure encodes a variant LukB protein or polypeptide comprising an amino acid substitution at the amino acid residue corresponding to Val53 of SEQ ID NO: 39 in combination with an amino acid residue substitution at one or more amino acid residues corresponding to Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 39. In any embodiment, the polynucleotide encodes the variant LukB protein or polypeptide having the amino acid sequence of SEQ ID NO: 19 (LukB CC8 Val53Leu, Glu45Cys, Glu109Cys, Thr121Cys, and Arg154Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 19. In any embodiment, the polynucleotide encodes a variant LukB protein or polypeptide having the amino acid sequence of SEQ ID NO: 20 (LukB CC45 Val53Leu, Glu45Cys, Thr122Cys, Glu110Cys, Arg155Cys), or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 20.

[0169] In any embodiment, an exemplary nucleic acid molecule of the immunogenic composition as described herein encodes a CC45 LukA variant sequence of SEQ ID NO: 4 and a CC45 LukB sequence of SEQ ID NO: 16. An exemplary nucleic acid molecule encoding this LukAB heterodimer (RARPR-15) comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 104 (CC45 LukA variant) operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 108 (CC45 LukB). An exemplary nucleic acid molecule encoding this LukAB heterodimer comprises the nucleotide sequence of SEQ ID NO: 104 operatively coupled to the nucleotide sequence of SEQ ID NO: 108.

[0170] In any embodiment, an exemplary nucleic acid molecule of the present disclosure encodes a CC45 LukA variant sequence of SEQ ID NO: 4 and a CC45 LukB variant sequence of SEQ ID NO: 18. An exemplary nucleic acid molecule encoding this LukAB heterodimer (RARPR-30) comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 104 (CC45 LukA variant) operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 110 (CC45 LukB variant). An exemplary nucleic acid

molecule encoding this LukAB heterodimer comprises the nucleotide sequence of SEQ ID NO: 104 operatively coupled to the nucleotide sequence of SEQ ID NO: 110.

[0171] In any embodiment, an exemplary nucleic acid molecule of the present disclosure encodes a CC8 LukA variant sequence of SEQ ID NO: 3 and a CC8 LukB sequence of SEQ ID NO: 15. An exemplary nucleic acid molecule encoding this LukAB heterodimer (RARPR-32) comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 (CC8 LukA variant) operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 107 (CC8 LukB). An exemplary nucleic acid molecule encoding this LukAB heterodimer comprises the nucleotide sequence of SEQ ID NO: 103 operatively coupled to the nucleotide sequence of SEQ ID NO: 107.

[0172] In any embodiment, an exemplary nucleic acid molecule of the present disclosure encodes a CC8 LukA variant sequence of SEQ ID NO: 3 and a CC45 LukB variant sequence of SEQ ID NO: 18. An exemplary nucleic acid molecule encoding this LukAB heterodimer (RARPR-33) comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 (CC8 LukA variant) operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 110 (CC45 LukB variant). An exemplary nucleic acid molecule encoding this LukAB heterodimer comprises the nucleotide sequence of SEQ ID NO: 103 operatively coupled to the nucleotide sequence of SEQ ID NO: 110.

[0173] In any embodiment, an exemplary nucleic acid molecule of the present disclosure encodes a CC8 LukA variant sequence of SEQ ID NO: 3 and a CC8 LukB variant sequence of SEQ ID NO: 17. An exemplary nucleic acid molecule encoding this LukAB heterodimer (RARPR-34) comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 (CC8 LukA variant) operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 109 (CC8 LukB variant). An exemplary nucleic acid molecule encoding this LukAB heterodimer comprises the nucleotide sequence of SEQ ID NO: 103 operatively coupled to the nucleotide sequence of SEQ ID NO: 109.

[0174] Exemplary LukA and LukB nucleic acid molecule sequences of the present disclosure are provided in Table 4 below.

Table 4. Exemplary LukA and LukB Polynucleotide Sequences

Construct Name	SEQ ID NO:	DNA Sequence
CC8 LukA ^{wt}	101	GCT CAC AAA GAT TCT CAG GAT CAA AAT AAG AAG GAG CAC GTC GAC AAG TCT CAG CAG AAA GAC AAG CGT AAT GTT ACA AAC AAG GAC AAA AAC AGC ACT GCT CCA GAC GAC ATT GGA AAA AAC GGT AAG ATT ACT AAA CGC ACC GAA ACG GTA TAT GAC GAA AAA ACG AAC ATT TTG CAA AAC TTG CAG TTC GAT TTC ATT GAC GAC CCC ACT TAT GAC AAG AAT GTC CTT CTG GTG AAG AAG CAG GGC AGC ATT CAC TCA AAC TTG AAA TTT GAG TCT CAC AAG GAG GAG AAG AAC TCC AAT TGG CTG AAA TAC CCA TCA GAG TAC CAC GTT GAT TTT CAA GTG AAA CGT AAC CGC AAA ACG GAA ATT TTG GAC CAA TTG CCG AAA AAC AAG ATC TCC ACC GCG AAA GTA GAC TCA ACA TTC AGT TAC TCT TCC GGC GGA AAG TTC GAC AGC ACT AAG GGG ATC GGG CGC ACT TCT TCC AAT TCG TAC TCG AAA ACG ATT TCT TAC AAT CAG CAG AAT TAT GAC ACT ATC GCA TCT GGT AAA AAT AAT AAC TGG CAC GTG CAT TGG TCG GTG ATT GCT AAT GAT TTA AAG TAT GGA GGT GAG GTA AAA AAT CGT AAT GAC GAG CTG CTG TTT TAC CGT AAC ACT CGC ATC GCA ACC GTT GAA AAC CCG GAA TTG TCC TTT GCC TCG AAA TAC CGC TAC CCT GCA TTA GTT CGT TCA GGC TTT AAT CCC GAG TTT TTG ACT TAT CTT TCC AAT GAA AAA TCG AAC GAG AAG ACT CAG TTC GAG GTT ACG TAC ACC CGC AAT CAG GAC ATT TTG AAG AAC CGT CCG GGA ATT CAC TAT GCG CCT CCC ATC TTA GAG AAG AAT AAG GAT GGA CAA CGT TTG ATC GTT ACA TAT GAA GTT GAC TGG AAA AAT AAG ACC GTA AAG GTT GTG GAT AAG TAT TCG GAT GAT AAT AAG CCC TAT AAA GAA GGG
CC45 LukA ^{wt}	102	GCG AAC AAA GAT TCT CAG GAC CAG ACC AAA AAG GAG CAC GTA GAC AAG GCC CAG CAA AAA GAG AAG CGT AAT GTG AAC GAC AAA GAT AAG AAT ACT CCG GGG CCA GAT GAT ATC GGC AAG AAC GGT AAA GTC ACG AAG CGT ACA GTG TCT GAG TAT GAC AAA GAA ACA AAC ATC CTG CAG AAC TTA CAA TTC GAC TTT ATT GAT GAT CCA ACT TAC GAT AAG AAT GTG TTG CTG GTT AAG AAA CAA GGT TCA ATC CAT TCT AAC TTG AAG TTC GAG TCA CAC CGT AAC GAA ACG AAC GCG TCG TGG TTG AAA TAT CCG TCA GAG TAT CAT GTT GAT TTT CAA GTA CAA CGT AAT CCC AAA ACG GAA ATT TTG GAC CAA TTA CCT AAA AAT AAG ATT AGC ACC GCC AAG GTT GAC TCA ACT TTC TCC TAC TCA TTA GGA GGA AAG TTC GAT TCG ACA AAA GGG ATC GGG CGT ACA TCT TCG AAT AGC TAC AGT AAG AGC ATT AGC TAT AAC CAG CAG AAC TAT GAT ACG ATT GCT TCA GGG AAA AAT AAC AAC CGT CAC GTA CAT TGG TCA GTG GTT GCG AAC GAT CTT AAA TAT GGA AAC GAG ATT AAG AAT CGT AAC GAC GAA TTT TTG TTT TAC CGC AAT

		ACA CGC CTT AGT ACC GTG GAA AAT CCC GAG CTG TCC TTC GCG TCG AAG TAT CGC TAT CCG GCC CTT GTG CGT TCG GGT TTC AAT CCC GAG TTC TTA ACA TAT ATT TCC AAT GAG AAA ACT AAC GAC AAG ACT CGC TTC GAA GTC ACC TAC ACT CGC AAC CAG GAC ATT CTG AAA AAC AAG CCT GGA ATT CAT TAC GGG CAA CCA ATT TTA GAG CAG AAT AAG GAT GGA CAG CGC TTT ATT GTG GTA TAT GAG GTG GAC TGG AAG AAT AAG ACA GTA AAA GTT GTG GAA AAG TAC TCT GAC CAG AAT AAG CCC TAT AAA GAA GGA
CC8 LukAΔ10C	111	GCT CAC AAA GAT TCT CAG GAT CAA AAT AAG AAG GAG CAC GTC GAC AAG TCT CAG CAG AAA GAC AAG CGT AAT GTT ACA AAC AAG GAC AAA AAC AGC ACT GCT CCA GAC GAC ATT GGA AAA AAC GGT AAG ATT ACT AAA CGC ACC GAA ACG GTA TAT GAC GAA AAA ACG AAC ATT TTG CAA AAC TTG CAG TTC GAT TTC ATT GAC GAC CCC ACT TAT GAC AAG AAT GTC CTT CTG GTG AAG AAG CAG GGC AGC ATT CAC TCA AAC TTG AAA TTT GAG TCT CAC AAG GAG GAG AAG AAC TCC AAT TGG CTG AAA TAC CCA TCA GAG TAC CAC GTT GAT TTT CAA GTG AAA CGT AAC CGC AAA ACG GAA ATT TTG GAC CAA TTG CCG AAA AAC AAG ATC TCC ACC GCG AAA GTA GAC TCA ACA TTC AGT TAC TCT TCC GGC GGA AAG TTC GAC AGC ACT AAG GGG ATC GGG CGC ACT TCT TCC AAT TCG TAC TCG AAA ACG ATT TCT TAC AAT CAG CAG AAT TAT GAC ACT ATC GCA TCT GGT AAA AAT AAT AAC TGG CAC GTG CAT TGG TCG GTG ATT GCT AAT GAT TTA AAG TAT GGA GGT GAG GTA AAA AAT CGT AAT GAC GAG CTG CTG TTT TAC CGT AAC ACT CGC ATC GCA ACC GTT GAA AAC CCG GAA TTG TCC TTT GCC TCG AAA TAC CGC TAC CCT GCA TTA GTT CGT TCA GGC TTT AAT CCC GAG TTT TTG ACT TAT CTT TCC AAT GAA AAA TCG AAC GAG AAG ACT CAG TTC GAG GTT ACG TAC ACC CGC AAT CAG GAC ATT TTG AAG AAC CGT CCG GGA ATT CAC TAT GCG CCT CCC ATC TTA GAG AAG AAT AAG GAT GGA CAA CGT TTG ATC GTT ACA TAT GAA GTT GAC TGG AAA AAT AAG ACC GTA AAG GTT GTG GAT AAG TAT
CC45 LukAΔ10C	112	GCAAATAAAGACTCTCAAGATCAGACTAAAAAGGAACAT GTTGATAAGGCGCAACAAAAAGAAAAGCGTAATGTCAAT GATAAGGACAAGAATACTCCGGGACCCGACGACATTGGC AAGAACGGAAAGGTGACAAAGCGTACCGTTAGTGAGTAC GACAAGGAAACAAATATCCTGCAGAACTTACAGTTCGAT TTTATTGACGATCCTACCTATGACAAGAATGTCCTGTTGG TGAAGAAACAGGGCAGCATTTCATTCCAATTTAAATTTG AAAGCCATCGTAACGAAACAAATGCATCTTGGCTTAAAT ACCCTTCTGAGTACCACGTAGATTTTCAGGTACAACGCAA CCCAAAAACCGAAATTCTGGATCAACTGCCCAAGAATAA AATTTCTACGGCTAAAGTTGACAGTACATTTAGCTACAGT TTAGGGGGAAAGTTTGATAGTACAAAAGGAATTGGTCGT ACTTCCAGTAACCTCTATTCGAAATCTATTTCTATAATC

		AACAGAATTACGACACCATCGCATCCGGTAAAAACAATA ATCGCCACGTACATTGGAGTGTGTCGCGAATGACTTAAA GTACGGTAACGAAATCAAGAACCGCAACGACGAATTCTT ATTCTATCGTAACACGCGTTTAAGCACCGTCGAGAACCCC GAGTTATCCTTTGCTAGCAAATATCGCTATCCTGCGTTAG TACGCTCAGGGTTCAATCCTGAGTTCTTAACCTACATCTC CAACGAGAAAATAATGATAAGACACGCTTCGAGGTGAC CTACACCCGTAATCAGGATATCCTTAAAAATAAACCGGG TATTCATTACGGGCAACCCATTTTAGAACAGAATAAGGA CGGCCAACGTTTTATCGTGGTCTATGAGGTTGATTGGAAG ACAAGACAGTGAAAGTGGTTGAAAAGTAT
CC8 LukA W95	103	CATAAAGATTTCGCAGGATCAAAATAAGAAGGAGCATGTT GACAAGAGCCAGCAGAAAGACAAGCGCAATGTTACAAA CAAAGATAAGAACTCTACAGCGCCCGATGACATTGGTAA GAACGGCAAGATAACTAAGCGGACGGAAACCGTGTATGA CGAGAAAATAACATTCTGCAAAATTTGCAATTTGACTTT ATCGACGATCCAACCTATGACAAGAATGTCTTGCTTGTC AAATGCAAGGTTTCGATTCATTCAAACCTTAAATTTGAATC CCACAAAGAGGAGAAAACTCTAATTGGTTAAAGTATCC TTCAGAATATCACATAGATTTCCAGGTAAAGAGAAACCG TAAAACGGAGATACTGGATCAACTGCCTAAAAACAAGAT CTCGACAGCTAAGGTGGACGCTACGTTCTCGTACTCGTCT GGTGGGAAGTTCGACTCGACCAAAGGCATTGGGCGTACA TCATCAAATAGCTATTCAAAGACTATTAGCTATAATCAGC AGAACTATGATACGATAGCTTCGGGTAAGAATAACAACT GGCACGTTTCATTGGTTCGATCATTGCAAATGACTTGAAGTA TGGCGGAGAGGTAAAGAATCGCAACGATGAGCTGTTATT CTATCGCAATACGAGAATTGCGACTGTAGAGAACCCGGA ATTGTCTTTTGCTCCAAATATCGGTACCCGGCATTGGTA CGCTCTGGTTTCAATCCTGAGTTTTTAACTTACCTTTCCAA CGAAAAGAGTAATGAGAAGACCCAATTTGAGGTTACCTA CACCCGTAACCAGGATATTTTGAAGAATCGGCCGGGCAT CCATTATGCCCCACCAATCCTGGAGAAAAATAAAGACGG TCAGCGGCTTATTGTGACTTACGAGGTCGATTGGAAAAAT AAGACGGTCAAGGTAGTGGACAAATATTCTGATGACAAT AAACCGTACAAAGCTGGC
CC45 LukA W95	104	GCTAATAAGGACTCCCAGGACCAGACAAAGAAGGAACA CGTCGACAAAGCCCAGCAAAAAGAAAAACGCAACGTAA ACGATAAGGACAAGAACACCCAGGACCCGATGATATTG GGAAGAACGGTAAAGTCACAAAACGCACAGTGAGCGAG TACGATAAAGAAACAAATATCCTGCAAAATCTGCAATTT GACTTCATCGATGACCCTACCTATGATAAGAATGTGTTGT TGGTTAAGATGCAGGGAAGTATTCATTCCAACCTGAAATT CGAGAGCCACCGTAACGAAACGAATGCGAGTTGGTTAAA GTACCCCTTCAGAATACCACATTGATTTTCAGGTGCAGCGT AACCCGAAAACCGAAATCTTAGACCAGCTGCCTAAAAAC AAGATTTCTACGGCCAAGGTGGACGCAACTTTCAGTTATA GTCTTGAGGAAAGTTTCGACAGTACCAAAGGTATCGGCC GCACATCCTCAAACAGCTATTCGAAATCCATTTCTTACAA CCAGCAAAATTATGACACGATCGCCTCAGGTAAGAACAA CAATCGTCATGTGCATTGGAGCATCGTGGCTAACGATTG

		AAATATGGTAACGAAATCAAAAATCGCAATGACGAGTTC TTGTTTTACCGCAATACTCGCCTTTCTACGGTAGAGAATC CTGAGCTTAGCTTTGCGAGCAAGTATCGTTACCCTGCTCT TGTACGTTTCGGGTTTCAACCCAGAGTTCCTTACTTATATC TCCAATGAGAAGACGAACGATAAAACCCGTTTTGAAGTT ACATACACGCGTAATCAGGACATCTTAAAGAATAAACCG GGGATTCATTATGGGCAGCCGATCTTAGAGCAAAATAAG GATGGACAGCGTTTCATTGTAGTGTATGAGGTTGACTGGA AGAACAAGACGGTAAAAGTAGTTGAAAAGTATTCCGACC AAAACAAGCCTTATAAGGCGGGT
CC8 LukA W97 W72	105	CACAAAGACAGCCAGGATCAAAACAAGAAAGAGCACGT GGACAAGAGCCAGCAAAAGGATAAACGTAAACGTTACCA ACAAGGACAAAAACAGCACCCGCGCCGGACGATATCGGC AAGAACGGCAAAATTACCAAGCGTACCGAGACCGTGTAC GATGAAAAAACCAACATCCTGCAGAACCTGCAATTTCGAC TTTATTGACGATCCGACCTGCGATAAAAACGTGCTGCTGG TTAAGATGCAGGGCAGCATCCACAGCAACCTGAAATTCG AAAGCCACAAAGAGGAAAAGAACAGCAACTGGCTGAAG TACCCGAGCGAGTATCACATTGACTTTCAGGTGAAACGT AACCGTAAGACCGAAATCCTGGATCAACTGCCGAAGAAC AAAATTAGCACCGCGAAGGTTTTCGCGACCTTCAGCTAC AGCAGCGGTTGCAAATTTGACAGCACCAAGTGCATCGGC CGTACCAGCAGCAACAGCTATAGCAAAACCATCAGCTAC AACCAGCAAAACTATGATACCATTGCGAGCGGCAAGAAC AACAACTGGCACGTGCACTGGAGCATCATTGCGAACGAC CTGAAATACGGTGGCGAGGTTAAGAACCGTAACGATGAA CTGCTGTTCTATCGTAACACCCGTATCGCGACCGTGGAGA ACCCGGAAGTGAAGCTTTGCGAGCAAATACCGTTATCCGG CGCTGGTGCCTAGCGGTTTCAACCCGGAGTTTCTGGTTTA CCTGAGCAACGAGAAAAGCAACGAAAAGACCCAGTTTCG AAGTTACCTACACCCGTAACCAAGACATCCTGAAGAACC GTCCGGGTATCCACTATGCTCCGCCGATTCTGGAGAAGA ACAAAGATGGCCAACGTCTGATTGTGACCTATGAAGTTG ACTGGAAGAACAAAACCGTTAAAGTGGTTGATAAGTACA GCGACGATAACAAACCGTATAAGGCGGGT
CC45 LukA W97 W72	106	GCAAACAAAGACTCACAAGATCAGACAAAGAAAGAGCA TGTAGACAAAGCTCAACAGAAGGAAAAGCGCAATGTGA ACGACAAGGATAAAAATACTCCTGGTCCAGATGACATTG GTAAGAATGGTAAAGTTACTAAGCGGACCGTCTCTGAAT ATGATAAGGAGACAAATATTCTCCAGAATTTGCAATTTCG ATTCATTGATGATCCGACGTGCGATAAGAACGTATTGCT CGTTAAAATGCAGGGCTCCATCCATTCTGAATCTCAAGTTC GAATCCCATCGCAACGAGACAAACGCTTCCTGGCTCAAA TATCCTAGCGAGTATCATATCGACTTCCAAGTTCAACGGA ACCCTAAAGTGAATCCTTGATCAACTCCCTAAGAACA AAATCTCAACTGCCAAGGTCTGTGCCACATTTTCTTATTC TCTTGGCTGCAAATTCGATTCAACAAAGTGTATTGGTCGT ACATCAAGTAATAGCTATAGTAAAAGCATCAGTTATAAC CAGCAAAACTATGATACAATCGCGTCAGGCAAAAACAAT AATCGTCATGTCCATTGGTCCATTGTCGCGAACGACCTTA AGTACGGTAACGAAATTAAGAATCGGAACGATGAGTTTT

		TGTTCTATCGCAACACCCGTCTGTCTACTGTCGAAAACCC GGAGTTGTCCTTCGCAAGTAAATATCGCTATCCTGCTTTG GTACGTTCTGGGTTTAACCCGGAATTTCTCGTCTACATCA GCAACGAGAAAACAAATGACAAAACGCGCTTTGAAGTCA CGTACACACGTAATCAGGACATCTTAAAAAATAAACCAG GGATTCACTATGGTCAGCCAATCTTGGAGCAGAATAAAG ACGGCCAGCGTTTCATTGTCGTTTATGAAGTGGACTGGAA AAACAAAACGTGTTAAGGTGGTTGAGAAATATTCCGACCA AAACAAACCGTATAAGGCCGGT
CC8 LukB ^{wt}	107	AAAATCAATTCTGAAATTAAGCAAGTGTCCGAAAAAAAT TTGGATGGAGACACGAAGATGTATACGCGTACTGCTACG ACGTCAGACTCCCAGAAGAACATTACACAGAGTCTGCAA TTTAATTTTCTGACAGAACCCTAATGACAAGGAACT GTCTTTATTAAGGCTAAAGGGACTATCGGAAGCGGCTTA CGCATTTTAGACCCCAACGGTTATTGGAATAGCACGCTGC GCTGGCCGGGCAGTTACTCAGTATCAATCCAAAATGTCTG ATGATAACAATAACACCAATGTTACCGATTTGCCCCCAA GAACCAGGATGAATCGCGCGAGGTTAAATACACATACGG CTACAAGACAGGCGGTGACTTTAGCATCAACCGTGGGGG CTTGACAGGGAATATTACTAAGGAATCAAATTATAGTGA GACTATCTCTTATCAACAACCGTCCTATCGTACCTTATTA GACCAGAGTACCTCCCACAAAGGTGTAGGGTGGAAAGTT GAAGCGCACCTGATTAATAATATGGGTCACGATCACACA CGCCAACTGACCAACGACAGTGACAACCGCACAAAAAGT GAAATTTTGTAGTCTTACCCGTAACGGAAATCTGTGGGCCA AAGACAATTTACACCGAAAGATAAGATGCCGGTCACTG TATCTGAGGGGTTCAATCCCGAGTTTTTAGCAGTAATGTC GCATGACAAAAAGGACAAAGGGAAATCCCAGTTTGTGTG CCACTATAAGCGTAGCATGGATGAATTCAAAATCGACTG GAACCGTCACGGTTTCTGGGGTTACTGGTCAGGTGAGAA CCACGTAGACAAGAAAGAGGAGAACTGAGCGCATTATA TGAGGTTGATTGGAAAACGCACAATGTGAAATTTGTTAA AGTCCTGAATGACAACGAGAAAAAG
CC45 LukB ^{wt}	108	GAAATTAAGTCTAAGATCACAACAGTATCGGAGAAAAAC CTGGATGGCGATACTAAGATGTATACACGCACCGCCACT ACTTCGGACACGGAGAAGAAGATCTCACAATCGTTACAG TTTAATTTTCTTACAGAACCGAACTACGACAAAGAGACC GTCTTCATTAAAGCTAAAGGTACGATTGGTTCGGGATTAA AAATTCTGAATCCGAATGGCTATTGGAACAGTACCTTACG TTGGCCGGGGTCATATTCTGTATCCATTCAAAACGTGGAC GACAATAACAACAGCACCAATGTGACAGATTTGCTCCA AAGAATCAGGATGAGTCCCGCGAGGTGAAATATACCTAT GGGTACAAAACAGGAGGTGACTTTAGCATTAAACCGTGGT GGCTTGACTGGTAATATCACGAAGGAAAAAAATTACTCT GAGACTATTTCTACCAACAGCCGTCGTATCGCACCTTGA TCGACCAACCAACGACTAACAAAGGGGTCGCGTGGAAG TTGAGGCCACAGTATTAACAATATGGGCCACGATCACA CTCGTCAGCTTACTAACGATTTCGGATGACCGCGTCAAGTC GGAAATTTTCAGCCTGACGCGTAACGGAAATTTGTGGGC TAAAGACAATTTCACTCCTAAGAACAAGATGCCCGTGAC TGTTTCCGAAGGCTTTAATCCCGAATTCTTAGCGGTGATG

		TCTCATGATAAAAAATGATAAAGGAAAATCGCGCTTCATT GTGCATTATAAGCGTTCTATGGACGACTTCAAATTGGATT GGAATAAGCACGGATTCTGGGGGTACTGGTCCGGGGAAA ATCACGTAGATCAAAAGGAAGAGAAGTTGTCCGCTTTGT ATGAAGTGGACTGGAAGACTCACGACGTTAAGTTGATCA AGACCTTCAATGACAAAGAGAAGAAA
CC8 LukB Val53Leu	109	AAGATCAATTCGGAAATTAAACAGGTAAGTGAGAAAAAT TTGGATGGCGATACCAAAATGTACACCCGCACCGCTACC ACGTCAGATTCACAAAAAATATTACACAGTCCTTGCAG TTCAATTTCTGACAGAACCGAATTACGACAAGGAGACT TTGTTCAATTAAAGCCAAGGGAACCATCGGGTCCGGATTG CGTATCTTGGACCCGAACGGATATTGGAACCTCGACCTTAC GTTGGCCCGGGTCTTACAGTGTTAGTATCCAAAACGTAG ATGATAACAATAACACAAACGTGACAGATTTTGCACCTA AAAACCAGGACGAAAGCCGCGAGGTAAAGTACACATAT GGGTATAAAACAGGGGGGGGACTTTTCCATCAACCGTGGT GGTTTGACCGGGAACATCACCAAGAGTCAAATTACAGT GAGACCATCAGTTATCAGCAGCCGTCCTATCGTACATTAT TGGATCAGTCGACTTCACATAAAGGGGTTCGGATGGAAAG TAGAGGCTCATTGTATCAACAACATGGGTCACGATCATA CACGTCAGTTAACGAACGATAGCGATAATCGCACGAAGT CAGAAATCTTTAGTCTGACTCGTAACGGTAACTTGTGGGC CAAGGACAATTTACGCCCCAAAGATAAGATGCCTGTGAC GGTATCGGAGGGGTTCAATCCAGAATTCCTTGCTGTAATG TCCCATGACAAAAAAGACAAGGGCAAATCGCAATTTGTA GTCCACTATAAGCGTTCTATGGACGAGTTCAAGATTGACT GGAACCGCCACGGCTTCTGGGGGTACTGGAGTGGTGAGA ATCATGTGGATAAAAAGGAGGAGAACTTAGCGCCCTGT ATGAGGTAGATTGGAAAACACACAATGTCAAGTTCGTGA AAGTTCTTAATGACAACGAAAAAAA
CC45 LukB Val53Leu	110	GAGATCAAGAGCAAAATTACCACCGTGAGCGAAAAGAA CCTGGACGGTGATACCAAAATGTATACCCGTACCGCGAC CACCAGCGACACCGAGAAGAAAATTAGCCAGAGCCTGCA ATTCAACTTTCTGACCGAGCCGAACCTACGATAAGGAAAC CCTGTTTCATCAAGGCGAAAGGCACCATTTGGTAGCGGCCT GAAAATCCTGAACCCGAACGGTTATTGGAACAGCACCCCT GCGTTGGCCGGGTAGCTACAGCGTGAGCATCCAGAACGT TGACGATAACAACAACAGCACCAACGTGACCGACTTCGC GCCGAAGAACCAAGATGAGAGCCGTGAAGTTAAATACAC CTATGGTTACAAAACCGGTGGCGACTTTAGCATTAACCGT GGTGGCCTGACCGGCAACATCACCAAGGAGAAAAACTAT AGCGAAACCATTAGCTATCAGCAACCGAGCTACCGTACC CTGATCGATCAGCCGACCACCAACAAGGGTGTGGCGTG AAAGTTGAGGCGCACAGCATTAAACAACATGGGCCACGAC CACACCCGTCAACTGACCAACGATAGCGACGATCGTGTG AAGAGCGAAATCTTCAGCCTGACCCGTAACGGTAACCTG TGGGCGAAAGACAACCTTTACCCCGAAGAACAATAATGCCG GTGACCGTTAGCGAGGGTTTCAACCCGGAATTTCTGGCG GTGATGAGCCACGACAAGAACGATAAGGGCAAAGCCG TTTCATTGTTCACTACAAACGTAGCATGGACGATTTCAAG CTGGACTGGAACAAACACGGTTTTTGGGGCTATTGGAGC

		GGCGAGAACCACGTTGATCAGAAAGAGGAGAACTGAG CGCGCTGTACGAAGTGGACTGGAAGACCCACGATGTAA GCTGATCAAAACCTTTAACGATAAAGAAAAGAAA
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[0175] In any embodiment, the immunogenic composition disclosed herein comprises a polynucleotide encoding a SpA polypeptide. In any embodiment, the polynucleotide encodes a wildtype or non-variant SpA polypeptide. In any embodiment, the polynucleotide encodes a SpA A domain comprising an amino acid sequence of SEQ ID NO: 55 or 48. In any embodiment, the polynucleotide encodes a SpA B domain comprising an amino acid sequence of SEQ ID NO: 56 or 49. In any embodiment, the polynucleotide encodes a SpA C domain comprising an amino acid sequence of SEQ ID NO: 57 or 50. In any embodiment, the polynucleotide encodes a SpA D domain comprising an amino acid sequence of SEQ ID NO: 58 or 51. In any embodiment, the polynucleotide encodes a SpA E domain comprising an amino acid sequence of SEQ ID NO: 59 or 52. In any embodiment, the polynucleotide encodes a SpA polypeptide comprises at least two of the SpA IgG domains, at least three of the SpA IgG domains, at least four of the SpA IgG domains, or all five of the SpA IgG domains. In any embodiment, the polynucleotide encodes a SpA polypeptide comprising an amino acid sequence of SEQ ID NO: 53 or a sequence having 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% sequence identity to SEQ ID NO: 53.

[0176] In any embodiment, the polynucleotide of the immunogenic composition encodes variant E, D, A, B, and/or C domains, which comprise an amino acid sequence having 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO:55 or 48, SEQ ID NO:56 or 49, SEQ ID NO:57 or 50, SEQ ID NO:58 or 51, and SEQ ID NO:59 or 52, respectively. Exemplary variant E, D, A, B, and C SpA domains are described *supra*.

[0177] In any embodiment, the polynucleotide encodes a SpA variant polypeptide having a variant E domain that comprises a substitution at amino acid position 6, 7, 33, and/or 34 of SEQ ID NO: 59. In any embodiment, the polynucleotide encodes a SpA variant polypeptide having a variant D domain that comprises a substitution at amino acid position 9, 10, 36, and/or 37 of SEQ ID NO:58. In any embodiment, the polynucleotide encodes a SpA variant polypeptide having a variant A domain that comprises a substitution at amino acid position 7, 8, 34, and/or 35 of SEQ ID NO: 55. In any embodiment, the polynucleotide encodes a SpA variant polypeptide having a variant B domain that comprises a substitution at

amino acid position 7, 8, 34, and/or 35 of SEQ ID NO:56. In any embodiment, the polynucleotide encodes a SpA variant polypeptide having a variant C domain that comprises a substitution at amino acid position 7, 8, 34, and/or 35 of SEQ ID NO:57.

[0178] In any embodiment, the polynucleotide of the immunogenic compositions encodes a SpA variant polypeptide comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90% (but not 100%) sequence identity to SEQ ID NO: 53 or 72. In any embodiment, the SpA variant polypeptide comprises an amino acid sequence having 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity to SEQ ID NO: 53 or 72 or a fragment thereof.

[0179] In any embodiment, the polynucleotide of the immunogenic composition encodes a SpA variant polypeptide comprising one or more amino acid substitutions in the SpA domain D, or at a corresponding amino acid position in the other SpA IgG domains, where the one or more amino acid substitutions disrupt or decrease the binding of the SpA variant polypeptide to the IgG Fc. In any embodiment, the polynucleotide encodes a SpA variant polypeptide further comprising one or more amino acid substitutions in a V_H3 binding sub-domain of the D domain, or at a corresponding amino acid position in the other IgG domains, that disrupt or decrease binding to V_H3.

[0180] In any embodiment, the polynucleotide encodes a SpA variant polypeptide comprising a variant A domain, for example, a variant A domain comprising an amino acid sequence of SEQ ID NO:62, 67, 88, or 93. In any embodiment, the polynucleotide encodes a SpA variant polypeptide that comprises a variant B domain, for example, a variant B domain comprising an amino acid sequence of SEQ ID NO:63, 68, 89, or 94. In any embodiment, the polynucleotide encodes a SpA variant polypeptide that comprises a variant C domain, for example, a variant C domain comprising an amino acid sequence of SEQ ID NO:64, 69, 90 or 95. In any embodiment, the polynucleotide encodes a SpA variant polypeptide that comprises a variant D domain, for example, a variant D domain comprising an amino acid sequence of SEQ ID NO:66, 71, 91, or 96. In any embodiment, the polynucleotide encodes a SpA variant polypeptide that comprises a variant E domain, for example, a variant E domain comprising an amino acid sequence of SEQ ID NO:65, 70, 92, or 97.

[0181] In any embodiment, the polynucleotide of the immunogenic composition encodes a SpA variant polypeptide that comprises a variant A, B, C, D, and E domain comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least

97%, at least 98%, or at least 99% identical to SEQ ID NO:62 or 67, SEQ ID NO:63 or 68, SEQ ID NO:64 or 69, SEQ ID NO:66 or 71, and SEQ ID NO:65 or 70, respectively.

[0182] In any embodiment, the polynucleotide of the immunogenic composition

5 encodes a SpA variant polypeptide that comprises a variant A, B, C, D, and E domain comprising an amino acid sequence having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to identical to SEQ ID NO:88 or 93, SEQ ID NO:89 or 94, SEQ ID NO:90 or 95, SEQ ID NO:91 or 96, and SEQ ID NO:92 or 97,
10 respectively.

[0183] In any embodiment, the polynucleotide of the immunogenic composition

encodes a SpA variant polypeptide comprising a variant D domain, where the variant D domain comprises a substitution at amino acid positions corresponding to positions 9, 10, and/or 33 of SEQ ID NO:58.

[0184] In any embodiment, the polynucleotide of the immunogenic composition

15 encodes a SpA variant polypeptide that comprises (i) lysine substitutions for glutamine amino acid residues in each of SpA A-E domains at the amino acid positions corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58); and (ii) a glutamate substitution for a serine amino acid residue in each of SpA A-E domains at the amino acid position

20 corresponding to position 33 of SpA D domain (SEQ ID NO:58). In any embodiment, the polynucleotide encodes a SpA E domain having an amino acid sequence of SEQ ID NO: 65. In any embodiment, the polynucleotide of the immunogenic composition encodes a SpA D domain having an amino acid sequence of SEQ ID NO: 66. In any embodiment, the polynucleotide encodes a SpA A domain having the amino acid sequence of SEQ ID NO: 62.

25 In any embodiment, the polynucleotide encodes a SpA B domain having the amino acid sequence of SEQ ID NO: 63. In any embodiment, the polynucleotide encodes a SpA C domain having the amino acid sequence of SEQ ID NO: 64. In any embodiment, polynucleotide of the immunogenic compositions encodes a SpA variant polypeptide having the amino acid sequence of SEQ ID NO: 60.

[0185] In any embodiment, the polynucleotide of the immunogenic composition

30 encodes a SpA variant polypeptide that comprises (i) lysine substitutions for glutamine amino acid residues in each of SpA A-E domains at the amino acid positions corresponding to positions 9 and 10 of SpA D domain (SEQ ID NO:58); and (ii) a threonine substitution for a serine amino acid residue in each of SpA A-E domains at the amino acid position

corresponding to position 33 of SpA D domain (SEQ ID NO:58). In any embodiment, the polynucleotide encodes a SpA E domain having an amino acid sequence of SEQ ID NO: 70. In any embodiment, the polynucleotide of the immunogenic composition encodes a SpA D domain having an amino acid sequence of SEQ ID NO: 71. In any embodiment, the polynucleotide encodes a SpA A domain having the amino acid sequence of SEQ ID NO: 67. In any embodiment, the polynucleotide encodes a SpA B domain having the amino acid sequence of SEQ ID NO: 68. In any embodiment, the polynucleotide encodes a SpA C domain having the amino acid sequence of SEQ ID NO: 69. In any embodiment, polynucleotide of the immunogenic compositions encodes a SpA variant polypeptide having the amino acid sequence of SEQ ID NO: 61.

[0186] In any embodiment, the polynucleotide of the immunogenic composition encodes a SpA variant polypeptide comprising a variant A, B, C, D, and/or E domain. In any embodiment, the polynucleotide encodes a SpA variant polypeptide comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:60 or 61. In any embodiment, the polynucleotide encodes a SpA variant polypeptide comprising an amino acid sequence that is at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identical to the amino acid sequence of SEQ ID NO:54.

[0187] In some embodiments, the nucleic acid molecules encoding the *S. aureus* polypeptide as described herein are codon optimized for expression in mammalian cells, preferably human cells. Methods of codon-optimization are known and have been described previously (e.g. International Patent Application Publication No. WO1996/09378 to Seed, which is hereby incorporated by reference in its entirety). A sequence is considered codon optimized if at least one non-preferred codon as compared to a wild-type sequence is replaced by a codon that is more preferred. Herein, a non-preferred codon is a codon that is used less frequently in an organism than another codon coding for the same amino acid, and a codon that is more preferred is a codon that is used more frequently in an organism than a non-preferred codon. The frequency of codon usage for a specific organism can be found in codon frequency tables that are well known and available in the art. Preferably more than one non-preferred codon, e.g. more than 10%, 40%, 60%>, 80%> of non-preferred codons, preferably most (e.g. at least 90%) or all non-preferred codons, are replaced by codons that are more preferred.

Preferably the most frequently used codons in an organism are used in a codon-optimized sequence. Replacement by preferred codons generally leads to higher expression.

[0188] Polynucleotide sequences of the present disclosure can be cloned using routine molecular biology techniques, or generated de novo by DNA synthesis, which can be performed using routine procedures by service companies having business in the field of DNA synthesis and/or molecular cloning (e.g. GeneArt, GenScript, Invitrogen, Eurofins).

[0189] In some embodiments, the aforementioned nucleic acid molecules are inserted into a vector, e.g., an expression vector for use in an immunogenic composition as described herein. Alternatively, these nucleic acid molecules may be inserted into an expression vector that is transformed or transfected into an appropriate host cell for expression and isolation of the encoded SpA polypeptide, LukA variant polypeptide, LukB protein, or LukAB complex (as a stable heterodimer) as disclosed herein.

[0190] In accordance with this aspect of the disclosure, the nucleic acid molecules encoding the *S. aureus* polypeptides as described herein can be incorporated into any expression vector capable of expressing the polypeptides encoded by the nucleic acid sequence construct. Suitable expression vectors comprise nucleic acid sequence elements that control, regulate, cause or permit expression of the polypeptides encoded by such a vector. Such elements may comprise transcriptional enhancer binding sites, RNA polymerase initiation sites, ribosome binding sites, and other sites that facilitate the expression of encoded polypeptides in a given expression system. Suitable vectors include, without limitation, DNA vectors, plasmid vectors, a linear nucleic acid, and a viral vector, e.g., adenoviral vectors.

[0191] In one embodiment, the expression vector is a circular plasmid (see, e.g., Muthumani et al., "Optimized and Enhanced DNA Plasmid Vector Based In vivo Construction of a Neutralizing anti-HIV-1 Envelope Glycoprotein Fab," *Hum. Vaccin. Immunother.* 9: 2253-2262 (2013), which is hereby incorporated by reference in its entirety). Plasmids can transform a target cell by integration into the cellular genome or exist extrachromosomally (e.g., autonomous replicating plasmid with an origin of replication). Exemplary plasmid vectors include, without limitation, pCEP4, pREP4, pVAX, pcDNA3.0, provax, or any other plasmid expression vector capable of expressing the variant LukA and/or variant LukB proteins or polypeptides encoded by the recombinant nucleic acid sequence construct.

[0192] In another embodiment, the expression vector is a linear expression cassette ("LEC"). LECs are capable of being efficiently delivered to a subject via electroporation to express the SpA, LukA, and/or LukB polypeptides encoded by the recombinant nucleic acid molecules described herein. The LEC may be any linear DNA devoid of a phosphate

backbone. In one embodiment, the LEC does not contain any antibiotic resistance genes and/or a phosphate backbone. In another embodiment, the LEC does not contain other nucleic acid sequences unrelated to the desired gene expression.

[0193] The LEC may be derived from any plasmid capable of being linearized. The plasmid may be capable of expressing the polypeptides encoded by the recombinant nucleic acid molecules as described herein. Exemplary plasmids include, without limitation, pNP (Puerto Rico/34), pM2 (New Caledonia/99), WLV009, pVAX, pcDNA3.0, or provax, or any other expression vector capable of expressing the polypeptides encoded by the recombinant nucleic acid sequence construct.

[0194] In another embodiment, the expression vector is a viral vector. Suitable viral vectors that are capable of expressing the polypeptides include, for example, an adeno-associated virus (AAV) vector (*see, e.g.*, Krause et al., "Delivery of Antigens by Viral Vectors for Vaccination," *Ther. Deliv.* 2(1):51-70 (2011); Ura et al., "Developments in Viral Vector-Based Vaccines," *Vaccines* 2: 624-641 (2014); Buning et al, "Recent Developments in Adeno-associated Virus Vector Technology," *J. Gene Med.* 10:717-733 (2008), each of which is incorporated herein by reference in its entirety), a lentivirus vector (*see, e.g.*, Ura et al., "Developments in Viral Vector-Based Vaccines," *Vaccines* 2: 624-641 (2014); and Hu et al., "Immunization Delivered by Lentiviral Vectors for Cancer and Infection Diseases," *Immunol. Rev.* 239: 45-61 (2011), which are hereby incorporated by reference in their entirety), a retrovirus vector (*see e.g.*, Ura et al., "Developments in Viral Vector-Based Vaccines," *Vaccines* 2: 624-641 (2014), which are hereby incorporated by reference in their entirety), a vaccinia virus, a replication deficient adenovirus vector, and a gutless adenovirus vector (*see e.g.*, U.S. Pat. No. 5,872,005, which is incorporated herein by reference in its entirety). Methods for generating and isolating adeno-associated viruses (AAVs) suitable for use as vectors are known in the art (*see, e.g.*, Grieger & Samulski, "Adeno-associated Virus as a Gene Therapy Vector: Vector Development, Production and Clinical Applications," *Adv. Biochem. Engin/Biotechnol.* 99: 119-145 (2005); Buning et al, "Recent Developments in Adeno-associated Virus Vector Technology," *J. Gene Med.* 10:717-733 (2008), each of which is incorporated herein by reference in its entirety).

[0195] The polynucleotides encoding the SpA, LukA and/or LukB polypeptides described herein are typically combined with sequences of a promoter, translation initiation, 3' untranslated region, polyadenylation, and transcription termination in the expression vector constructs to achieve maximal expression. Promoter sequences suitable for driving expression of the polypeptides described herein include, without limitation, the elongation factor 1-alpha

(EF1a) promoter, a phosphoglycerate kinase-1 (PGK) promoter, a cytomegalovirus immediate early gene promoter (CMV), a chimeric liver-specific promoter (LSP), a cytomegalovirus enhancer/chicken beta-actin promoter (CAG), a tetracycline responsive promoter (TRE), a transthyretin promoter (TTR), a simian virus 40 promoter (SV40) and a CK6 promoter. Other promoters suitable for driving gene expression in host cells that are known in the art are also suitable for incorporation into the expression constructs disclosed herein.

[0196] Another aspect of the present disclosure is directed to a host cell comprising the nucleic acid molecules encoding the *S. aureus* polypeptides described herein, or a vector containing these polynucleotides. Expression constructs encoding the SpA, LukA, and LukB proteins or polypeptides as described herein can be co-transfected, serially transfected, or separately transfected into host cells. Suitable host cells include, without limitation, primary cells, cells of a cell line, a mixed cell line, an immortalized cell population, or a clonal population of immortalized cells, as well known in the art (*see e.g.*, Ausubel et al., ed., Current Protocols in Molecular Biology, John Wiley & Sons, Inc., NY, N.Y. (1987-2001); Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Cold Spring Harbor, N.Y. (1989); Harlow and Lane, Antibodies, a Laboratory Manual, Cold Spring Harbor, N.Y. (1989); Colligan et al., eds., Current Protocols in Immunology, John Wiley & Sons, Inc., NY (1994-2001); Colligan et al., Current Protocols in Protein Science, John Wiley & Sons, NY, N.Y., (1997-2001), which are hereby incorporated by reference in their entirety). Such host cells may be eukaryotic cells, bacterial cells, plant cells or archaeal cells.

[0197] In some embodiments, a suitable host cell for the *S. aureus* polynucleotides described herein is a bacterial cell. Suitable bacterial host cells include, without limitation, *Escherichia* host cells, *Pseudomonas* host cells, *Staphylococcus* host cells, *Streptomyces* host cells, *Mycobacterium* host cells, and *Bacillus* host cells. In some embodiments, the host cell is an *Escherichia coli* host cell. In some embodiments, the host cell is a *S. aureus* host cell.

[0198] In some embodiments, a suitable host cell for the *S. aureus* polynucleotides described herein is a eukaryotic cell. Exemplary eukaryotic cells may be of mammalian, insect, avian or other animal origins. Mammalian eukaryotic cells include immortalized cell lines such as hybridomas or myeloma cell lines such as SP2/0 (American Type Culture Collection (ATCC), Manassas, Va., CRL-1581), NSO (European Collection of Cell Cultures (ECACC), Salisbury, Wiltshire, UK, ECACC No. 85110503), FO (ATCC CRL-1646) and Ag653 (ATCC CRL-1580) murine cell lines. An exemplary human myeloma cell line is U266 (ATCC CRL-TIB-196). Other useful cell lines include those derived from Chinese Hamster Ovary (CHO)

cells such as CHO-K1SV (Lonza Biologics, Walkersville, Md.), CHO-K1 (ATCC CRL-61) or DG44.

[0199] The SpA, LukA, and LukB polypeptides described herein can be prepared by any of a variety of techniques using the isolated polynucleotides, vectors, and host cells described *supra*. In general, proteins are produced by standard cloning and cell culture techniques commonly used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells, and recover the proteins or polypeptides from the culture medium. Transfecting the host cell can be carried out using a variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., by electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like.

[0200] The polypeptides described herein can be post-translationally modified by processes such as glycosylation, isomerization, deglycosylation or non-naturally occurring covalent modification such as the addition of polyethylene glycol (PEG) moieties (pegylation) and lipidation. Such modifications may occur *in vivo* or *in vitro*.

[0201] In some embodiments, the SpA, LukA, and LukB polynucleotides and/or polypeptides as described herein are preferably “isolated” polynucleotides and/or polypeptides. “Isolated” when used to describe the polynucleotides and polypeptides disclosed herein, means that the polynucleotide or polypeptide has been identified, separated and/or recovered from a component of its production environment. Preferably, the isolated polynucleotide or polypeptide is free of association with other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that could typically interfere with pharmaceutical use, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. The polynucleotides or polypeptides are recovered and purified from recombinant cell cultures by known methods including, but not limited to, protein A purification, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxyapatite chromatography and lectin chromatography. High performance liquid chromatography (“HPLC”) can also be used for purification.

Adjuvants of the Immunogenic Composition

[0202] As used herein, the term “adjuvant” refers to a compound that when administered in conjunction with or as part of the immunogenic composition described herein

augments, enhances and/or boosts the immune response to the SpA polypeptides, LukA polypeptides, the LukB polypeptides, and/or polynucleotides encoding the same. However, when the adjuvant compound is administered alone it does not generate an immune response to the aforementioned polypeptides or polynucleotides. Adjuvants can enhance an immune response by several mechanisms including, *e.g.*, lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of antigen presenting cells.

[0203] The immunogenic compositions described herein comprising the SpA, LukA, and LukB polypeptides and/or polynucleotides encoding the same, comprise an adjuvant or are administered in combination with an adjuvant. The adjuvant for administration in combination with an immunogenic composition of the invention can be administered before, concomitantly with, or after administration of the immunogenic compositions.

[0204] Specific examples of adjuvants include, but are not limited to, aluminum salts (alum) (such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, and aluminum oxide, including nanoparticles comprising alum or nanoalum formulations), calcium phosphate (*e.g.*, Masson JD et al, Expert Rev Vaccines 16: 289-299 (2017), which is hereby incorporated by reference in its entirety), monophosphoryl lipid A (MPL) or 3-de-O-acylated monophosphoryl lipid A (3D-MPL) (*see e.g.*, United Kingdom Patent GB2220211, EP0971739, EP1194166, US6491919, which are hereby incorporated by reference in their entirety), AS01, AS02, AS03 and AS04 (*see e.g.* EP1126876, US7357936 for AS04, EP0671948, EP0761231, US5750110 for AS02, which are hereby incorporated by reference in their entirety), imidazopyridine compounds (*see* WO2007/109812, which is hereby incorporated by reference in its entirety), imidazoquinoxaline compounds (*see* WO2007/109813, which is hereby incorporated by reference in its entirety), delta-inulin (*e.g.* Petrovsky N and PD Cooper, Vaccine 33: 5920-5926 (2015), which is hereby incorporated by reference in its entirety), STING-activating synthetic cyclic-di-nucleotides (*e.g.* US20150056224, which is hereby incorporated by reference in its entirety), combinations of lecithin and carbomer homopolymers (*e.g.* US6676958), and saponins, such as Quil A and QS21 (*see e.g.* Zhu D and W Tuo, 2016, Nat Prod Chem Res 3: e113 (doi:10.4172/2329-6836.1000e113), which is hereby incorporated by reference in its entirety), optionally in combination with QS7 (*see* Kensil et al., in Vaccine Design: The Subunit and Adjuvant Approach (eds. Powell & Newman, Plenum Press, NY, 1995); US 5,057,540, which are hereby incorporated by reference in their entirety). In any embodiment, the adjuvant is Freund's adjuvant (complete or incomplete). In any embodiment, the adjuvant comprises Quil-A, such as for instance commercially obtainable from Brenntag (now Croda) or Invivogen. QuilA

contains the water-extractable fraction of saponins from the *Quillaja saponaria* Molina tree. These saponins belong to the group of triterpenoid saponins, that have a common triterpenoid backbone structure. Saponins are known to induce a strong adjuvant response to T-dependent as well as T-independent antigens, as well as strong cytotoxic CD8+ lymphocyte responses and potentiating the response to mucosal antigens. They can also be combined with cholesterol and phospholipids, to form immunostimulatory complexes (ISCOMs), wherein QuilA adjuvant can activate both antibody-mediated and cell-mediated immune responses to a broad range of antigens from different origins. In certain embodiments, the adjuvant is AS01, for example AS01B. AS01 is an adjuvant system containing MPL (3-O-desacyl-4'-monophosphoryl lipid A), QS21 (*Quillaja saponaria* Molina, fraction 21), and liposomes. In certain embodiments, the AS01 is commercially available or can be made as described in WO 96/33739, which is hereby incorporated by reference in its entirety. Certain adjuvants comprise emulsions, which are mixtures of two immiscible fluids, e.g. oil and water, one of which is suspended as small drops inside the other and are stabilized by surface-active agents. Oil-in-water emulsions have water forming the continuous phase, surrounding small droplets of oil, while water-in-oil emulsions have oil forming the continuous phase. Certain oil-in-water emulsions comprise squalene (a metabolizable oil). Certain adjuvants comprise block copolymers, which are copolymers formed when two monomers cluster together and form blocks of repeating units. An example of a water in oil emulsion comprising a block copolymer, squalene and a microparticulate stabilizer is TiterMax®, which can be commercially obtained from Sigma-Aldrich.

[0205] Optionally emulsions can be combined with or comprise further immunostimulating components, such as a TLR4 agonist. Suitable, but non-limiting examples of adjuvant combinations for use in the compositions disclosed herein include, oil in water emulsions (such as squalene or peanut oil) also used in MF59 (*see e.g.* EP0399843, US 6299884, US6451325) and AS03, optionally in combination with immune stimulants, such as monophosphoryl lipid A and/or QS21 such as in AS02 (*see* Stoute et al., 1997, N. Engl. J. Med. 336, 86-91, which is hereby incorporated by reference in its entirety). Further examples of adjuvants are liposomes containing immune stimulants such as MPL and QS21, such as in AS01E and AS01B (e.g. US 2011/0206758, which is hereby incorporated by reference in its entirety). Other examples of adjuvants are CpG and imidazoquinolines (such as imiquimod and R848) (*see e.g.*, Reed G, et al., 2013, Nature Med, 19: 1597-1608, which is hereby incorporated by reference in its entirety). In certain preferred embodiments according to the invention, the adjuvant is a Th1 adjuvant.

[0206] In any embodiment, the adjuvant comprises saponins, preferably the water-extractable fraction of saponins obtained from *Quillaja saponaria*. In certain embodiments, the adjuvant comprises QS-21.

[0207] In any embodiment, the adjuvant of the immunogenic composition disclosed herein contains a toll-like receptor 4 (TLR4) agonist alone or in combination with another adjuvant. TLR4 agonists are well known in the art, *see e.g.* Ireton GC and SG Reed, 2013, Expert Rev Vaccines 12: 793-807, which is hereby incorporated by reference in its entirety. In any embodiment, the adjuvant is a TLR4 agonist comprising lipid A, or an analog or derivative thereof.

[0208] As used herein, the term “lipid A” refers to the hydrophobic lipid moiety of an LPS molecule that comprises glucosamine and is linked to keto-deoxyoctulosonate in the inner core of the LPS molecule through a ketosidic bond, which anchors the LPS molecule in the outer leaflet of the outer membrane of Gram-negative bacteria. Lipid A, as used herein includes naturally occurring lipid A, mixtures, analogs, derivatives and precursors thereof. The term includes monosaccharides, *e.g.*, the precursor of lipid A referred to as lipid X; disaccharide lipid A; hepta-acyl lipid A; hexa-acyl lipid A; penta-acyl lipid A; tetra-acyl lipid A, *e.g.*, tetra-acyl precursor of lipid A, referred to as lipid IVA; dephosphorylated lipid A; monophosphoryl lipid A; diphosphoryl lipid A, such as lipid A from *Escherichia coli* and *Rhodobacter sphaeroides*. Several immune activating lipid A structures contain 6 acyl chains. Four primary acyl chains attached directly to the glucosamine sugars are 3-hydroxy acyl chains usually between 10 and 16 carbons in length. Two additional acyl chains are often attached to the 3-hydroxy groups of the primary acyl chains. *E. coli* lipid A, as an example, typically has four C14 3-hydroxy acyl chains attached to the sugars and one C12 and one C14 attached to the 3-hydroxy groups of the primary acyl chains at the 2' and 3' position, respectively.

[0209] As used herein, the term “lipid A analog or derivative” refers to a molecule that resembles the structure and immunological activity of lipid A, but that does not necessarily naturally occur in nature. Lipid A analogs or derivatives can be modified to be shortened or condensed, and/or to have their glucosamine residues substituted with another amine sugar residue, *e.g.* galactosamine residues, to contain a 2-deoxy-2-aminogluconate in place of the glucosamine-1-phosphate at the reducing end, to bear a galacturonic acid moiety instead of a phosphate at position 4'. Lipid A analogs or derivatives can be prepared from lipid A isolated from a bacterium, *e.g.*, by chemical derivation, or chemically synthesized, *e.g.* by first determining the structure of the preferred lipid A and synthesizing analogs or derivatives thereof. Lipid A analogs or derivatives are also useful as TLR4 agonist adjuvants (*see, e.g.*

Gregg KA et al, 2017, MBio 8, eDD492-17, doi: 10.1128/mBio.00492-17, which is hereby incorporated by reference in its entirety).

[0210] For example, a lipid A analog or derivative can be obtained by deacylation of a wild- type lipid A molecule, *e.g.*, by alkali treatment. Lipid A analogs or derivatives can for instance be prepared from lipid A isolated from bacteria. Such molecules could also be chemically synthesized. Another example of lipid A analogs or derivatives are lipid A molecules isolated from bacterial cells harboring mutations in, or deletions or insertions of enzymes involved in lipid A biosynthesis and/or lipid A modification.

[0211] MPL and 3D-MPL are lipid A analogs or derivatives that have been modified to attenuate lipid A toxicity. Lipid A, MPL, and 3D-MPL have a sugar backbone onto which long fatty acid chains are attached, wherein the backbone contains two 6-carbon sugars in glycosidic linkage, and a phosphoryl moiety at the 4 position. Typically, five to eight long chain fatty acids (usually 12-14 carbon atoms) are attached to the sugar backbone. Due to derivation of natural sources, MPL or 3D-MPL can be present as a composite or mixture of a number of fatty acid substitution patterns, *e.g.* hepta-acyl, hexa-acyl, penta-acyl, etc., with varying fatty acid lengths. This is also true for some of the other lipid A analogs or derivatives described herein, however synthetic lipid A variants can also be defined and homogeneous. MPL and its manufacture are for instance described in US 4,436,727, which is hereby incorporated by reference in its entirety. 3D-MPL is for instance described in US 4,912,094B1 (which is hereby incorporated by reference in its entirety), and differs from MPL by selective removal of the 3-hydroxymyristic acyl residue that is ester linked to the reducing-end glucosamine at position 3. Examples of lipid A (analog, derivatives) suitable for inclusion in the immunogenic compositions described herein include MPL, 3D-MPL, RC529 (*e.g.* EP1385541, which is hereby incorporated by reference in its entirety), PET-lipid A, GLA (glycopyranosyl lipid adjuvant, a synthetic disaccharide glycolipid; *see e.g.* US20100310602 and US8722064, which are hereby incorporated by reference in their entirety), SLA (*see e.g.* Carter D et al, 2016, Clin. Transl. Immunology. 5: e108 (doi:10.1038/cti.2016.63), which is hereby incorporated by reference in its entirety which describes a structure- function approach to optimize TLR4 ligands for human vaccines), PHAD (phosphorylated hexaacyl disaccharide; the structure of which is the same as that of GLA), 3D-PHAD, 3D-(6- acyl)-PHAD (3D(6A)-PHAD) (PHAD, 3D-PHAD, and 3D(6A)PHAD are synthetic lipid A variants, which also provide structures of these molecules), E6020 (CAS Number 287180-63-6), ONO4007, OM-174, and the like. In certain preferred embodiments, the TLR4 agonist adjuvant comprises a

lipid A analog or derivative chosen from 3D-MPL, GLA, or SLA. In certain embodiments the lipid A analog or derivative is formulated in liposomes.

[0212] The adjuvant, preferably including a TLR4 agonist, may be formulated in various ways, *e.g.* in emulsions such as water-in-oil (w/o) emulsions or oil-in-water (o/w) emulsions (examples are MF59, AS03), stable (nano-)emulsions (SE), lipid suspensions, liposomes, (polymeric) nanoparticles, virosomes, alum adsorbed, aqueous formulations (AF), and the like, representing various delivery systems for immunomodulatory molecules in the adjuvant and/or for the immunogens (*see e.g.* Reed et al, 2013, *supra*; and Alving CR et al, 2012, Curr Opin Immunol 24: 310-315, which are hereby incorporated by reference in their entirety).

[0213] In any embodiment, the immunostimulatory TLR4 agonist may optionally be combined with other immunomodulatory components, such as squalene oil-in-water emulsion (*e.g.* MF59; AS03); saponins (*e.g.* QuilA, QS7, QS21, Matrix M, Iscoms, Iscomatrix, etc); aluminum salts; activators for other TLRs (*e.g.* imidazoquinolines, flagellin, dsRNA analogs, TLR9 agonists, such as CpG, etc); and the like (*see e.g.* Reed et al, 2013, *supra*).

[0214] In any embodiment, the adjuvant of the immunogenic composition disclosed herein is a combination of a TLR4 agonist, *e.g.*, GLA, formulated as a stable emulsion (*i.e.*, GLA-SE). The stable emulsion used in GLA-SE is an oil-in-water emulsion, wherein the oil is squalene (*see e.g.* WO2013/119856). In any embodiment, the adjuvant of the immunogenic composition disclosed herein in a combination of a TLR4 agonist *e.g.*, GLA, in combination with a saponin (*e.g.*, GLA-QS21). In any embodiment, the aforementioned adjuvants can be formulated as liposomes. An exemplary adjuvant thus also includes GLA-LSQ, which comprises a synthetic TLR4 agonist (*e.g.*, MPL [GLA]) and a saponin (*e.g.*, QS21), formulated as liposomes.

[0215] Additional exemplary adjuvants for use in the immunogenic compositions described herein comprising a lipid A analog or derivative include SLA-SE (synthetic MPL [SLA], squalene oil/water emulsion), SLA- Nanoalum (synthetic MPL [SLA], aluminum salt), GLA-Nanoalum (synthetic MPL [GLA], aluminum salt), SLA-AF (synthetic MPL [SLA], aqueous suspension), GLA-AF (synthetic MPL [GLA], aqueous suspension), SLA-alum (synthetic MPL [SLA], aluminum salt), GLA-alum (synthetic MPL [GLA], aluminum salt), AS01 (MPL, QS21, liposomes), AS02 (MPL, QS21, oil/water emulsion), AS25 (MPL, oil/water emulsion), AS04 (MPL, aluminum salt), and AS15 (MPL, QS21, CpG, liposomes). *See, e.g.*, WO2008/153541; WO2010/141861; WO2013/119856; WO2019/051149; WO 2013/119856; WO 2006/116423; US Patent No. 4,987,237; U.S. Patent No. 4,436,727; U.S.

Patent No. 4,877,611; U.S. Patent No. 4,866,034; U.S. Patent No. 4,912,094; U.S. Patent No. 4,987,237; U.S. Patent No. 5,191,072; U.S. Patent No. 5,593,969; U.S. Patent No. 6,759,241; U.S. Patent No. 9,017,698; U.S. Patent No. 9,149,521; U.S. Patent No. 9,149,522; U.S. Patent No. 9,415,097; U.S. Patent No. 9,415,101; U.S. Patent No. 9,504,743; Reed G, et al.,

2013, supra, Johnson et al., 1999, J Med Chem, 42:4640-4649, and Ulrich and Myers, 1995, Vaccine Design: The Subunit and Adjuvant Approach; Powell and Newman, Eds.; Plenum: New York, 495-524, which are hereby incorporated by reference in their entirety.

***S. aureus* Immunogenic Compositions and Methods of Use**

[0216] In one aspect, the immunogenic compositions as disclosed herein comprise any one or more of the SpA polypeptides and LukA variant polypeptides as described herein, or one or more nucleic acid molecules encoding the polypeptides as described herein. In another aspect, the immunogenic compositions as disclosed herein comprise any one or more of the SpA polypeptides and LukB variant polypeptides as described herein, or one or more nucleic acid molecules encoding the polypeptides as described herein. In another aspect, the immunogenic compositions as disclosed herein comprise any one or more of the SpA polypeptides, the LukA variant polypeptides, and the LukB polypeptides as described herein, or one or more nucleic acid molecules encoding the polypeptides as described herein.

[0217] In any embodiment, the immunogenic composition comprises one or more SpA polypeptides (variant or non-variant), CC45 LukA variant polypeptides, CC45 LukB polypeptides (variant or non-variant), or polynucleotides encoding the same. For example, an immunogenic composition of the present disclosure may comprise, a SpA variant polypeptide, a CC45 LukA variant polypeptide, a CC45 LukB non-variant polypeptide or polynucleotides encoding the same as described herein. An exemplary immunogenic composition in accordance with this embodiment comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC45 LukA variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 81 of SEQ ID NO: 2, a serine to alanine substitution at the amino acid position corresponding to position 139 of SEQ ID NO: 2, valine to isoleucine substitutions at the amino acid positions corresponding to positions 111

and 191 or SEQ ID NO: 2, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 321 of SEQ ID NO: 2. In any embodiment, this Luka variant polypeptide has the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 4. This composition further comprises a CC45 LukB polypeptide, such as the polypeptide of SEQ ID NO: 16, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 16.

[0218] Another exemplary immunogenic composition according to the aforementioned embodiment comprises a SpA variant polypeptide, a CC45 Luka variant polypeptide e, a CC45 LukB variant polypeptide or polynucleotides encoding the same as described herein. An exemplary immunogenic composition comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC45 Luka variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 81 of SEQ ID NO: 2, a serine to alanine substitution at the amino acid position corresponding to position 139 of SEQ ID NO: 2, valine to isoleucine substitutions at the amino acid positions corresponding to positions 111 and 191 or SEQ ID NO: 2, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 321 of SEQ ID NO: 2. In some embodiments, this Luka variant polypeptide has the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 4. This composition further comprises a CC45 LukB variant polypeptide comprising an amino acid substitution corresponding to Val53Leu in SEQ ID NO: 16. In some embodiments, this LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 18.

[0219] Other immunogenic compositions according to this embodiment comprise a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at

least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO:2 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 16. In some embodiments, the CC45 LukB variant sequence comprises the amino acid sequence selected from SEQ ID NOs: 18, 20, and 22. For example, in some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 4 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 6 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 8 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 10 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 11 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 12 in combination with a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant selected from SEQ ID NOs: 18, 20, and 22. In one embodiment, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant having an amino acid sequences of SEQ ID NO: 4 in combination with a CC45 LukB having the amino acid sequence of SEQ ID NO: 16. In one embodiment, the immunogenic composition comprises a

SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant having an amino acid sequences of SEQ ID NO: 11 in combination with a CC45 LukB having the amino acid sequence of SEQ ID NO: 16. In one embodiment, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant having an amino acid sequences of SEQ ID NO: 12 in combination with a CC45 LukB having the amino acid sequence of SEQ ID NO: 16. In one embodiment, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant having an amino acid sequences of SEQ ID NO: 8 in combination with a CC45 LukB having the amino acid sequence of SEQ ID NO: 16. In one embodiment, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant having an amino acid sequences of SEQ ID NO: 4 in combination with a CC45 LukB variant having the amino acid sequence of SEQ ID NO: 18.

[0220] In another embodiment, the immunogenic composition comprises a SpA polypeptide, a CC8 LukA variant polypeptide, and a CC8 LukB polypeptide (variant or non-variant), or polynucleotides encoding the polypeptides as disclosed herein. For example, the immunogenic composition comprises a SpA variant polypeptide, CC8 LukA variant polypeptide, and a CC8 LukB polypeptide or polynucleotides encoding the same as described herein. An exemplary composition comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC8 LukA variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, a serine to alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, valine to isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO: 1, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1. In any embodiment, this LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3. This composition further comprises a CC8 LukB polypeptide, such as the polypeptide of SEQ ID NO: 15, or an amino acid sequence

having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 15.

[0221] Another immunogenic composition in accordance with this embodiment comprises a SpA variant polypeptide, CC8 LukA variant polypeptide, a CC8 LukB variant polypeptide or polynucleotide encoding the same as disclosed herein. An exemplary immunogenic composition comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC8 LukA variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, a serine to alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, valine to isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO: 1, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1. In any embodiment, this LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3. This composition further comprises a CC8 LukB variant polypeptide comprising a valine to leucine amino acid substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 15. In any embodiment, this LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 17.

[0222] Other immunogenic compositions according to this embodiment comprise a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 1 in combination with a CC8 LukB sequence of SEQ ID NO: 15 or a variant sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 15. In some embodiments, the CC8 LukB sequence variant sequence comprises an amino acid sequence selected from SEQ ID NOs: 17, 19, and 21. For example, in

some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 3, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having 85% or more sequence identity to CC8 LukB of SEQ ID NO: 15, *e.g.*, a CC8 LukB variant sequence selected from SEQ ID NOs: 17, 19 and 21. In

5 some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 5 in combination with a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB of SEQ ID NO: 15, *e.g.*, a CC8 LukB variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide
10 of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 7, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB of SEQ ID NO: 15, *e.g.*, a CC8 LukB variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 9, and a CC8 LukB sequence of SEQ ID NO: 15
15 or a variant thereof having >85% sequence identity to CC8 LukB of SEQ ID NO: 15, *e.g.*, a CC8 LukB variant sequence selected from SEQ ID NOs: 17, 19 and 21.

[0223] In another embodiment, the immunogenic composition comprises a SpA polypeptide (variant or non-variant), a CC8 LukA variant polypeptide, and a CC45 LukB polypeptide (variant or non-variant) or polynucleotides encoding the same as described herein.

20 For example, the composition comprises a SpA variant polypeptide, a CC8 LukA variant polypeptide, and a CC45 LukB polypeptide or polynucleotide encoding the same. An exemplary immunogenic composition according to this embodiment comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10
25 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC8 LukA variant polypeptide
30 comprising a lysine to methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, a serine to alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, valine to isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO: 1, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1.

In any embodiment, this LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3. This composition further comprises a CC45 LukB polypeptide, such as the polypeptide of SEQ ID NO: 16, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 16.

[0224] Another immunogenic composition according to this embodiment comprises a SpA variant polypeptide, a CC8 LukA variant polypeptide, and a CC45 LukB variant polypeptide or polynucleotides encoding the same as disclosed herein. An exemplary immunogenic composition according to this embodiment comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC8 LukA variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, a serine to alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, valine to isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO: 1, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1. In any embodiment, this LukA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 3. This composition further comprises a CC45 LukB variant polypeptide comprising an amino acid substitution corresponding to Val53Leu in SEQ ID NO: 16. In some embodiments, this LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 18.

[0225] Other immunogenic compositions according to this embodiment comprise a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 1, and a CC45 LukB

sequence of SEQ ID NO: 16 or a variant sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 16. In some embodiments, the CC45 LukB variant sequence comprises the amino acid sequence selected from SEQ ID NOs: 18, 20, and 22. For example, in some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 3, and a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 5, and a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 7, and a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant of SEQ ID NO: 9, and a CC45 LukB sequence of SEQ ID NO: 16 or a variant thereof having >85% sequence identity to CC45 LukB of SEQ ID NO: 16, *e.g.*, a CC45 LukB variant sequence selected from SEQ ID NOs: 18, 20, and 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant having the amino acid sequence of SEQ ID NO: 5 and a CC45 LukB variant having the amino acid sequence of SEQ ID NO: 16. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant having the amino acid sequence of SEQ ID NO: 5 and a CC45 LukB variant having the amino acid sequence of SEQ ID NO: 22. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant having the amino acid sequence of SEQ ID NO: 5 and a CC45 LukB variant having the amino acid sequence of SEQ ID NO: 18. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC8 LukA variant having the amino acid sequence of SEQ ID NO: 5 and a CC45 LukB variant having the amino acid sequence of SEQ ID NO: 20.

[0226] In another embodiment, the immunogenic composition comprises a SpA polypeptide (variant or non-variant), a CC45 LukA variant polypeptide, and a CC8 LukB

polypeptide (variant or non-variant), or polynucleotides encoding the same as described herein. For example, an immunogenic composition of the present disclosure may comprise, a SpA variant polypeptide, a CC45 LukA variant polypeptide, and a CC8 LukB polypeptide. An exemplary immunogenic composition comprises a SpA variant polypeptide comprising at least one SpA A, B, C, D, or E domain, where the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58. An exemplary SpA variant polypeptide comprises the amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 60. This composition further comprises a CC45 LukA variant polypeptide comprising a lysine to methionine substitution at the amino acid position corresponding to position 81 of SEQ ID NO: 2, a serine to alanine substitution at the amino acid position corresponding to position 139 of SEQ ID NO: 2, valine to isoleucine substitutions at the amino acid positions corresponding to positions 111 and 191 or SEQ ID NO: 2, and a glutamic acid to alanine substitution at the amino acid position corresponding to position 321 of SEQ ID NO: 2. In some embodiments, this Luka variant polypeptide has the amino acid sequence of SEQ ID NO: 4, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 4. This composition further comprises a CC8 LukB polypeptide, for example the LukB polypeptide of SEQ ID NO: 15, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 15. Alternatively, the composition comprises a CC8 LukB variant polypeptide comprising a valine to leucine amino acid substitution at the amino acid position corresponding to position 53 SEQ ID NO: 15. In any embodiment, this LukB variant polypeptide comprises the amino acid sequence of SEQ ID NO: 17, or an amino acid sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the amino acid sequence of SEQ ID NO: 17.

[0227] Other immunogenic compositions according to this embodiment comprise a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO:2 in combination with a CC8 LukB sequence of SEQ ID NO: 15 or a variant sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 15. In some embodiments, the CC8 LukB variant sequence comprises the

amino acid sequence selected from SEQ ID NOs: 17, 19 and 21. For example, in some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 4, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 6, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 8, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 9, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 10, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 11, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21. In some embodiments, the immunogenic composition comprises a SpA variant polypeptide of SEQ ID NO: 60, a CC45 LukA variant of SEQ ID NO: 12, and a CC8 LukB sequence of SEQ ID NO: 15 or a variant thereof having >85% sequence identity to CC8 LukB sequence of SEQ ID NO: 15, *e.g.*, a variant sequence selected from SEQ ID NOs: 17, 19 and 21.

[0228] Another aspect of the present disclosure is directed to an immunogenic composition comprising a SpA polypeptide as described herein and any of the variant LukB proteins or polypeptides as described herein, or nucleic acid molecules encoding the SpA and LukB variant as described herein. In particular, the variant LukB protein or polypeptide of the immunogenic composition comprises one or more amino acid residue insertions, substitutions,

and/or deletions described herein. In some embodiments, the composition comprises a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, and a LukB variant of SEQ ID NO: 15 (CC8).

5 Exemplary CC8 LukB variants include, without limitation, the LukB variants of SEQ ID NOs: 17, 19, and 21. In some embodiments, the composition comprises a SpA variant polypeptide comprising the sequence of SEQ ID NO: 60, or a sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 60, and a LukB variant of SEQ ID NO: 16 (CC45). Exemplary CC45 LukB
10 variants include, without limitation, the LukB variants of SEQ ID NOs: 18, 20, and 22.

[0229] An immunogenic composition in accordance with this embodiment can further comprise a LukA protein or polypeptide. For example, a composition comprising a SpA variant and LukB variant as described in the preceding paragraph further comprises a CC8 LukA sequence of SEQ ID NO: 1 or a variant sequence having at least 85%, 86%, 87%, 88%,
15 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 1. Alternatively, the immunogenic composition comprising a SpA variant and LukB variant as described in the preceding paragraph further comprises a CC45 LukA sequence of SEQ ID NO: 2 or a variant sequence having at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 2.

[0230] The immunogenic compositions as described herein may further include one or more additional *S. aureus* antigens. Suitable *S. aureus* antigen include, without limitation, serotype 336 polysaccharide antigen, clumping factor A, clumping factor B, a fibrinogen binding protein, a collagen binding protein, an elastin binding protein, a MHC analogous protein, a polysaccharide intracellular adhesion, beta hemolysin, delta hemolysin, Pantone-
25 Valentine leukocidin, leukocidin M, exfoliative toxin A, exfoliative toxin B, V8 protease, hyaluronate lyase, lipase, staphylokinase, an enterotoxin, an enterotoxin superantigen SEA, an enterotoxin superantigen SAB, toxic shock syndrome toxin-1, poly-N-succinyl beta-1→6 glucosamine, catalase, beta-lactamase, teichoic acid, peptidoglycan, a penicillin binding protein, chemotaxis inhibiting protein, complement inhibitor, Sbi, Type 5 antigen, Type 8
30 antigen, and lipoteichoic acid. Other suitable *S. aureus* antigens to include in the immunogenic composition include, without limitation, CP5, CP8, Eap, Ebh, Emp, EsaB, EsaC, EsxA, EsxB, EsxAB(fusion), IsdA, IsdB, IsdC, MntC, rTSST-1, rTSST-1v, TSST-1, SasF, vWbp, vWh vitronectin binding protein, Aaa, Aap, Ant, autolysin glucosaminidase, autolysin amidase, Can, collagen binding protein, Csa1A, EFB, Elastin binding protein, EPB, FbpA, fibrinogen binding

protein, Fibronectin binding protein, FhuD, FhuD2, FnbA, FnbB, GehD, HarA, HBP, Immunodominant ABC transporter, IsaA/PisA, laminin receptor, Lipase GehD, MAP, Mg²⁺ transporter, MHC II analog, MRPII, NPase, RNA III activating protein (RAP), SasA, SasB, SasC, SasD, SasK, SBI, SEA exotoxins, SEB exotoxins, mSEB, SitC, Ni ABC transporter, SitC/MntC/saliva binding protein, SsaA, SSP-1, SSP-2, Spa5, SpAKKAA, SpAkR, Sta006, and Sta011.

[0231] The immunogenic compositions of the present disclosure are prepared by formulating the SpA, LukA, and/or LukB polypeptides as described herein with a pharmaceutically acceptable carrier and optionally a pharmaceutically acceptable excipient. As used herein, the terms “pharmaceutically acceptable carrier” and “pharmaceutically acceptable excipient” (e.g., additives such as diluents, immunostimulants, adjuvants, antioxidants, preservatives and solubilizing agents) are non-toxic to the subject administered the composition at the dosages and concentrations employed. Examples of pharmaceutically acceptable carriers include water, *e.g.*, buffered with phosphate, citrate and another organic acid. Representative examples of pharmaceutically acceptable excipients that may be useful in the present disclosure include antioxidants such as ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt forming counterions such as sodium; and/or nonionic surfactants.

[0232] The formulation of pharmaceutically active ingredients with pharmaceutically acceptable carriers is known in the art, *e.g.*, Remington: The Science and Practice of Pharmacy (e.g. 21st edition (2005), and any later editions). Non-limiting examples of additional ingredients include: buffers, diluents, solvents, tonicity regulating agents, preservatives, stabilizers, and chelating agents. One or more pharmaceutically acceptable carrier can be used in formulating the pharmaceutical compositions of the invention.

[0233] In any embodiment, the immunogenic composition as described herein is a liquid formulation. A preferred example of a liquid formulation is an aqueous formulation, *i.e.*, a formulation comprising water. The liquid formulation can comprise a solution, a suspension, an emulsion, a microemulsion, a gel, and the like. An aqueous formulation typically comprises at least 50% w/w water, or at least 60%, 70%, 75%, 80%, 85%, 90%, or at least 95% w/w of water.

[0234] In any embodiment, the immunogenic composition can be formulated as an injectable which can be injected, for example, via an injection device (e.g., a syringe or an infusion pump). The injection can be delivered intramuscularly, intraperitoneally, intravitreally, or intravenously, for example.

5 **[0235]** The immunogenic composition of the present disclosure may be formulated for parenteral administration. Solutions, suspensions, or emulsions of the composition can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof in oils. Illustrative oils are those of petroleum, animal, vegetable, or synthetic origin, for
10 example, peanut oil, soybean oil, or mineral oil. In general, water, saline, aqueous dextrose and related sugar solution, and glycols, such as propylene glycol or polyethylene glycol, are preferred liquid carriers, particularly for injectable solutions. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

15 **[0236]** Pharmaceutical immunogenic compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
20 microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

[0237] In any embodiment, the immunogenic composition as described herein is a solid formulation, e.g., a freeze-dried or spray-dried composition, which can be used as is, or
25 whereto the physician or the patient adds solvents, and/or diluents prior to use. Solid dosage forms can include tablets, such as compressed tablets, and/or coated tablets, and capsules (e.g., hard or soft gelatin capsules). The immunogenic composition can also be in the form of sachets, dragees, powders, granules, lozenges, or powders for reconstitution, for example.

[0238] The dosage forms of the immunogenic composition may be immediate release,
30 in which case they can comprise a water-soluble or dispersible carrier, or they can be delayed release, sustained release, or modified release, in which case they can comprise water-insoluble polymers that regulate the rate of dissolution of the dosage form in the gastrointestinal tract or under the skin.

[0239] In other embodiments, the immunogenic composition can be delivered intranasally, intrabuccally, or sublingually.

[0240] The pH in an aqueous formulation of the immunogenic composition can be between pH 3 and pH 10. In one embodiment, the pH of the immunogenic composition is from about 7.0 to about 9.5. In another embodiment, the pH of the immunogenic composition is from about 3.0 to about 7.0.

[0241] Another aspect of the present disclosure relates to methods of using the immunogenic composition as described herein. Accordingly, one aspect is directed to a method for treating or preventing a Staphylococcus infection in a subject in need thereof that involves administering an effective amount of an immunogenic composition as disclosed herein. Another aspect is directed to a method for eliciting an immune response to a Staphylococcus bacterium in a subject in need thereof, that involves administering an effective amount of an immunogenic composition as disclosed herein. Another aspect is directed to a method for decolonization or preventing colonization or recolonization of a Staphylococcus bacterium in a subject in need thereof that involves administering an effective amount of an immunogenic composition as disclosed herein. In accordance with this aspect, the methods described herein are suitable for preventing short term and persistent colonization or recolonization of a Staphylococcus bacterium in a subject in need thereof.

[0242] The methods of the present disclosure involve administering any one of the immunogenic compositions described *supra*. A suitable subject for treatment in accordance with these aspects of the present disclosure is a subject at risk of developing a *S. aureus* infection, a subject at risk of exposure to *S. aureus* bacterium, and/or a subject exposed to *S. aureus* bacterium.

[0243] In accordance with this aspect of the present disclosure, a prophylactically effective amount of the immunogenic composition is administered to the subject to generate an immune response against *S. aureus* infection. A prophylactically effective amount is the amount necessary to generate or elicit a humoral (*i.e.*, antibody mediated) and cellular (T-cells) immune response. The elicited humoral response is sufficient to prevent or at least reduce the extent of *S. aureus* infection that would otherwise develop in the absence of such response. Preferably, administration of a prophylactically effective amount of the immunogenic composition described herein induces a neutralizing immune response against *S. aureus* in the subject. To effectuate an effective immune response in a subject, the composition may further contain one or more additional *S. aureus* antigens or an adjuvant as described *supra*. In an alternative embodiment, the adjuvant is administered separately from the composition to the

subject, either before, after, or concurrent with administration of the composition of the present disclosure.

[0244] For purposes of this aspect of the disclosure, the target “subject” encompasses any animal, preferably a mammal, more preferably a human. In the context of administering an immunogenic composition for purposes of preventing, inhibiting, or reducing the severity of a *S. aureus* infection and *S. aureus* colonization in a subject, the target subject encompasses any subject that is at risk of being infected by *S. aureus*. Particularly susceptible subjects include immunocompromised infants, juveniles, adults, and elderly adults. However, any infant, juvenile, adult, or elderly adult at risk for *S. aureus* infection can be treated in accordance with the methods and immunogenic composition described herein. Particularly suitable subjects include those at risk of infection with methicillin-resistant *S. aureus* (MRSA) or methicillin sensitive *S. aureus* (MSSA). Other suitable subjects include those subjects which may have or are at risk for developing a condition resulting from a *S. aureus* infection, *i.e.*, a *S. aureus* associated condition, such as, for example, skin wounds and infections, tissue abscesses, folliculitis, osteomyelitis, pneumonia, scalded skin syndrome, septicemia, septic arthritis, myocarditis, endocarditis, and toxic shock syndrome.

[0245] In some embodiments, the subject is at least or at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 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, 85, or 90 years old (or any range derivable therein). In certain embodiments, the subject or patient described herein, such as the human subject, is a pediatric subject. A pediatric subject is one that is defined as less than 18 years old. In some embodiments, the pediatric subject is 2 years old or less. In some embodiments, the pediatric subject is less than 1-year-old. In some embodiments, the pediatric subject is less than 6 months old. In some embodiments, the pediatric subject is 2 months old or less. In some embodiments, the human patient is 65 years old or older. In some embodiments, the human patient is a health care worker. In some embodiments, the patient is one that will receive a surgical procedure.

[0246] Numerous other factors may also be accounted for when administering the immunogenic composition under conditions effective to induce a robust immune response. These factors include, for example and without limitation, the concentration of the active agents in the composition, the mode and frequency of administration, and the subject details, such as age, weight and overall health and immune condition. General guidance can be found, for example, in the publications of the International Conference on Harmonization and in REMINGTON'S PHARMACEUTICAL SCIENCES (Mack Publishing Company 1990), which

is hereby incorporated by reference in its entirety. A clinician may administer an immunogenic composition as described herein until a dosage is reached that provides the desired or required prophylactic effect, e.g., the desired antibody titers. The progress of the prophylactic response can be easily monitored by conventional assays.

5 [0247] In one embodiment of the present disclosure, the immunogenic composition as described herein is administered prophylactically to prevent, delay, or inhibit the development of *S. aureus* infection in a subject at risk of being infected with *S. aureus* or at risk of developing an associated condition. In some embodiments of the present disclosure, prophylactic administration of the immunogenic composition is effective to fully prevent *S.*
10 *aureus* infection in an individual. In other embodiments, prophylactic administration is effective to prevent the full extent of infection that would otherwise develop in the absence of such administration, *i.e.*, substantially prevent or inhibit *S. aureus* infection in an individual.

[0248] In the context of using prophylactic compositions to prevent *S. aureus* infection, the dosage of the composition is one that is adequate to generate an antibody titer capable of
15 neutralizing *S. aureus* LukAB mediated cytotoxicity and SpA mediated virulent activity, and is capable of achieving a reduction in a number of symptoms, a decrease in the severity of at least one symptom, or a delay in the further progression of at least one symptom, or even a total alleviation of the infection.

[0249] Prophylactically effective amounts of the immunogenic compositions described
20 herein will depend on whether an adjuvant is co-administered, with higher dosages being required in the absence of adjuvant. The amount of SpA, LukA, and LukB polypeptides and/or polynucleotides encoding the same for administration can vary from 1 µg-500 µg per patient. In some embodiments, 5, 10, 20, 25, 50 or 100 µg is used for each human injection. Occasionally, a higher dose of 1-50 mg per injection is used. In some embodiments, about 10,
25 20, 30, 40 or 50 mg is used for each human injection. The timing of injections can vary significantly from once a year to once a decade. Generally, an effective dosage can be monitored by obtaining a fluid sample from the subject, generally a blood serum sample, and determining the titer of antibody developed against SpA, LukA, LukB or LukAB, using methods well known in the art and readily adaptable to the specific antigen to be measured.
30 Ideally, a sample is taken prior to initial dosing and subsequent samples are taken and titered after each immunization. Generally, a dose or dosing schedule which provides a detectable titer at least four times greater than control or “background” levels at a serum dilution of 1:100 is desirable, where background is defined relative to a control serum or relative to a plate background in ELISA assays.

[0250] The immunogenic composition of the present disclosure can be administered by parenteral, topical, intravenous, oral, intraperitoneal, intranasal or intramuscular means for prophylactic treatment.

EMBODIMENTS

5 [0251] The invention provides also the following non-limiting embodiments.

[0252] Embodiment 1 is an immunogenic composition comprising:

- (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
- (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptide

10 comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.

[0253] Embodiment 2 is a combination of two or more immunogenic compositions, together comprising:

- (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
- (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptide

15 comprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.

[0254] Embodiment 3 is the immunogenic composition of embodiment 1 or the combination of immunogenic compositions of embodiment 2, wherein the LukA variant polypeptide comprises an amino acid substitution at the amino acid residue corresponding to

20 Glu323 of SEQ ID NO: 25.

[0255] Embodiment 4 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1-3, wherein said LukA variant polypeptide comprises amino acid substitutions at each amino acid residue corresponding to amino acid residues Lys83, Ser141, Val113, Val193, and Glu323 of SEQ ID NO: 25.

25 [0256] Embodiment 5 is the immunogenic composition or the combination of immunogenic compositions of embodiment 4, wherein the amino acid substitutions comprise Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala.

[0257] Embodiment 6 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1-5, wherein said LukA variant

30 polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4.

[0258] Embodiment 7 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1–6, wherein said LukA variant polypeptide further comprises: an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25.

5 [0259] Embodiment 8 is the immunogenic composition or the combination of immunogenic compositions of embodiment 7, wherein the amino acid substitutions comprise Tyr74Cys, Asp140Cys, Gly149Cys, and Gly156Cys.

[0260] Embodiment 9 is the immunogenic composition or the combination of immunogenic compositions of embodiments 7 or 8, wherein said LukA variant polypeptide
10 comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 6.

[0261] Embodiment 10 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1–9, wherein said variant LukA protein
15 or polypeptide further comprises: an amino acid substitution at the amino acid residue corresponding to amino acid residue Thr249 of SEQ ID NO: 25.

[0262] Embodiment 11 is the immunogenic composition or the combination of immunogenic compositions of embodiment 10, wherein said LukA variant polypeptide
20 comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 7, an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 8, an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 9, or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 10.

[0263] Embodiment 12 is the immunogenic composition or the combination of
25 immunogenic compositions of any one of embodiments 1–11, wherein the SpA polypeptide is a SpA variant polypeptide.

[0264] Embodiment 13 is the immunogenic composition or the combination of immunogenic compositions of embodiment 12, wherein the SpA variant polypeptide has at least one amino acid substitution that disrupts Fc binding and at least a second amino acid
30 substitution that disrupts VH3 binding.

[0265] Embodiment 14 is the immunogenic composition or the combination of immunogenic compositions of embodiments 12 or 13, wherein the SpA variant polypeptide comprises a SpA D domain, said SpA D domain comprising an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 58.

[0266] Embodiment 15 is the immunogenic composition or the combination of immunogenic compositions of embodiment 14, wherein the SpA variant polypeptide further comprises a SpA E, A, B, and/or C domain.

[0267] Embodiment 16 is the immunogenic composition or the combination of
 5 immunogenic compositions of embodiment 15, wherein the SpA E domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:59, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:55, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:56; the SpA C domain
 10 comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:57.

[0268] Embodiment 17 is the immunogenic composition or the combination of immunogenic compositions of embodiment 12 or 13, wherein the SpA variant polypeptide comprises a SpA E, D, A, B, or C domain wherein the SpA E domain comprises an amino acid
 15 sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:52, wherein the SpA D domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:51, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:48, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of
 20 SEQ ID NO:49; the SpA C domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:50.

[0269] Embodiment 18 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 12–17, wherein the SpA variant polypeptide consecutively comprises SpA E, D, A, B, and C domains.

[0270] Embodiment 19 is the immunogenic composition or the combination of immunogenic compositions of embodiment 18, wherein the SpA variant polypeptide comprises SpA E, D, A, B, and C domains and has an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:53.

[0271] Embodiment 20 is the immunogenic composition or the combination of
 30 immunogenic compositions of any one of embodiments 12–17, wherein each SpA E, D, A, B, and C domain has an amino acid substitution at one or both amino acid positions corresponding to amino acid positions 9 and 10 of SEQ ID NO: 58.

[0272] Embodiment 21 is the immunogenic composition or the combination of immunogenic compositions of embodiment 20, wherein the amino acid substitution at one or

both amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 is a lysine residue for a glutamine residue.

[0273] Embodiment 22 is the immunogenic composition or the combination of immunogenic compositions of embodiment 21, wherein the SpA variant polypeptide comprises SpA E, D, A, B, and C domains and has an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:54.

[0274] Embodiment 23 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 12–22, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

[0275] Embodiment 24 is the immunogenic composition or the combination of immunogenic compositions of embodiment 23, wherein the SpA E domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:65, wherein the SpA D domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:66, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:62, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:63; the SpA C domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:64.

[0276] Embodiment 25 is the immunogenic composition or the combination of immunogenic compositions of embodiment 23, wherein the SpA E domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:92, wherein the SpA D domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:91, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:88, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:89; the SpA C domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:90.

[0277] Embodiment 26 is the immunogenic composition or the combination of immunogenic compositions of embodiment 23, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60.

[0278] Embodiment 27 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 12–22, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) a threonine substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

[0279] Embodiment 28 is the immunogenic composition or the combination of immunogenic compositions of embodiment 27, wherein the SpA E domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:70, wherein the SpA D domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:71, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:67, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:68 ; the SpA C domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:69.

[0280] Embodiment 29 is the immunogenic composition or the combination of immunogenic compositions of embodiment 27, wherein the SpA E domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:97, wherein the SpA D domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:96, the SpA A domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:93, the SpA B domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:94, and the SpA C domain comprises an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:95.

[0281] Embodiment 30 is the immunogenic composition or the combination of immunogenic compositions of embodiment 27, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 61, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 61.

[0282] Embodiment 31 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 12–22, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) an amino acid substitution at the amino acid position corresponding to position 29 of SEQ ID NO: 58.

[0283] Embodiment 32 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1–31, wherein said compositions further comprise a *S. aureus* Leukocidin B (LukB) polypeptide or variant thereof.

[0284] Embodiment 33 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein the LukB polypeptide is a LukB polypeptide of SEQ ID NO: 15 or a LukB polypeptide of SEQ ID NO: 16.

[0285] Embodiment 34 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein the LukB polypeptide is a LukB variant polypeptide.

[0286] Embodiment 35 is the immunogenic composition or the combination of immunogenic compositions of embodiment 34, wherein the LukB variant polypeptide comprises an amino acid sequence having at least 85% sequence similarity to the amino acid sequence of SEQ ID NO:15 or an amino acid sequence having at least 85% sequence similarity to the amino acid sequence of SEQ ID NO: 16.

[0287] Embodiment 36 is the immunogenic composition or the combination of immunogenic compositions of embodiment 35, wherein the LukB variant polypeptide comprises an amino acid substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 15 and SEQ ID NO: 16.

[0288] Embodiment 37 is the immunogenic composition or the combination of immunogenic compositions of embodiment 36, wherein the amino acid substitution is a valine to leucine substitution.

[0289] Embodiment 38 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 34–37, wherein said LukB variant polypeptide comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 15.

[0290] Embodiment 38 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 34–37, wherein said LukB variant polypeptide comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu110, Thr122, and Arg155 of SEQ ID NO: 16.

[0291] Embodiment 40 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 34–39, wherein the LukB variant polypeptide comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from SEQ ID NOs: 17-22.

[0292] Embodiment 41 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 4 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 4 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 16, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 16.

[0293] Embodiment 42 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 3 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 15.

[0294] Embodiment 43 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 3 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 18.

[0295] Embodiment 44 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 41–43, wherein the SpA polypeptide is a SpA variant polypeptide.

[0296] Embodiment 45 is the immunogenic composition or the combination of immunogenic compositions of embodiment 44, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

[0297] Embodiment 46 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32 wherein (i) the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC8 LukA variant polypeptide comprising a methionine substitution at the amino acid position corresponding to position 80 of

SEQ ID NO: 1, an alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO:1, and an alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1; and (iii) the LukB polypeptide is a CC45

5 LukB variant polypeptide comprising a leucine substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 16.

[0298] Embodiment 47 is the immunogenic composition or the combination of immunogenic compositions of embodiment 46, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60; the LukA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 3; and the LukB variant polypeptide comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO:18.

[0299] Embodiment 48 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein (i) the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC8 LukA variant polypeptide comprising a methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, an alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO:1, and an alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1; and (iii) the LukB polypeptide is a CC8 LukB variant polypeptide comprising a leucine substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 15.

[0300] Embodiment 49 is the immunogenic composition or the combination of immunogenic compositions of embodiment 48, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60; the LukA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 3; and the

LukB variant polypeptide comprises an amino acid sequence of SEQ ID NO: 17, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO:17.

[0301] Embodiment 50 is the immunogenic composition or the combination of

5 immunogenic compositions of embodiment 32, wherein (i) the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC8 LukA variant polypeptide
10 comprising a methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, an alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of SEQ ID NO:1, and an alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1; and (iii) the LukB polypeptide is a CC8 LukB
15 polypeptide comprising an amino acid sequence of SEQ ID NO: 15.

[0302] Embodiment 51 is the immunogenic composition or the combination of immunogenic compositions of embodiment 50, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60; the LukA variant
20 polypeptide comprises an amino acid sequence of SEQ ID NO: 3, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 3; and the LukB polypeptide comprises an amino acid sequence of SEQ ID NO: 15, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO:15.

25 **[0303]** Embodiment 52 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein (i) the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of
30 SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC45 LukA variant polypeptide comprising a methionine substitution at the amino acid position corresponding to position 81 of SEQ ID NO: 2, an alanine substitution at the amino acid position corresponding to position 139 of SEQ ID NO: 2, isoleucine substitutions at the amino acid positions corresponding to positions 111 and 191 of SEQ ID NO:2, and an alanine substitution at the amino acid position

corresponding to position 321 of SEQ ID NO: 2; and (iii) the LukB polypeptide is a CC45 LukB variant polypeptide comprising a leucine substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 16.

[0304] Embodiment 53 is the immunogenic composition or the combination of

5 immunogenic compositions of embodiment 52, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60; the LukA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 4, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 4; and the
10 LukB variant polypeptide comprises an amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO:18.

[0305] Embodiment 54 is the immunogenic composition or the combination of immunogenic compositions of embodiment 32, wherein (i) the SpA variant polypeptide

15 comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC45 LukA variant polypeptide comprising a methionine substitution at the amino acid position corresponding to position 81 of
20 SEQ ID NO: 2, an alanine substitution at the amino acid position corresponding to position 139 of SEQ ID NO: 2, isoleucine substitutions at the amino acid positions corresponding to positions 111 and 191 of SEQ ID NO:2, and an alanine substitution at the amino acid position corresponding to position 321 of SEQ ID NO: 2; and (iii) the LukB polypeptide is a CC45 LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 16.

25 **[0306]** Embodiment 55 is the immunogenic composition or the combination of

immunogenic compositions of embodiment 54, wherein the SpA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 60, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 60; the LukA variant polypeptide comprises an amino acid sequence of SEQ ID NO: 4, or an amino acid sequence
30 having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO: 4; and the LukB polypeptide comprises an amino acid sequence of SEQ ID NO: 16, or an amino acid sequence having at least 90% sequence similarity to the amino acid sequence of SEQ ID NO:16.

[0307] Embodiment 56 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1 to 55, further comprising an adjuvant.

[0308] Embodiment 57 is the immunogenic composition or the combination of immunogenic compositions of embodiment 56, wherein the adjuvant comprises aluminum salts, such as aluminum hydroxide, aluminum phosphate, aluminum sulfate, and aluminum oxide.

[0309] Embodiment 58 is the immunogenic composition or the combination of immunogenic compositions of embodiment 56, wherein the adjuvant comprises aluminum hydroxide or aluminum phosphate.

[0310] Embodiment 59 is the immunogenic composition or the combination of immunogenic compositions of embodiment 56, wherein the adjuvant comprises a stable oil-in-water emulsion.

[0311] Embodiment 60 is the immunogenic composition or the combination of immunogenic compositions of embodiment 56, wherein the adjuvant comprises a saponin.

[0312] Embodiment 61 is the immunogenic composition or the combination of immunogenic compositions of embodiment 60, wherein the saponin is QS21.

[0313] Embodiment 62 is the immunogenic composition or the combination of immunogenic compositions of embodiment 56, wherein the adjuvant comprises a TLR4 agonist.

[0314] Embodiment 63 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the TLR4 agonist is lipid A or an analog or derivative thereof.

[0315] Embodiment 64 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the TLR4 agonist comprises MPL, 3D-MPL, RC529, GLA, SLA, E6020, PET-lipid A, PHAD, 3D-PHAD, 3D-(6-acyl)-PHAD, ONO4007, or OM-174.

[0316] Embodiment 65 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the TLR4 agonist is glycopyranosyl lipid adjuvant (GLA).

[0317] Embodiment 66 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the adjuvant comprises a TLR4 agonist in combination with a stable oil-in-water emulsion.

[0318] Embodiment 67 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the adjuvant comprises a TLR4 agonist formulated in a stable oil-in-water emulsion.

[0319] Embodiment 68 is the immunogenic composition or the combination of immunogenic compositions of embodiment 65, wherein the adjuvant comprises GLA-SE.

[0320] Embodiment 69 is the immunogenic composition or the combination of immunogenic compositions of embodiment 62, wherein the adjuvant comprises a TLR-4 agonist in combination with a saponin.

[0321] Embodiment 70 is the immunogenic composition or the combination of immunogenic compositions of embodiment 65, wherein the adjuvant comprises GLA-LSQ.

[0322] Embodiment 71 is an immunogenic composition or a combination of immunogenic compositions, wherein said compositions comprises one or more isolated nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or variant thereof, the LukA variant polypeptide, and the LukB polypeptide or variant thereof of the immunogenic compositions of any one of embodiments 1-55.

[0323] Embodiment 72 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71, wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or a variant thereof and a nucleic acid molecule encoding the LukAB heterodimer (RARPR-15), wherein the nucleic acid molecule encoding the LukAB heterodimer comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 104 operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 108.

[0324] Embodiment 73 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71, wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or a variant thereof and a nucleic acid molecule encoding the LukAB heterodimer (RARPR-30), wherein the nucleic acid molecule encoding the LukAB heterodimer comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 104 operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 110.

[0325] Embodiment 74 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71 wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or a variant thereof and a nucleic acid molecule encoding the LukAB heterodimer (RARPR-32), wherein the nucleic acid molecule encoding the LukAB heterodimer comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 107.

[0326] Embodiment 75 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71, wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or variant thereof and a nucleic acid molecule encoding the LukAB heterodimer (RARPR-33), wherein the nucleic acid molecule encoding the LukAB heterodimer comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 110.

[0327] Embodiment 76 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71, wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or variant thereof and a nucleic acid molecule encoding the LukAB heterodimer (RARPR-34), wherein the nucleic acid molecule encoding the LukAB heterodimer comprises a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 103 operatively coupled to a nucleotide sequence having at least 85%, at least 90%, at least 95%, at least 97%, or at least 99% sequence similarity to the nucleotide sequence of SEQ ID NO: 109.

[0328] Embodiment 77 is the immunogenic composition or the combination of immunogenic compositions of embodiment 71, wherein the one or more nucleic acid molecules are contained in one or more vectors.

[0329] Embodiment 78 is the immunogenic composition of embodiments 71 or 77, wherein said composition comprises a host cell, wherein said host cell comprises said one or more nucleic acid molecules or said one or more vectors.

[0330] Embodiment 79 is a method for treating or preventing a *Staphylococcus* infection in a subject in need thereof, the method comprising: administering to the subject in need thereof an effective amount of the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1 to 78.

5 [0331] Embodiment 80 is a method for eliciting an immune response to a *Staphylococcus* bacterium in a subject in need thereof, the method comprising: administering to the subject in need thereof an effective amount of the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1 to 78.

[0332] Embodiment 81 is a method for decolonization or preventing colonization or
10 recolonization of a *Staphylococcus* bacterium in a subject in need thereof, the method comprising: administering to the subject in need thereof an effective amount of the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1 to 78.

[0333] Embodiment 82 is the immunogenic composition or the combination of
15 immunogenic compositions of any one of embodiments 1–78 for use in a method of generating an immune response against *S. aureus* in a subject.

[0334] Embodiment 83 is the immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1–78 for use as a medicament.

20 EXAMPLES

[0335] The following examples are provided to illustrate embodiments of the present disclosure but are by no means intended to limit its scope.

Example 1: Exemplary LukA Variant Polypeptides, LukB Variant Polypeptides, and Stable LukAB Heterodimer Complexes

25 [0336] For expression of LukAB heterodimeric proteins, *E. coli* BL21(DE3) cells were co-transformed with a *lukA* construct cloned into pCDFDuet-1 and a *lukB* construct cloned into pETDuet-1. Transformants were cultured in 50 µg/mL ampicillin and 50 µg/mL spectinomycin to select for pETDuet-1 and pCDFDuet-1, respectively, in Luria-Bertani broth at 37°C, with shaking at 190 rpm, overnight. For expression, fresh Terrific Broth media was inoculated with
30 1:50 dilution of the overnight culture at 37°C, with shaking at 190 rpm until cultures reached an OD₆₀₀ = 2. Expression was then induced through the addition of isopropyl β-d-1-thiogalactopyranoside to a final concentration of 1 mM, and induction continued at 37°C for an additional 5 hours. The expression of LukAB heterodimers including pairs of cysteine substitutions in LukA and/or LukB were expressed in the cytoplasm of *E. coli* Origami 2(DE3)

cells to support disulfide bond formation. The expression of LukA monomers in the periplasm of *E. coli* BL21(DE3) was performed through the transformation of *lukA* constructs in pD861-CH, with induction in Terrific Broth (supplemented with 30 µg/mL kanamycin) using a final concentration of 4 mM rhamnose at 37°C for 4 hours. After induction of both cytoplasmic and periplasmic expression constructs, the cells were harvested through centrifugation at 4000 rpm at 4°C for 15 min and then resuspended in lysis buffer (94% Bugbuster [EMD Millipore] + 6% 5 M NaCl + 0.4% 4 M imidazole + protease inhibitor cocktail [ProteaseArrest, G-Biosciences]). Following lysis at room temperature for 20 minutes, the lysates were incubated on ice for 45 minutes and then centrifuged at 16100 x g, 4°C for 35 minutes. Proteins were purified through the 6xHis-tag at the N-terminus of LukA using an AKTA Pure 25M FPLC and HisTrap columns and eluted using an imidazole gradient (50 – 500 mM imidazole in 50 mM sodium phosphate buffer, pH 7.4, 200 mM NaCl). Fractions containing purified protein, as determined by SDS-PAGE, were pooled, and dialyzed in 50 mM sodium phosphate buffer, pH 7.4, 200 mM NaCl, 10% glycerol at 4°C overnight. Purified proteins were quantified through the bicinchoninic acid (BCA) protein assay (Pierce).

Table 5. Exemplary LukA, LukB, and LukAB Heterodimer Complexes Utilized in the Studies Described Herein

Toxoid/ Toxin Name	LukA	LukA Substitutions	SEQ ID NO:	LukB	LukB Substitutions	SEQ ID NO:
RARPR-013	CC45 W94	E321A, Lys81Leu, Ser139Ala, Val111Ile, Val191Ile	11	CC45	none	16
RARPR-015	CC45 W95	E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile	4	CC45	none	16
RARPR-017	CC45 W96	E321A, Lys81Leu, Ser139Ala, Val111Ile, Val191Ile, Thr247Val	12	CC45	none	16
RARPR-019	CC45 W97	E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val	8	CC45	none	16
RARPR-30	CC45 W95	E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile	4	CC45	Val53Leu	18
RARPR-31	CC8 W95	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile	3	none		
RARPR-32	CC8 W95	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile	3	CC8	none	15
RARPR-33	CC8 W95	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile	3	CC45	Val53Leu	18
RARPR-34	CC8 W95	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile	3	CC8	Val53Leu	17
LukA monomer	CC8 W97 W72	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	9	none		
LukA monomer	CC45 W97 W72	E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys	10	none		

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Toxoid/ Toxin Name	LukA	LukA Substitutions	SEQ ID NO:	LukB	LukB Substitutions	SEQ ID NO:
	CC8 W95 W72	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	5	CC45	none	16
	CC8 W95 W72	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	5	CC45	Glu45Cys, Thr122Cys, Glu110Cys, Arg155Cys	22
	CC8 W95 W72	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	5	CC45	Val53Leu	18
	CC8 W95 W72	E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys	5	CC45	Val53Leu, Glu45Cys, Thr122Cys, Glu110Cys, Arg155Cys	20
CC8delta10	CC8	Deletion of C-terminal residues 312-321	113	CC8	none	15
CC45delta10	CC45	Deletion C-terminal residues 313-322	114	CC45	none	16
CC8 toxin	CC8	none	1	CC8	none	15
CC45 toxin	CC45	none	2	CC45	none	16

Example 2: Cytotoxicity of Wild-type, LukA, and LukAB Toxoids

[0337] The cytotoxicity of LukAB toxoid proteins (as defined in Table 5) was assessed in comparison with wild-type LukAB toxin using either the promonocytic cell line THP-1, or freshly isolated primary human polymorphonuclear leukocytes (hPMNs).

5 [0338] THP-1 cells were differentiated in the presence of phorbol 12-myristate 13-acetate prior to testing cytotoxicity. For THP-1 cytotoxicity assays, a total of 1×10^5 cells in 50 μ L RPMI were added to each well of a 96-well plate. LukAB toxins and toxoid proteins were adjusted to a standard concentration of protein, serially diluted in ice-cold RPMI medium, and 50 μ L volumes of each were added to appropriate wells. In addition to RPMI-only
10 negative controls, Triton X-100 was added to a final concentration of 0.1% as a positive control. Plates were incubated for 2 hours at 37°C, 5% CO₂, prior to assessing release of the cytoplasmic enzyme lactate dehydrogenase, which served as a marker of membrane integrity, using the CytoTox-ONE assay (Promega).

[0339] The cytotoxicity of LukA and LukAB toxoids against differentiated THP-1 cells
15 is provided in Table 6 below. Differentiated THP-1 cells were sensitive to the wild-type toxins, as both the CC8 and CC45 LukAB wild-type toxins killed 30% or more of the cell population at toxin concentrations as low as 0.313 μ g/mL. Deletion of the final 10 amino acid residues in the C-terminus of LukA (delta10) reduced the cytotoxicity of the CC8delta10 toxin to less than 5% cell death at 40 μ g/mL but did not reduce the cytotoxicity of the CC45delta10 toxin toward
20 differentiated THP-1 cells. Neither of the LukA monomers displayed cytotoxicity toward differentiated THP-1 cells. This result was expected, as LukA should not form an active pore complex in the absence of LukB. Each of the LukAB dimer toxoids, including RARPR-33, RARPR-34, and RARPR-15, displayed markedly reduced cytotoxicity toward differentiated THP-1 cells, with cell death at 1% or less for each of the toxoids tested at the highest tested
25 concentration, 40 μ g/mL.

Table 6. Cytotoxicity of LukA or LukAB proteins against differentiated THP-1 cells, a human monocytic cell line, using a standardized amount of toxin. Data are presented as the percent of dead cells.

Toxin	LukAB Concentration (µg/mL)											
	40	20	10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.04	0.02
RARPR-15	-12	-12	-12	-14	-14	-15	-10	-14	-12	-11	-9	-7
RARPR-30	0	-1	0	0	0	0	0	0	0	0	0	0
RARPR-31	0	0	-1	0	0	0	-1	-1	0	0	-1	-1
RARPR-32	0	-1	-1	-1	0	0	0	0	0	0	0	0
RARPR-33	1	1	1	0	1	1	1	0	1	1	1	1
RARPR-34	1	0	1	1	1	1	0	1	1	0	0	1
CC8 LukA W97 (monomer)	1	1	0	0	1	0	0	0	0	0	1	1
CC45 LukA W97 (monomer)	1	1	0	0	0	0	0	0	0	1	1	2
CC8delta10	4	3	1	1	0	0	0	0	0	-1	0	0
CC45delta10	53	39	32	31	33	41	54	55	53	35	13	5
CC8 toxin	41	44	43	39	39	38	37	30	15	6	2	1
CC45 toxin	42	34	31	34	40	46	46	36	18	4	1	0

[0340] For hPMNs, prior to intoxication, all toxins were normalized to 2.5 µg/mL (per subunit) and then 20 µL of toxin was pipetted into the top wells of a 96-well plate and serially diluted 2-fold in 10 µL of 1X PBS. PMNs were isolated and normalized to 200,000 cells per 90 µL RPMI (10 mM HEPES + 0.1% HSA). 90 µL of PMNs were then pipetted into each well and the toxin-PMN mixture was incubated in a 37°C + 5% CO₂ incubator for 1 hour. To assess toxicity, 10 µL of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hours. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance of 492 nm.

[0341] The cytotoxicity of LukA monomers and LukAB dimer toxoids against human primary PMN cells is provided in Table 7 below. The wild-type CC8 and CC45 toxins displayed greater than 90% killing of primary human PMNs at toxin concentrations of 0.313 µg/mL and 1.25 µg/mL, respectively. In comparison, each of the LukAB toxoids and the LukA monomers were considerably reduced in cytotoxicity toward these cells. Deletion of the 10 C-terminal residues in CC8 LukA essentially eliminated cytotoxicity toward differentiated THP-1 cells, whereas this toxin retained cytotoxicity against hPMNs, with greater than 20% killing observed at concentrations equal to or higher than 5 µg/mL. The CC8 and CC45 LukA monomers displayed little cytotoxicity toward hPMNs, as expected for toxoids lacking the LukB component critical for the formation of the active pore complex. Each of the LukAB dimer toxoids displayed notably reduced cytotoxicity toward hPMN cells in comparison with the CC8 and CC45 wild-type LukAB toxins. The RARPR-33 LukAB toxoid, as well as related toxoids RARPR-32 and -34, displayed less cytotoxicity than CC8delta10, with RARPR-33 killing only 15% of the cell population at the highest tested concentration, 20 µg/mL.

Table 7. Cytotoxicity of LukA or LukAB proteins against human primary polymorphonuclear leukocytes using a standardized amount of toxin. Data are presented as the percent of dead cells

Toxoid/Toxin	LukAB Concentration (µg/mL)										
	20	10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.04	0.02
RARPR-15	42	36	31	20	14	11	11	11	6	10	11
RARPR-30	27	17	14	10	5	0	0	0	0	0	0
RARPR-31	12	2	1	0	0	3	0	0	0	0	0
RARPR-32	17	6	4	4	2	1	0	0	0	0	0
RARPR-33	15	8	6	3	1	0	0	0	0	0	0
RARPR-34	16	9	5	3	0	0	0	0	0	0	0
CC8 LukA W97 (monomer)	5	3	2	1	2	1	1	2	0	2	1
CC45 LukA W97 (monomer)	7	6	2	3	4	1	0	1	2	0	0
CC8delta10	29	26	22	18	11	11	4	4	3	4	3
CC8 toxin	93	96	97	96	97	93	90	87	75	52	29
CC45 toxin	97	97	97	97	96	85	72	57	49	30	19

Example 3: Cytotoxicity of RARPR-33 LukAB Toxoids and Other Variants of WT LukAB Toxins

[0342] An additional experiment was performed to evaluate the cytotoxicity and immunogenicity of RARPR-33 and different variants of WT LukAB toxin and LukA monomers. Mice were used for the immunogenicity studies.

[0343] The cytotoxicity of LukAB toxins, toxoids, and monomers was assessed on human PMNs. Prior to intoxication, all toxins were normalized to 100 µg/mL (per subunit) and then 20 µL of toxin was pipetted into the top wells of a 96-well plate and serially diluted 2-fold in 10 µL of 1X PBS. PMNs were isolated from different donors and normalized to 200,000 cells per 90 µL RPMI (10 mM HEPES + 0.1% HSA). 90 µL of PMNs were pipetted into each well and the toxin-PMN mixture was incubated in a 37°C + 5% CO₂ incubator for 1 hour. To assess toxicity, 10 µL of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hours. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance of 492 nm. The percentage of dead cells was calculated by subtracting out background (healthy cells + PBS) and normalizing to Triton X100-treated cells which are set at 100% dead.

[0344] The cytotoxicity of LukA monomers and LukAB toxins against human primary PMN cells is provided in FIG. 3. The wild-type LukAB CC8 and CC45 toxins displayed greater than 90% killing of primary human PMNs at toxin concentrations of 2.5 µg/mL and 5 µg/mL, respectively. Maximum killing was also observed for the LukAB hybrid toxins CC8/CC45 and CC45/CC8 at 2.5 µg/mL. In comparison, the LukAB toxoids and the LukA monomers were considerably reduced in cytotoxicity toward these cells. Deletion of the 10 C-terminal residues in CC8 LukA retained cytotoxicity against hPMNs, with greater than 20% killing observed at concentrations equal to or higher than 5 µg/mL. The CC8 and CC45 LukA monomers and the combination of these monomers displayed little cytotoxicity toward hPMNs. The RARPR-33 LukAB toxoid displayed less cytotoxicity than CC8Δ10C, with RARPR-33 killing only 15% of the cell population at the highest tested concentration, 20 µg/mL.

Example 4: Immunogenicity of LukAB Variants in Mice

[0345] To determine immunogenicity of the different LukAB variants, Envigo Hsd:ND4 (4 week old) mice (n=5/antigen) were subcutaneously administered 20 µg of LukAB in 50 µL of 10% glycerol 1X TBS mixed with 50 µL of the adjuvant, TiterMax® Gold. A cohort of 5 mice also received a mock immunization consisting of an equal volume of 10% glycerol 1X TBS and TiterMax® Gold. Following two boosts of the same antigen-adjuvant cocktail, with two weeks apart, mice were bled via cardiac puncture and serum was obtained.

[0346] To determine anti-LukAB antibody titers, ELISAs were performed. WT LukAB CC8 or CC45 was diluted to 2 µg/ml in 1X PBS and coated in 100 µl in 96 well Immulon 2HB plates (Thermo Fisher, cat no. 3455) and incubated at 4°C overnight. Plates were then washed 3X with wash buffer (1X PBS + 0.05% Tween) and then blocked with 200 µl of blocking buffer (2.5% milk in 1X PBS) for 1hr. Five-fold serial dilutions starting at 1:500 of serum into blocking buffer were generated and allowed to incubate on the rocker for 1 hr. The plates were then washed again 3X and mouse IgG-HRP (Biorad) antibody diluted 1:5,000 in blocking buffer was added and allowed to incubate for 1 hr at room temperature. Unbound secondary antibody was washed out in three successive washes with wash buffer. TMB (100 µl) that was brought up to room temperature was added to each well and incubated covered for 25 mins. After the reaction was completed, an equal amount of 2N Sulfuric acid was then added to each reaction well to stop the reaction. The plates were then read on an Envision plate reader for 450 nm absorbance. The heatmaps depicted in Figures 4A and 4B show average absorbance values from duplicate measurements, with black representing high absorbance and antibody binding to the coating antigen and white representing low absorbance and no antibody binding.

[0347] RARPR-33 elicited robust anti-CC8 and anti-CC45 LukAB IgG antibody titers (Fig. 4A and Fig. 4B). RARPR-33 immunization elicited comparable anti-CC8 IgG responses as immunization with the CC8 WT toxin, CC8/CC45 hybrid toxin and the CC8Δ10C toxoid. The anti-CC8 LukAB IgG titers induced by the individual CC8 LukA monomer were not as high as those induced by CC8 LukAB toxin or the CC8/CC45 hybrid toxin, CC45/CC8 hybrid toxin, and RARPR 33 hybrid antigens (FIG. 4A).

[0348] The anti-CC45 LukAB titers in RARPR-33 immunized mice were higher than those elicited by the CC8/CC45 WT hybrid antigen and were on par with those elicited by the CC45 WT antigen. Combining the CC8 and CC45 LukA monomers elicited antibody titers to both CC8 and CC45 LukAB (FIG. 4B). However, these anti-CC8 and anti-CC45 LukAB titers elicited by the combined CC8 and CC45 LukA monomers were not as high as those elicited by RARPR 33. The individual CC45 LukA monomer elicits very high anti-CC45 LukAB titers - similar to the levels elicited by the CC45/CC8 hybrid and only slightly lower than those elicited by RARPR-33 or CC45 WT toxin. These results show that upon RARPR-33 immunization antibody responses towards both LukAB CC8 and CC45 are induced that are high in magnitude.

Example 5: Antibody Mediated Neutralization of Toxin Cytotoxicity

[0349] Antibody mediated neutralization of toxin cytotoxicity was assessed with serum obtained from mice immunized as described above in Example 4. Heat-inactivated pooled sera was normalized to 40% serum in PBS and then 20 μ L of serum was pipetted into the top wells of a 96-well plate and serially diluted 2-fold in 10 μ L of 1X PBS. An LD₉₀ of each of the LukAB toxin clonal complex sequence variants were added to the plate (10 μ L/well) for 15 min at room temperature. Freshly isolated human primary polymorphonuclear leukocytes (hPMNs) normalized to 200,000 cells per 80 μ L RPMI (10 mM HEPES + 0.1% HSA) were then added to the serum-toxin mixture and incubated for 1 hr at 37°C + 5% CO₂. To assess toxicity, 10 μ L of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hours. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance of 492 nm. The antibody neutralization data is presented FIG. 5.

[0350] Sera from mice immunized with RARPR-33 exhibited the most potent, broadly LukAB-neutralizing capacity of all the antigens (FIG. 5). The sera from RARPR 33-immunized mice strongly neutralized the cytotoxic effect of all 11 LukAB variants tested at as low as 0.25% serum, and for most LukAB variants also provided protection at as low as 0.063-0.125% serum (FIG. 5). Immunization with the individual CC8 and CC45 LukA monomers resulted in sera with LukAB-neutralizing capacity that is highly biased to the antigen backbone (FIG. 5). The combination of CC8 LukA monomer with CC45 LukA administered at 20 μ g of each monomer (40 μ g of total protein) for each immunization yielded sera with both broad and potent LukAB-neutralizing capacity, neutralizing all 11 LukAB variants tested at as low as 0.5% serum (FIG. 5), however, this is lower than to what was observed with sera from RARPR-33 immunized mice.

[0351] Combined, the data presented in Examples 3-5 show that the attenuating and stabilizing mutations incorporated into the CC8/CC45 LukAB backbone of RARPR-33 improves the broad immunogenic effects of the CC8/CC45 WT LukAB hybrid (FIGs. 4 and 5) while also rendering RARPR-33 highly attenuated compared to the CC8/CC45 WT LukAB toxin (FIG. 3).

Example 6: Antisera Toxin Neutralization

[0352] Antibody mediated neutralization of toxin cytotoxicity was assessed with serum obtained from mice immunized with wild-type LukAB, wild-type LukAB hybrids (*i.e.*, CC8 LukA/CC45 LukB and CC45 LukA/CC8 LukB), LukA monomers, or LukAB toxoids. Heat-

inactivated pooled sera were normalized to 40% serum in PBS and then 20 μ L of serum was pipetted into the top wells of a 96-well plate and serially diluted 2-fold in 10 μ L of 1X PBS. An LD₉₀ of each of the LukAB toxin clonal complex sequence variants were then added to the wells of the plate (10 μ L/well) containing either 2%, 1% or 0.5% serum for 15 min at room temperature. Freshly isolated human primary polymorphonuclear leukocytes (hPMNs) from different donors normalized to 200,000 cells per 80 μ L RPMI (10 mM HEPES + 0.1% HSA) were then added to the serum-toxin mixture and incubated for 1 hr at 37°C + 5% CO₂. To assess toxicity, 10 μ L of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hours. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance of 492 nm. The antibody neutralization data is presented in the Tables of FIG. 6A (2% antibody serum), FIG. 6B (1% antibody serum), and FIG. 6C (0.5% antibody serum).

[0353] Immunization with wild-type CC8 and CC45 LukAB elicited antibodies that neutralized the naturally occurring sequence variants of LukAB toxins in a pattern that reflected the sequence composition of the immunized antigen. Antibodies elicited by CC8 LukAB toxin potently neutralized toxins derived from CC8, CC1, CC5, and other *S. aureus* lineages, but they did not provide complete neutralization of toxins derived from CC30, CC45, or ST22A *S. aureus*. Likewise, immunization with CC45 LukAB toxin elicited antibodies that potently neutralized toxins derived from CC30, CC45, or ST22A *S. aureus* lineages, but not toxins derived from other lineages.

[0354] Immunization of mice with a non-natural hybrid LukAB, either CC8 LukA combined with CC45 LukB or CC45 LukA combined with CC8 LukB, elicited antibodies that displayed broader neutralization of LukAB sequence variants in comparison with the naturally occurring dimer combinations. Of the non-natural hybrid dimers, CC8 LukA and CC45 LukB displayed a slightly better neutralization profile than the opposite combination, a pattern that was retained in proteins carrying the Glu to Ala substitution in the penultimate residue of LukA (E323A). As observed for antibodies elicited against the wild-type toxins, the LukA monomers elicited antibodies that displayed a neutralization pattern indicative of their sequence compositions. A combination of CC8 LukA and CC45 LukA monomers (RARPR-31 + CC45 LukA W97) elicited antibodies that displayed a broad neutralizing pattern, but the potency of neutralization was reduced in comparison with the dimer antigens, as is evident by the reduced level of neutralization at 1% or 0.5% sera.

[0355] Of the dimer toxoids, RARPR-15, RARPR-33, and RARPR-34 displayed a broadly neutralizing antibody response against all tested LukAB sequence variants. The non-

natural wild-type dimer combinations also displayed a broad neutralization profile, although the potency of the neutralizing response was inferior to that observed for several toxoids. Both the hybrid wild-type and the toxoid antigens displayed a broadly neutralizing profile when tested at 2% (FIG. 6A) and 1% (FIG. 6B) sera, but the improved potency of the response to the toxoids was evident when tested at 0.5% sera (FIG. 6C). At this lowest tested concentration, RARPR-15, RARPR-32 RARPR-33, and RARPR-34 each displayed a broad neutralizing response. RARPR-33, in particular, elicited sera that retained a broadly neutralizing response, whereas the hybrid wild-type antigens and the E323A toxoids failed to elicit a broadly protective response at 0.5% sera, and the neutralization pattern elicited by the CC45 toxoid RARPR-15 at the lowest tested concentration reflected its sequence composition, as high levels of neutralization were only observed for CC30, CC45, and ST22A LukAB toxins. The hybrid dimer toxoid RARPR-33 elicited a potent and broadly neutralizing immune response.

Example 7: Cytotoxicity of RARPR-33 at High Concentrations.

Methods:

[0356] Cytotoxicity assay: To evaluate the cytotoxicity of each respective LukAB protein complex, freshly isolated primary human polymorphonuclear leukocytes (PMNs) were intoxicated with *S. aureus* toxins. PMNs were isolated from different donors and normalized to 200,000 cells per 50 μ l RPMI (10 mM HEPES + 0.1% HSA). 50 μ l of toxin in PBS was added to the cells and the toxin-PMN mixture was incubated in a 37°C + 5% CO₂ incubator for 1 hr. To assess toxicity, 10 μ l of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hrs. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance of 492 nm. % Dead cells are calculated by subtracting out background (healthy cells + PBS) and normalizing to TritonX-treated cells which are set at 100% dead.

[0357] LDH assay: To evaluate whether each respective LukAB protein complex can cause cell lysis, freshly isolated primary human polymorphonuclear leukocytes (PMNs) from different donors were intoxicated with *S. aureus* LukAB toxins and LDH release was measured. WT toxins were serially diluted 2-fold in PBS and tested at concentrations ranging from 5-0.0024 μ g/ml. LukAB toxoids were diluted in PBS and tested at 2.5, 2, 1, 1.5, and 0.5 mg/ml. PMNs were isolated and normalized to 200,000 cells per 50 μ l RPMI (10 mM HEPES + 0.1% HSA). 50 μ l of PMNs were then pipetted into each well and 50 μ l of diluted toxin was added per well. The toxin-PMN mixture was incubated in a 37°C + 5% CO₂ incubator for 2 hr. To assess LDH release, the plates were centrifuged at 1500 rpm for 5 min, then 25 μ l of

supernatant was removed from each well and transferred to 96-well black clear- bottom plates. 25 µl of CytoTox-ONE homogeneous membrane integrity reagent (Promega) was added to the black clear-bottom 96-well plate, and the mixture was incubated for 10 min at room temperature in the dark. Cell lysis was assessed with a PerkinElmer EnVision 2103 Multilabel Reader by recording fluorescence with an excitation wavelength of 560nm and an emission wavelength of 590nm. % Dead cells were calculated by subtracting out background (healthy cells + PBS) and normalizing to TritonX-treated cells which were set at 100% dead.

Results:

[0358] In previous examples, cytotoxicity of the LukAB toxoid RARPR-33 on hPMNs was determined up to a concentration of 20 µg/ml. Next, cytotoxicity of human PMN was monitored in presence of higher concentrations (up to 2.5mg/ml) of RARPR-33. Maximum cytotoxicity of human PMNs (4-6 donors) based on CellTiter measurements was observed for the WT LukAB CC8, CC45 and CC8/CC45 toxins upon 1 hour intoxication with ~0.156 µg/ml toxin (FIG. 7A). For RARPR-33 and the CC8 LukA monomer, the percentage of dead cells measured with CellTiter was ~10% at a concentration of 0.5 mg/ml (FIG. 7B). Incubating PMNs with concentrations up to 2.5 mg/ml of RARPR-33 or the CC8 LukA monomer did not further increase the percentage of dead cells determined by CellTiter measurements.

[0359] The LD₁₅ value indicates the concentration of an antigen which induces 15% cell death. The LD₁₅ was determined using linear regression. For CC8 WT LukAB the LD₁₅ was 0.013 µg/ml, for CC45 WT LukAB the LD₁₅ was 0.004 µg/ml, and for CC8/CC45 LukAB hybrid the LD₁₅ was 0.002 µg/ml. The LD₁₅ for LukAB RARPR-33 was at 2.5 mg/ml. The LD₁₅ values were compared by dividing the LD₁₅ concentrations of RARPR-33 by the LD₁₅ concentration of the WT antigens. Based on these observations LukAB RARPR-33 toxicity is >192,308 fold less than LukAB CC8 WT, >625,000 fold less than LukAB CC45 WT, and >1,250,000 fold less than the LukAB CC8/CC45 hybrid.

[0360] In addition, a LDH assay was performed to assess plasma membrane damage after two hours of incubation with the different WT toxins, CC8 LukA monomer or RARPR-33. Cytotoxicity of human PMN was induced after 2 hours of exposure to WT toxins, CC8 WT, CC45 WT, or the CC8/CC45 toxin hybrid (FIG. 7C). Maximum cell death, determined by LDH, was observed at a concentration of 0.625 µg/ml toxin. In contrast, no plasma membrane damage of human PMNs was observed following two hours of exposure to RARPR-33 or the CC8 LukA monomer at concentrations up to 2.5mg/ml (FIG. 7D). These data show that RARPR-33 is detoxified and unable to induce cell death of human PMNs at concentrations up

to 2.5 mg/ml. The mutations incorporated into the CC8/CC45 LukAB backbone of RARPR-33, highly attenuated the cytotoxicity compared to the CC8/CC45 WT LukAB toxin.

Example 8: Comparison of RARPR-33 vs D39A/R23E Toxoid

5 [0361] A LukAB toxoid based on a CC8 backbone was generated in which LukA has a D39A mutation and LukB has a R23E point mutation. This “D39A/R23E toxoid” was described in Kailasan, S. et al, “Rational Design of Toxoid Vaccine Candidates for *Staphylococcus aureus* Leukocidin AB (LukAB),” *Toxins* 11(6): (2019), which is hereby incorporated by reference in its entirety. This toxoid was generated on a LukAB CC8
10 backbone and was described to be > 36,000-fold attenuated in toxicity as compared to WT CC8 LukAB toxin. The cytotoxicity was determined using the HL-60 cell line differentiated to be PMN-like. In the present experiment a comparison was made between the D39A/R23E toxoid and RARPR-33. The cytotoxicity on human polymorphonuclear leukocytes (PMNs) was determined and the ability to induce broadly toxin neutralizing antibodies upon immunization
15 was assessed.

Methods:

[0362] **Cytotoxicity assays:** To evaluate the cytotoxicity of each respective LukAB protein complex, freshly isolated primary human polymorphonuclear leukocytes (PMNs) from different donors were intoxicated with *S. aureus* toxins. PMNs were isolated and normalized to
20 200,000 cells per 50 µl RPMI (10 mM HEPES + 0.1% HSA). To the cells, 50 µl of toxin in PBS was added and the toxin-PMN mixture was incubated in a 37°C + 5% CO₂ incubator for 2 hrs. To assess toxicity, 10 µl of CellTiter 96 Aqueous One Solution (CellTiter; Promega) was added to the 96-well plate, and the mixture was incubated at 37°C in 5% CO₂ for 1.5 hrs. PMN viability was assessed with a PerkinElmer EnVision 2103 Multilabel Reader at an absorbance
25 of 492 nm. The percentage of dead cells are calculated by subtracting out background (healthy cells + PBS) and normalizing to TritonX-treated cells which are set at 100% dead.

[0363] **LDH assay:** To evaluate whether each respective LukAB protein complex causes cell lysis, freshly isolated primary human polymorphonuclear leukocytes (PMNs) from different donors were intoxicated with *S. aureus* LukAB toxins and LDH release was
30 measured. WT toxins were serially diluted 2-fold in PBS and tested at concentrations ranging between 0.5 µg/ml – 0.00024 µg/ml. LukAB toxoids were diluted in PBS to a concentration ranging between 1 mg/ml – 0.03125 mg/ml and tested. PMNs were isolated and normalized to 200,000 cells per 50 µl RPMI (10 mM HEPES + 0.1% HSA). PMNs (50 µl) were then pipetted into each well and 50 µl of diluted toxin was added per well. The toxin-PMN mixtures

were incubated in a 37°C + 5% CO₂ incubator for 2 hr. To assess LDH release, the plates were centrifuged at 1500 rpm for 5 min, then 25 µl of supernatant was removed from each well and transferred to 96-well black clear-bottom plates. 25 µl of CytoTox-ONE homogeneous membrane integrity reagent (Promega) was added to the black clear-bottom 96-well plate, and the mixture was incubated for 10 min at room temperature in the dark. Cell lysis was assessed with a PerkinElmer EnVision 2103 Multilabel Reader by recording fluorescence with an excitation wavelength of 560nm and an emission wavelength of 590nm. Percentage of dead cells was calculated by subtracting out background (healthy cells + PBS) and normalizing to TritonX-treated cells which were set at 100% death.

[0364] Mouse immunizations. Envigo Hsd:ND4 (4 week old) mice (n=5/antigen) were subcutaneously administered 20 µg of LukAB in 50 µl of 10% glycerol 1X TBS mixed with 50 µl of the adjuvant, TiterMax® Gold. Following two boosts of the same antigen/adjuvant cocktail, mice were bled via cardiac puncture and serum was obtained for toxin neutralization studies.

[0365] Toxin neutralization assay. Sera from immunized mice was pooled from each group and heat inactivated in a water bath at 55°C for 30 min. The pooled, heat-inactivated sera were then diluted to 40% with PBS. Further dilutions of the sera were then achieved by serially diluting the 40% stocks 2-fold in 10 µl of PBS in a 96 well plate. Toxin (10 µl) was added into the serum wells at a final concentration of 0.156 µg/ml toxin (LD90). 80 µl of hPMNs at a concentration of 200,000 cells in RPMI + 0.1% HSA + 10 mM HEPES were added into each well. Plates were then incubated in a 37°C + 5% CO₂ incubator for 1 hr. Following the incubation, Cell Titer was added to the intoxications and incubated for 1.5 hrs. Following the incubation, plates were then read on the plate reader at 492 nm absorbance. Percentages of dead cells were calculated by subtracting out background (healthy cells +PBS) and normalizing to TritonX-treated cells which are set at 100% death.

Results:

[0366] The cytotoxicity of the D39A/R23E toxoid has been reported to be tested up to ~12 µg/ml. Here the cytotoxicity of RARPR-33 and the D39A/R23E toxoid were determined on human PMNs up to a concentration of 1 mg/ml. In addition, WT LukAB CC8, CC45 and CC8/CC45 were tested for comparison. Maximum cytotoxicity of human PMNs based on CellTiter measurements was observed upon 1-hour intoxication with ~0.02 µg/ml WT LukAB CC8/CC45, ~0.03 µg/ml LukAB CC8 and 0.125 µg/ml LukAB CC45 (FIG. 8A). The average of 5 donors is shown. For the D39A/R23E toxoid, around 22% of cytotoxicity was observed

upon incubation with 1 mg/ml. For RARPR-33, the percentage of dead cells measured with CellTiter was around 3% at a concentration of 1 mg/ml (FIG. 8B).

[0367] In addition, a LDH assay was performed to assess plasma membrane damage after two hours of incubation with the different WT toxins, the D39A/R23E toxoid and RARPR-33. Cytotoxicity of human PMN was induced after 2 hours upon exposure to WT toxins, CC8 WT, CC45 WT and the combination of CC8/CC45 toxin hybrids (FIG. 8C). Maximum cell death, determined by LDH, was observed at a concentration of 0.25 µg/ml toxin. Upon two hours exposure of human PMN with concentrations up to 1 mg/ml of the D39A/R23E toxoid, around 8% of cell death was observed. When incubating human PMNs with a similar concentration of RARPR-33, no plasma membrane damage was observed, indicating no cell death (FIG. 8D). These results indicate that RARPR-33 is attenuated to below the limit of detection in the assay and is more attenuated than the D39A/R23E toxoid.

[0368] Sera from mice immunized with RARPR-33 or the D39A/R23E toxoid was tested in a toxin neutralization assay to assess the ability of the sera to prevent toxin induced cell death of human PMNs. Neutralization towards sixteen different LukAB toxins was tested on PMNs isolated from 4 donors.

[0369] In the presence of 0.125% sera from RARPR 33-immunized mice, the cytotoxic effect of all 16 LukAB variants tested was neutralized (FIG. 9). At a similar serum concentration, sera from D39A/R23E toxoid immunized mice only protected equally as sera from RARPR-33 immunized mice against the cytotoxic effect of LukAB CC8. Against all other toxins, no protection or a much lower protection was observed for sera from D39A/R23E toxoid immunized mice. These results show that RARPR-33 immunization induced a much broader toxin neutralizing response than immunization with the D39A/R23E toxoid.

Example 9: Thermal Stabilization of LukAB Toxoids

[0370] Stability of the LukAB toxoids in comparison to the wild-type protein was assessed through thermal unfolding experiments using intrinsic tryptophan or tyrosine fluorescence to estimate the melting temperature (T_m), corresponding to the midpoint of the transition of the protein from the folded to unfolded state. Thermal stability was assessed using the NanoTemper's PromethiusNT.Plex instrument (NanoTemper Inc., Germany). Thermal unfolding measurements were made on protein samples of 0.3 to 1 mg/mL (20 µL, buffer: 50 mM sodium phosphate buffer, 200 mM NaCl, pH 7.4, 10% glycerol) in duplicate runs for each sample. Prometheus NanoDSF user interface (Melting Scan tab) was used to set up the experimental parameters for the run. The thermal scans for a typical sample span from 20°C to 95°C at a rate of 1.0°C/min. A standard mAb (CNT05825 or NIST) in the same buffer used

for the samples was included as a control, and the runs were performed in duplicate. Thermal melting profiles were analyzed with the vendor software PR.ThermControl to determine the temperature at which 50% of the protein unfolds (T_m).

[0371] Tables 8A and 8B show the thermal stability of LukA and LukAB toxoid proteins as assessed by nanoDSF. The temperature of the start of protein unfolding (T_{onset}) and the midpoint of the transition (T_{m1}) of protein unfolding are presented, along with the difference in T_m between comparable constructs with and without stabilizing substitutions (ΔT_m)

Table 8A. Single substitutions in the CC45 genetic background and combination substitutions in the hybrid CC8 / CC45 genetic background.

Toxin Name	LukA	LukB	Tonset	Tm1	ΔT_m^a
CC45 toxin	CC45 LukA ^{E321A}	CC45 LukB ^{wt}	40.3	47.3	--
CC8 / CC45 toxin	CC8 LukA ^{E320A}	CC45 LukB ^{wt}	37.7	43.9	--
	CC45 LukA ^{E321A, Lys81Met}	CC45 LukB ^{wt}	40.5	47.4	0.1
	CC45 LukA ^{E321A, Ser139Ala}	CC45 LukB ^{wt}	40.5	47.4	0.1
	CC45 LukA ^{E321A, Val111Ile}	CC45 LukB ^{wt}	40.0	47.7	0.4
	CC45 LukA ^{E321A, Val190Ile}	CC45 LukB ^{wt}	40.5	47.5	0.2
	CC45 LukA ^{E321A, Thr247Val}	CC45 LukB ^{wt}	41.3	47.3	0
	CC45 LukA ^{E321A}	CC45 LukB ^{Val53Leu}	40.7	47.8	0.5
RARPR-15	CC45 LukA ^{E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile}	CC45 LukB ^{wt}	41.2	48.9	1.6
RARPR-33	CC8 LukA ^{E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile}	CC45 LukB ^{Val53Leu}	40.0	47.9	4.0
LukA monomer	CC8 LukA ^{E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val, Tyr71Cys, Asp137Cys, Gly146Cys, Gly153Cys}	No LukB	45.2	61.8	--
LukA monomer	CC45 LukA ^{E321A, Lys81Met, Ser139Ala, Val111Ile, Val191Ile, Thr247Val, Tyr72Cys, Asp138Cys, Gly147Cys, Gly154Cys}	No LukB	51.9	58.1	--

^a ΔT_m represents the difference between the T_m values for the CC45 or the CC8 / CC45 toxins without stabilizing substitutions and disulfide bonds in comparison with the corresponding LukAB proteins carrying one or more substitutions.

LukA monomers included an N-terminal PelB signal sequence to direct expression to the periplasm of *E. coli* to support disulfide bond formation.

LukAB dimers carrying pairs of cysteine substitutions to support disulfide bond formation were expressed in the cytoplasm of *E. coli* Origami 2(DE3) cells.

Table 8B. Single substitutions in the CC8 genetic background and combination substitutions in the hybrid CC8 / CC45 genetic background.

Toxin Name	LukA	LukB	Tonset	Tm1	ΔTm^a
CC45 toxin	CC45 LukA ^{E321A}	CC45 LukB ^{wt}	37.8	45.3	--
CC8 / CC45 toxin	CC8 LukA ^{E320A}	CC45 LukB ^{wt}	35.3	42.2	--
	CC8 LukA ^{E320A, Lys80Met}	CC45 LukB ^{wt}	38.8	44.9	2.7
	CC8 LukA ^{E320A, Ser138Ala}	CC45 LukB ^{wt}	38.9	44.7	2.5
	CC8 LukA ^{E320A, Val110Ile}	CC45 LukB ^{wt}	38.0	44.0	1.8
	CC8 LukA ^{E320A, Val190Ile}	CC45 LukB ^{wt}	37.2	43.5	1.3
	CC8 LukA ^{E320A, Thr246Ile}	CC45 LukB ^{wt}	36.4	43.1	0.9
	CC8 LukA ^{E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile, Thr246Val}	CC45 LukB ^{wt}	40.5	46.8	4.6
RARPR-33	CC8 LukA ^{E320A, Lys80Met, Ser138Ala, Val110Ile, Val190Ile}	CC45 LukB ^{Val53Leu}	38.8	46.3	4.1

5 **[0372] Results:** Thermal stability analysis (Table 8A), revealed that the CC45 LukA^{E321A} / CC45 LukB protein displayed a Tm value 3°C higher than the Tm for the CC8 LukA^{E321A} / CC45 LukB hybrid protein. Individual substitutions in CC45 LukA, in combination with CC45 LukB^{wt}, resulted in modest increases in Tm of 0 to 0.4°C. The Val53Leu substitution in LukB resulted in a 0.5°C increase in Tm. As the hybrid LukAB

10 toxoids included the CC8 LukA background, individual amino acid substitutions were tested in CC8 LukA, combined with wild-type CC45 LukB (Table 8B). As seen with CC45 LukA, the individual substitutions in CC8 LukA also increased Tm values above the wild-type LukAB. Combinations of substitutions in LukA (RARPR-15) produced a Tm value 1.6°C higher than the CC45 LukA^{E321A} / CC45 LukB protein, and a combination of CC8 LukA substitutions with

15 LukB^{Val53Leu} (RARPR-33) resulted in a Tm value that was 4°C higher than the CC8 LukA^{E321A} / CC45 LukB hybrid. The increased thermal stability of RARPR-33 was observed in both datasets (Tables 8A and 8B). Although nanoDSF may produce some variability for proteins that unfold at less than 50°C, the ΔTm , determined using controls run within each set, was consistent across datasets at 4.0 and 4.1°C, respectively. The LukA monomers included both

20 combinations of substitutions and pairs of cysteine substitutions and displayed elevated Tm values of $\geq 58^\circ\text{C}$, indicating the further contribution of disulfide bonds to increased thermal stability.

Discussion of Examples 1–9:

25 **[0373]** The stable LukAB variant heterodimer toxoids described herein possess several characteristics that render them highly suitable as *S. aureus* vaccine antigen candidate.

[0374] Firstly, the LukA monomers and the LukAB dimer toxoids, including RARPR-30, RARPR-31, RARPR-32, RARPR-33, RARPR-34, and RARPR-15, displayed markedly reduced cytotoxicity toward differentiated human THP-1 and human PMNs as compared to wildtype toxins and other known toxoids (*i.e.*, CC8delta10 and CC45delta10 toxoids). Even at concentrations of up to 2.5 mg/ml, RARPR-33 remained non-cytotoxic, demonstrating its full attenuation.

[0375] Secondly, the combination of substitutions introduced in the LukA and LukB variant proteins significantly enhanced the thermal stability of the heterodimer RARPR complexes relative to corresponding toxoids containing only a single substitution. In particular, the combinations of substitutions in LukA (RARPR-15) produced a T_m value 1.6°C higher than the CC45 LukA^{E321A} / CC45 LukB protein, and a combination of CC8 LukA substitutions with LukB^{Val53Leu} (RARPR-33) resulted in a T_m value that was 4°C higher than the CC8 LukA^{E321A} / CC45 LukB hybrid.

[0376] In addition to the attenuated cytotoxicity and enhanced thermal stability, the LukAB RARPR toxoids described herein, particularly RARPR-15, RARPR-33, and RARPR-34 induced comparable or broader toxin neutralizing response and higher titers of neutralizing antibodies than wildtype CC45 and CC8 toxins, wildtype hybrid toxins, and toxoids, including the E323A toxoids and D39A/R23E toxoid.

[0377] In summary, the attenuated cytotoxicity, improved thermal stability, robust immunogenicity, and broadly neutralizing antibody profile renders the LukAB RARPR toxoids described herein ideal vaccine antigen candidates.

Example 10: Efficacy of LukAB RARPR-33, SpA*, and GLA-SE in a Surgical-Wound Minipig Infection Model

[0378] The aim of the experiment was to evaluate whether a combination of a Spa variant antigen and a RARPR LukAB dimer with or without a glucopyranosyl lipid adjuvant (GLA), a toll like receptor 4 (TLR) agonist, can provide protection in a *S. aureus* surgical-wound infection model in Göttingen minipigs. The Spa variant antigen (Spa*) that was tested had an amino acid sequence of SEQ ID NO:60. The mutant LukAB dimer RARPR-33 that was tested comprises a LukA variant polypeptide comprising the amino acid sequence of SEQ ID NO: 3 and a LukB variant polypeptide comprising the amino acid sequence of SEQ ID NO: 18. The GLA adjuvant was formulated in a stable emulsion (SE) and contained 10 µg GLA and 2% SE.

[0379] ***In vivo* Experiment.** Male Göttingen Minipigs (3 pigs per group) were immunized intramuscularly on 3 separate occasions at 3-week intervals according to the schedule shown in FIG. 10, with the following compositions or combinations of compositions:

1. Buffer control (no adjuvant, no LukAb RARPR-33, no Spa*)
- 5 2. LukAB RARPR-33 (100 µg) + Spa* (100 µg) + adjuvant GLA-SE (10 µg)
3. LukAB RARPR-33 (100 µg) + Spa* (100 µg), no adjuvant
4. Adjuvant only GLA-SE (10 µg)

[0380] Following vaccination, the pigs were challenged with a clinically-relevant *S. aureus* strain, i.e. Clonal Complex (CC) 398. At day +8 post-infection, pigs were euthanized and the bacterial burden at the surgical site was determined.

[0381] The primary endpoint of the study was the reduction in bacterial burden (cfu) at surgical site in animals vaccinated with the LukAB + Spa variant with or without the adjuvant. Vaccination with the adjuvant alone or with formulation buffer alone were used as controls.

15 **Materials and Methods:**

[0382] **Minipig Surgical Wound Infection Methods:** Five to eight-month-old male Göttingen minipigs (Marshall Biosciences, North Rose, NY) were group-housed and maintained on a 12-hour light/dark cycle with access to water ad libitum. On the morning of surgery, fasted minipigs were sedated, intubated, and placed under isoflurane anesthesia for the duration of the surgery. Surgery was performed on the left thigh whereby the muscle layer was exposed and a 5- mm bladeless trocar (Endopath® Xcel, Ethicon Endo-Surgery, Guaynabo, Puerto Rico) was advanced to the depth of the femur. A bacterial challenge consisting of 20µl inoculum (approx. 6 log₁₀ CFU/ml *S. aureus*) was injected into the wound (top of femur) via a 6-inch MILA spinal needle (Mila International, Inc., Florence, KY) through the trocar, which was then removed. After administration of the bacterial challenge, the muscle was closed with a single silk suture, and the skin closed with absorbable PDS suture. Eight days later while under sedation, minipigs were euthanized with a barbiturate. Once death was confirmed, organs were processed separately for microbiology. Samples were homogenized in saline using a Bead Ruptor Elite (Omni International, Kennesaw, GA, USA), then diluted and plated on TSA plates using an Autoplate 5000 Spiral Plater (Spiral Biotech, Norwood, MA, USA). Plates were incubated 18-24h at 37°, then read on a QCount colony counter (Spiral Biotech, Norwood, MA, USA).

[0383] One-way ANOVA with Dunnett's multiple comparison test was performed to test statistical significance between multiple groups. The ANOVA model contained group and surgery date as explanatory factors. All animal studies were reviewed and approved by the

Janssen Spring House Institutional Animal Care and Use Committee and housed in an AAALAC-accredited facility.

Results:

[0384] **Efficacy in the minipig surgical wound infection model.** To test efficacy of the vaccine with and without the adjuvant, the number of colony forming units (cfu) was determined in the mid and deep muscle after three immunizations and a challenge with a *S. aureus* strain belonging to clonal complex CC398.

[0385] In the mid muscle, immunization with the combination of LukAB, Spa* + adjuvant (GeoMean log10 cfu/g mid muscle = 1.61), resulted in a significant decrease of cfu compared to the group that was immunized with LukAB and Spa* without the adjuvant (GeoMean log10 cfu/g mid muscle = 6.03, $P=0.0018$) or compared to the group that only received the adjuvant (GeoMean log10 cfu/g mid muscle = 6.08, $P=0.0017$) (FIG. 11A). No significant differences in cfu were found when comparing the buffer control group (GeoMean log10 cfu/g mid muscle = 5.77) to the adjuvant only group ($P=0.8711$) or the LukAB RARPR-33 + Spa* group ($P=0.9392$). However, a significant decrease in cfu was found between the buffer control group and the animals receiving LukAB RARPR-33, Spa* + adjuvant ($P=0.0006$). These results indicate that the adjuvant by itself does not provide a protective effect.

[0386] In the deep muscle, immunization with the combination of LukAB and Spa* without the adjuvant resulted in a decrease of cfu (GeoMean log10 cfu/g deep muscle = 4.18, $P=0.1389$). Immunization with the combination of LukAB RARPR-33, Spa* + adjuvant, resulted in an even larger decrease of cfu compared to the group that was immunized with LukAB and Spa* without the adjuvant (GeoMean log10 cfu/g deep muscle = 1.58) and a significant decrease compared to the group that only received the adjuvant (GeoMean log10 cfu/g deep muscle = 6.37, $P=0.0360$) (FIG. 11B). No significant differences in cfu were found when comparing the buffer control group (GeoMean log10 cfu/g deep muscle = 6.14) to the adjuvant only group ($P=0.9931$) or the LukAB RARPR-33 + Spa* group ($P=0.3953$). However, a significant decrease in cfu was found between the buffer control group and the animals receiving LukAB RARPR-33, Spa* + adjuvant ($P=0.0137$).

[0387] These results show that the LukAB RARPR-33 and Spa* combination was efficacious in reducing the bacterial burden in the minipig surgical site infection model and that the addition of the GLA-SE adjuvant further enhanced the reduction of the bacterial load at the surgical site.

[0388] **Conclusion:** To test the efficacy of the vaccine composition, the ability of the vaccine to reduce the bacterial burden in the minipig surgical wound infection model was

determined using a relevant *S. aureus* strain. Immunization of minipigs with the LukAB RARPR-33 + Spa* combination of vaccine compositions resulted in a reduction of the number of colony forming units in the muscle after challenge with a relevant clinical *S. aureus* strain. The addition of the GLA-SE adjuvant to the vaccine combination further reduced the bacterial burden. Therefore, the *S. aureus* vaccine candidate containing the LukAB toxoid and the Spa* mutant, together with the GLA-SE adjuvant, effectively protected against deep-seated *S. aureus* infection in a minipig surgical site infection model.

Example 11: Immune Responses Induced by Immunogenic Compositions in a Surgical-wound Minipig Infection Model

[0389] The aim of the experiment was to evaluate whether a combination of a Spa variant antigen and a LukAB RARPR-33 dimer further combined with two different adjuvants provide protection in a *S. aureus* surgical-wound infection model in Göttingen minipigs. The Spa variant antigen (SpA*) that was tested had an amino acid sequence of SEQ ID NO:60. The LukAB RARPR dimer that was tested comprises a LukA polypeptide comprising the amino acid sequence of SEQ ID NO:3 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO:18. In one arm of this experiment, the AS01b adjuvant, which is part of the licensed Shingrix vaccine (Leroux et al. 2016) and which contains a TLR4 agonist MPL and QS-21 was tested. In another arm of the experiment, the GLA-SE adjuvant, containing the TLR4 agonist GLA formulated in a stable emulsion was tested. The stable emulsion was an oil-in-water emulsion wherein the oil was squalene.

[0390] The minipig model was used to evaluate both immunogenicity (with respect to generation of antigen-specific IgG) and efficacy of the vaccine candidates. Minipigs have been widely used in infectious disease research as their immune system and organ and skin structure are largely similar to those of humans. In this model, after infection of a wound with *S. aureus* bacteria, a local infection develops throughout the layers of muscle and skin at the surgical site. Dissemination of infection to other internal organs is also observed, and the progression of the disease is highly similar to that in humans.

[0391] LukAB toxicity to minipig polymorphonuclear neutrophils (PMNs) is similar to what has been observed in human PMNs. This is in contrast to the highly reduced LukAB toxicity observed in mouse and rabbit PMNs due to species-specificity of the target of the toxin. Furthermore, due to frequent carriage of Staphylococcal species by pigs, minipigs, similar to adult humans (but not laboratory rodents), often have high levels of pre-existing antibodies to Staphylococcal antigens (including LukAB and other *S. aureus* proteins). Therefore, this model is likely to be a more reliable indicator of potential vaccine protection

in humans, particularly for vaccines containing LukAB and Spa variant, than previously available rodent models.

[0392] ***In vivo* experiment.** Male Göttingen Minipigs (3 pigs per group) were immunized intramuscularly on three separate occasions at 3-week intervals according to the schedule shown in FIG. 12, with the following compositions or combinations of compositions:

1. Buffer control (no adjuvant, no LukAB RARPR, no Spa variant)
2. LukAB RARPR-33 (100 µg) + SpA* (100 µg) + adjuvant AS01b (25 µg MPL + 25 µg QS-21)
3. LukAB RARPR-33 (100 µg) + SpA* (100 µg) + adjuvant GLA-SE (10 µg GLA)

[0393] Following vaccination, the pigs were challenged with a clinically relevant *S. aureus* strain, Clonal Complex (CC) 398. At day +8 post-infection, pigs were euthanized and the bacterial burden at the surgical site and internal organs was determined.

[0394] Blood samples were taken prior to the start of the study and at regular intervals during the vaccination period, as shown in FIG. 12. Serum analysis was performed to evaluate serum immunoglobulin quantity and function.

[0395] The primary endpoint of the study was the reduction in bacterial burden (CFU) at surgical site/organs in animals vaccinated with the LukAB + SpA* and the different adjuvants. Vaccination with buffer only was used as control.

Materials and Methods:

[0396] **Antibody responses against LukAB and SpA measured by enzyme linked immunosorbent assay (ELISA):** To measure IgG antibody levels against LukAB CC8 and LukAB CC45, 384-well Nunc plates (Thermo Fisher Scientific) were coated with 1.0 µg/ml LukAB CC8 or LukAB CC45 in PBS and incubated for 1h at 2-8°C. After washing with PBS + 0.05% Tween-20, plates were blocked with 2.5% skimmed milk, washed and serial 3-fold dilutions of serum prepared in diluent buffer (2.5% (w/v) skimmed milk powder in 1xPBS) starting at 1:10 were added to the wells. Plates were incubated for 1 hour at room temperature, washed and anti-Pig IgG-HRP secondary antibody (Sigma Aldrich) diluted 1:10,000 was added. After incubation at room temperature for 1 hour, plates were developed with TMB substrate (Leinco Technologies). The reaction was stopped by adding 1M sulphuric acid. Absorbance was read at 450nm. EC₅₀ titers, defined as half maximal effective concentration, were calculated based on duplicate 12-step titration curves that were analyzed with a 4-parameter logistic (PL) nonlinear regression model. Samples with an EC₅₀ titer below 30 were censored to 30. A Tobit model for potentially censored values was used to test statistical significance between the vaccine + adjuvant groups vs the buffer only group after three

immunizations. A Bonferroni correction was used to correct for multiple comparisons. To measure antibodies against SpA*, 96-well maxisorp plates were coated with 0.25 µg/ml SpA* in PBS and incubated over night at 2-8°C. Secondary antibody was a 1:10,000 dilution of anti-Pig IgG-HRP in blocking buffer. The other steps were similar as described above for the measurement of anti-LukAB antibody responses. A Tobit model for potentially censored values was used to test statistical significance between the vaccine + adjuvant groups vs the buffer only group after three immunizations. A Bonferroni correction was used to correct for multiple comparisons.

[0397] LukAB toxin neutralization assay. Cyto-Tox-One kit (Promega) was used to measure the release of lactate dehydrogenase (LDH) from cells with a damaged membrane. THP-1 cells were centrifuged and resuspended with RPMI to a density of 2×10^6 cells/mL. Cells (50 µL) were added to the 96 well culture plates containing serial 3-fold dilutions of serum or a 3-fold serial dilution of a reference LukAB monoclonal antibody with a starting concentration of 2,500 ng/mL. LukAB toxin CC8, CC45, CC22a, or CC398 was added to the test wells to a final concentration of 40 ng/mL (CC8, CC22a, CC398) or 20 ng/mL (CC45). Lysis solution (Promega) was added to the lysis control wells. The plates were incubated for 2 hours at 37°C in presence of 5% CO₂. The plates were centrifuged, 25 µL of the supernatant was transferred to a new plate and 25 µL CytoTox-ONE reagent (Promega) was added. Plates were incubated for 15 minutes at room temperature and stop solution (Promega) was added to the wells. Plates were read with the Biotek Synergy Neo 2 reader in monochromatic with an excitation wavelength of 560 and bandwidth of 5nm and an emission wavelength of 590 and bandwidth of 10nm. Gain is set at 120-130. IC₅₀ titers, representing the concentration at which 50% cytotoxicity was observed, were determined for all serum samples and the LukAB monoclonal reference antibody. Relative potency titers, representing the difference in IC₅₀ titers between serum samples and the reference monoclonal antibody were used as output value. Relative potency titers of the vaccine groups were compared to the buffer group after three immunizations. One-way ANOVA with Dunnett's multiple comparison test was performed to test statistical significance between the vaccine groups vs the buffer group.

[0398] Minipig Surgical Wound Infection Methods: Five to eight-month-old male Göttingen minipigs (Marshall Biosciences, North Rose, NY) were group-housed and maintained on a 12-hour light/dark cycle with access to water ad libitum. On the morning of surgery, fasted minipigs were sedated, intubated, and placed under isoflurane anesthesia for the duration of the surgery. Surgery was performed on the left thigh whereby the muscle layer was exposed and a 5-mm bladeless trocar (Endopath® Xcel, Ethicon Endo-Surgery, Guaynabo,

Puerto Rico) was advanced to the depth of the femur. A bacterial challenge consisting of 20 μ L inoculum (approx. 6 log₁₀ CFU/ml *S. aureus*) was injected into the wound (top of femur) via a 6-inch MILA spinal needle (Mila International, Inc., Florence, KY) through the trocar, which was then removed. After administration of the bacterial challenge, the muscle was closed with a single silk suture, and the skin closed with absorbable PDS suture. Eight days later while under sedation, minipigs were euthanized with a barbiturate. Once death was confirmed, organs were processed separately for microbiology. Samples were homogenized in saline using a Bead Ruptor Elite (Omni International, Kennesaw, GA, USA), then diluted and plated on TSA plates using an Autoplate 5000 Spiral Plater (Spiral Biotech, Norwood, MA, USA). Plates were incubated 18-24h at 37°C, then read on a QCount colony counter (Spiral Biotech, Norwood, MA, USA).

[0399] One-way ANOVA with Dunnett's multiple comparison test was performed to test statistical significance in cfu between the buffer group and groups that were immunized with LukAB RARPR-33 + SpA* + different adjuvants. The ANOVA model contains group and surgery dates as explanatory factors. All animal studies were reviewed and approved by the Janssen Spring House Institutional Animal Care and Use Committee and housed in an AAALAC-accredited facility.

Results:

[0400] **Antibody responses induced against LukAB and SpA*.** The groups of minipigs mentioned above were immunized on three occasions, three weeks apart with the combination of LukAB RARPR-33 (100 μ g) and SpA* (100 μ g) + adjuvant AS01b (25 μ g MPL and 25 μ g QS-21) or GLA-SE (10 μ g GLA, stable emulsion). A control group of animals was included that received only buffer. Animals were challenged with *S. aureus* three weeks after the third immunization. Blood samples were taken before each immunization and before challenge (FIG. 12) and analyzed for antibody responses against LukAB and SpA* by ELISA. In animals immunized with buffer only, low levels of anti-LukAB CC8 and CC45 IgG antibodies were measured, indicating the presence of pre-existing antibodies to LukAB (FIGs.13A and 13B). Antibody levels in the serum did not increase in time throughout the course of the experiment. Immunization with LukAB RARPR-33, SpA* adjuvanted with AS01b or GLA-SE resulted in higher geometric mean (Geomean) anti LukAB CC8 and LukAB CC45 IgG titers compared to the control group after three immunizations (FIGs.13A and 13B, Geomean IgG titers LukAB CC8 post three immunizations: LukAB RARPR-33 + SpA* + AS01b: 64637; $P=0.0034$ LukAB RARPR-33 + SpA* + GLA-SE: 116357, $P = 0.0003$; buffer control group: 2931. Geomean IgG titers LukAB CC45 post three immunizations: LukAB

RARPR-33 + SpA* + AS01b: 19764, $P < 0.0001$; LukAB RARPR-33 + SpA* + GLA-SE: 11620, $P < 0.0001$; buffer control group: 129).

[0401] Minipigs immunized with the buffer only had no measurable antibodies against SpA* at any time point (FIG. 13C). Immunization with LukAB RARPR-33, SpA* adjuvanted with AS01b or GLA-SE resulted in higher geometric mean (Geomean) anti SpA* IgG titers compared to the control group after three immunizations. (FIG. 13C, Geomean IgG SpA* post three immunizations: LukAB RARPR-33 + SpA* + AS01b: 7013, $P < 0.0001$; LukAB RARPR-33 + SpA* + GLA-SE: 1770, $P < 0.0001$; buffer control group: 30). These results indicate an induction of SpA* specific antibodies by the LukAB + SpA* + adjuvant vaccines.

[0402] Neutralization of the Cytotoxic Activity of LukAB Toxin. LukAB is a toxin that binds to receptors on neutrophils where it forms pores in the membrane and results in lysis of the cell. To assess the functionality of antibodies induced by the test vaccines, the ability of the sera from the vaccinated minipigs to inhibit LukAB toxin induced lysis of THP-1 cells was measured. The wild type LukAB toxin in the assay was from the clonal complex CC8 or CC45. LukA in LukAB RARPR-33 is derived from clonal complex CC8, LukB in LukAB RARPR-33 is derived from clonal complex CC45. A reference monoclonal LukAB specific antibody was also used in the assay. Difference in IC50 titers, representing the dilution at which 50% of the cytotoxicity is measured, between serum samples and the reference antibody were determined and plotted as relative potency titers (RP-titers). Neutralizing antibodies towards LukAB CC8 and LukAB CC45 were detected in the sera of minipigs at the start of the experiment (LukAB CC8 Geomean RP titer pre-immunization buffer control group: 1126; LukAB RARPR-33 + SpA* + AS01b: 1483; LukAB RARPR-33 + SpA* + GLA-SE: 896. LukAB CC45 Geomean RP titer pre-immunization buffer control group: 616; LukAB RARPR-33 + SpA* + AS01b: 954; LukAB RARPR-33 + SpA* + GLA-SE: 637). In animals vaccinated with the buffer only the RP-titers did not change over the course of the experiment (post three immunizations Geomean RP titer LukAB CC8: 1497; LukAB CC45: 884). In animals vaccinated with LukAB + SpA* + adjuvant, significantly higher GeoMean RP titers were measured in the sera after three immunizations (LukAB CC8 Geomean RP titer LukAB RARPR-33 + SpA* + AS01b: 17095, $P = 0.0007$; LukAB RARPR-33 + SpA* + GLA-SE: 10285, $P = 0.0116$. LukAB CC45 Geomean RP titer LukAB RARPR-33 + SpA* + AS01b: 20019, $P = 0.0022$; LukAB RARPR-33 + SpA* + GLA-SE: 16612, $P = 0.0047$). These results, shown in FIGs. 14A and 14B, indicate that LukAB in the vaccine (RARPR-33) induces functional antibodies that block the cytotoxic activity of the LukAB toxin.

[0403] **Cross neutralization of the cytotoxic activity of LukAB toxin.** Next, it was assessed whether the antibodies induced in minipigs upon immunization with LukAB RARPR-33 + SpA* and different adjuvants were able to cross neutralize the cytotoxicity of LukAB sequence variants that were not present in the backbone of RARPR-33. For this purpose, LukAB sequence variants CC22a and CC398 were used. Cross neutralization was measured by assessing the ability of the serum to inhibit LukAB toxin induced lysis of THP-1 cells. A reference monoclonal LukAB specific antibody was also used in the assay and relative potency titers were determined as described above. Cross neutralizing antibodies towards LukAB CC22a and LukAB CC398 were detectable in minipig sera at the start of the experiment (CC22a Geomean RP titer pre-immunization buffer control group: 541; LukAB RARPR-33 + SpA* + AS01b: 846; LukAB RARPR-33 + SpA* + GLA-SE: 436. LukAB CC398 Geomean RP titer pre-immunization buffer control group: 1061; LukAB RARPR-33 + SpA* + AS01b: 1090; LukAB RARPR-33 + SpA* + GLA-SE: 608). In animals vaccinated with the buffer only the RP-titers did not change over the course of the experiment (post three immunizations Geomean RP titer LukAB CC22a: 761; LukAB CC398: 1270). In animals vaccinated with LukAB + SpA* + adjuvant (AS01b or GLA-SE), significantly higher GeoMean RP titers were measured in the sera after three immunizations (LukAB CC22a Geomean RP titer LukAB RARPR-33 + SpA* + AS01b: 7524, $P = 0.0040$; LukAB RARPR-33 + SpA* + GLA-SE: 5025, $P = 0.0312$. LukAB CC398 Geomean RP titer LukAB RARPR-33 + SpA* + AS01b: 14396, $P = 0.0005$; LukAB RARPR-33 + SpA* + GLA-SE: 8051, $P = 0.0146$). These results, shown in FIGs. 14C and 14D, indicate that LukAB in the vaccine (RARPR-33) induces cross neutralizing antibodies that block the cytotoxic activity of various LukAB toxin sequence variants.

[0404] **Efficacy in the Minipig Surgical Wound Infection Model:** To test vaccine efficacy, the number of colony forming units (cfu) was determined at two sites of the muscle (mid and deep) and the spleen after three immunizations and a challenge with *S. aureus* from clonal complex CC398. Immunization with LukAB RARPR-33 + SpA* + AS01b adjuvant (GeoMean log₁₀ cfu/g muscle (mid) = 0.98, $P=0.0057$) or LukAB RARPR-33 + SpA* + GLA-SE (GeoMean log₁₀ cfu/g muscle (mid) = 0.83, $P=0.0046$) resulted in a significant decrease of cfu in the mid muscle compared to the adjuvant only group (GeoMean log₁₀ cfu/g muscle (mid) = 5.99) (FIG. 15A). Immunization with LukAB RARPR-33 + SpA* + AS01b adjuvant (GeoMean log₁₀ cfu/g muscle (deep) = 0.58, $P=0.0024$) or LukAB RARPR-33 + SpA* + GLA-SE (GeoMean log₁₀ cfu/g muscle (deep) = 0.76, $P=0.0031$) resulted also in a significant decrease of cfu in the deep muscle compared to the adjuvant only group (GeoMean

$\log_{10}$ cfu/g muscle (deep) = 6.10) (FIG. 15B). In the spleen, higher levels of cfu were observed in the control group immunized with adjuvant only (GeoMean $\log_{10}$ cfu/g spleen = 2.20) compared to immunization with LukAB + SpA* + AS01b or LukAB + SpA* + GLA-SE (GeoMean $\log_{10}$ cfu/g spleen = 0.51, $P=0.0138$ and 0.45, $P=0.0120$, respectively) (FIG. 15C).

These results, shown in FIGs. 15A–15C, indicate that the tested vaccine combination is efficacious in the minipig surgical site infection model. The vaccines also reduce the spread of the bacteria to organs like the spleen.

[0405] Conclusion: A vaccine composition containing the antigens LukAB RARPR-33 and SpA* with an adjuvant was shown to be immunogenic in minipigs as IgG antibodies against LukAB CC8, LukAB CC45 and SpA* were induced. The increase of anti-LukAB IgG antibody was associated with an increased cross-neutralization of the cytotoxic activity of the LukAB toxin, indicating that the induced IgG antibodies are functional. To test the efficacy of the vaccine composition, the ability of the vaccine to reduce the bacterial burden in the minipig surgical wound infection model was determined using a relevant *S. aureus* strain. Immunization of minipigs with the LukAB RARPR-33 + SpA* + adjuvant vaccine composition resulted in a significant reduction of the number of colony forming units in the muscle after challenge with the test strain. The vaccine composition also resulted in a significant reduction of cfu in the spleen. Therefore, the tested *S. aureus* vaccine candidate containing LukAB and SpA toxoid mutants effectively protected against deep-seated *S. aureus* infection and dissemination in a minipig surgical site infection model.

Example 12: Efficacy of LukAB RARPR-33 and Spa* in a Surgical-Wound Minipig Infection Model against a *S. aureus* USA300 strain

[0406] In example 10, it was shown that the combination of LukAB RARPR-33 and Spa*, without an adjuvant, provided some protection against a *S. aureus* challenge with a CC398 strain in the surgical site infection model in minipigs. The aim of this experiment was to evaluate whether Spa* in combination with LukAB RARPR-33 could provide protection in a *S. aureus* surgical-wound infection model in Göttingen minipigs, against a different challenge strain, in the absence of an adjuvant. The clinically relevant USA300 *S. aureus* strain was used.

[0407] The Spa variant antigen (Spa*) that was tested had an amino acid sequence of SEQ ID NO:60. The mutant LukAB dimer RARPR-33 that was tested comprises a LukA variant polypeptide comprising the amino acid sequence of SEQ ID NO: 3 and a LukB variant polypeptide comprising the amino acid sequence of SEQ ID NO: 18.

[0408] In vivo Experiment. Male Göttingen Minipigs (3 pigs per group) were immunized intramuscularly on 3 separate occasions at 3-week intervals according to the

schedule shown in FIG. 16A, with the following compositions or combinations of compositions (FIG. 16B):

1. Buffer control
2. LukAB RARPR-33 (100 µg) + Spa* (100 µg)

5 Following vaccination, the pigs were challenged with a clinically relevant *S. aureus* USA300 strain. At day +8 post-infection, pigs were euthanized and the bacterial burden at the surgical site was determined. The primary endpoint of the study was the reduction in bacterial burden (cfu) at a surgical site in animals vaccinated with LukAB and Spa variants. Vaccination with formulation buffer was used as a control.

10 **Materials and Methods:**

[0409] Minipig Surgical Wound Infection Methods: Göttingen minipigs were challenged with a *S. aureus* USA300 strain in the minipig surgical wound infection model. The challenge and determination of bacterial burden at the surgical site was performed according to the description in examples 10 and 11.

15 **Results:**

[0410] Efficacy in the minipig surgical wound infection model. To test the efficacy of the combination of the vaccine antigens, the number of colony forming units (cfu) was determined in the mid and deep muscle after three immunizations and a challenge with *S. aureus* USA300 strain.

20 **[0411]** In the mid muscle, immunization with the combination of LukAB RARPR-33 and Spa* (GeoMean log₁₀ cfu/g mid muscle = 2.15), resulted in a decrease in cfu compared to the group that only received the buffer (GeoMean log₁₀ cfu/g mid muscle = 5.73, *P*=0.2790) (FIG. 16C).

[0412] In the deep muscle, immunization with the combination of LukAB RARPR-33, 25 Spa* (GeoMean log₁₀ cfu/g deep muscle = 3.65), resulted in a significant decrease of cfu compared to the buffer control group (GeoMean log₁₀ cfu/g deep muscle = 6.21, *P*=0.0245) (FIG. 16D). These results show that, in absence of an adjuvant, the tested vaccine combination, is efficacious against a USA300 strain in the minipig surgical site infection model.

[0413] Conclusion: To test the efficacy of the combination of the vaccine antigens, the 30 ability of the vaccine combination to reduce the bacterial burden in the minipig surgical wound infection model was determined using a *S. aureus* USA300 strain. No adjuvant was used in this study. Immunization of minipigs with LukAB RARPR-33 + Spa* resulted in a reduction of the number of colony forming units in the muscle after challenge with the test strain compared to the buffer control group. These results show that, in the absence of an adjuvant, the

combination of LukAB RARPR-33 and Spa*, provides some level of protection against a *S. aureus* USA300 strain in the SSI model in minipigs.

Example 13: Efficacy of LukAB RARPR-33, Spa*, and GLA-SE in a Surgical-Wound Minipig Infection Model against a USA100 *S. aureus* strain

[0414] The aim of the experiment was to evaluate whether a combination of a Spa variant antigen and a RARPR LukAB dimer together with a glucopyranosyl lipid adjuvant (GLA), a toll like receptor 4 (TLR) agonist, can provide protection in a surgical-wound infection model in Göttingen minipigs against a challenge with a methicillin resistant *S. aureus* (MRSA) USA100 strain. USA100 isolates are responsible for a large portion of health care associated MRSA infections. The Spa variant antigen (Spa*) that was tested had an amino acid sequence of SEQ ID NO:60. The mutant LukAB dimer RARPR-33 that was tested comprises a LukA variant polypeptide comprising the amino acid sequence of SEQ ID NO: 3 and a LukB variant polypeptide comprising the amino acid sequence of SEQ ID NO: 18. The GLA adjuvant was formulated in a stable emulsion (SE) and contained 10 µg GLA and 2% SE.

[0415] ***In vivo* Experiment.** Male Göttingen Minipigs (3 pigs per group) were immunized intramuscularly on 3 separate occasions at 3-week intervals according to the schedule shown in FIG. 17A, with the following compositions or combinations of compositions (FIG. 17B):

1. Adjuvant GLA-SE (10 µg, 2% SE) (no LukAb RARPR-33, no Spa*)
2. LukAB RARPR-33 (100 µg) + Spa* (100 µg) + adjuvant GLA-SE (10 µg, 2% SE)

Following vaccination, the pigs were challenged with a clinically relevant *S. aureus* USA100 strain (ST5). At day +8 post-infection, pigs were euthanized and the bacterial burden at the surgical site was determined. The primary endpoint of the study was the reduction in bacterial burden (cfu) at a surgical site, in animals vaccinated with the LukAB + Spa variant combination together with GLA-SE, as compared to the animals vaccinated with GLA-SE alone.

Materials and Methods:

[0416] **Minipig Surgical Wound Infection Methods:** Göttingen minipigs were challenged with a *S. aureus* USA100 strain in the minipig surgical wound infection model. The challenge and determination of bacterial burden at the surgical site was performed according to the description in example 10 and 11. To test statistical significance between the two groups an ANOVA model was used.

Results:

[0417] Efficacy in the minipig surgical wound infection model. To test the efficacy of the vaccine combination, the number of colony forming units (cfu) was determined in the mid and deep muscle after three immunizations and challenge with *S. aureus* USA100.

5 **[0418]** In the mid muscle, immunization with the combination of LukAB RARPR-33, Spa* + GLA-SE (GeoMean log10 cfu/g mid muscle = 0.88), resulted in a significant decrease of cfu compared to the group that was immunized with GLA-SE alone (GeoMean log10 cfu/g mid muscle = 5.27, $P=0.0013$) (FIG. 17C). Also in the deep muscle, immunization with the combination of LukAB RARPR-33, Spa* + GLA-SE (GeoMean log10 cfu/g deep muscle =
10 0.30), resulted in a significant decrease of cfu compared to the group that was immunized with GLA-SE alone (GeoMean log10 cfu/g deep muscle = 5.37, $P<0.0001$) (FIG. 17D). These results indicate the combination of LukAB RARPR-33, Spa* and GLA-SE provide protection from a *S. aureus* USA100 strain in the SSI model and show that the test vaccine is efficacious in minipigs.

15 **[0419] Conclusion:** To test the efficacy of the combination of LukAB RARPR-33, Spa* and GLA-SE, the ability of the vaccine to reduce the bacterial burden in the minipig surgical wound infection model was determined using a relevant *S. aureus* USA100 strain. Immunization of minipigs with the LukAB RARPR-33 + Spa* + GLA-SE adjuvant vaccine composition resulted in a reduction of the number of colony forming units in the muscle after
20 challenge with the test strain. Combined with the results from the previous examples, it shows that the *S. aureus* vaccine combination containing a LukAB toxoid and a Spa mutant can effectively protect against a deep-seated infection caused by various clinically relevant *S. aureus* strain in a minipig surgical site infection model.

25 **Example 14: Immunogenicity of LukAB RARPR-33 and Spa* in combination with different adjuvants**

[0420] The aim of the experiment was to evaluate whether different adjuvants would improve the immunogenicity of a combination of a Spa variant antigen and a RARPR LukAB dimer. The Spa variant antigen (Spa*) that was tested had an amino acid sequence of SEQ ID NO:60. The mutant LukAB dimer RARPR-33 that was tested comprises a LukA variant
30 polypeptide comprising the amino acid sequence of SEQ ID NO: 3 and a LukB variant polypeptide comprising the amino acid sequence of SEQ ID NO: 18. Two adjuvants containing a TLR4 agonist were tested; AS01b (containing 5 µg MPL and 5 µg QS-21) and GLA formulated in a stable emulsion (GLA-SE, containing 1 µg GLA, 2% SE). In addition, two

Alum-based adjuvants were included: Alhydrogel adjuvant 2% (Aluminium hydroxide gel) and Adju-phos adjuvant (Aluminium phosphate gel).

[0421] In vivo Experiment. Female Swiss Webster mice (5-10 mice per group) were immunized subcutaneously on 3 separate occasions at 2-week intervals according to the schedule shown in FIG. 18A, with the following compositions or combinations of compositions (FIG. 18B):

1. LukAB RARPR-33 (5 µg) + SpA* (5 µg) + AS01b (5 µg MPL + 5 µg QS-21)
2. LukAB RARPR-33 (5 µg) + SpA* (5 µg) + GLA-SE (1 µg GLA, 2% SE)
3. LukAB RARPR-33 (5 µg) + SpA* (5 µg) + Alhydrogel adjuvant (50 µl)
4. LukAB RARPR-33 (5 µg) + SpA* (5 µg) + Adju-Phos adjuvant (50 µl)
5. LukAB RARPR-33 (5 µg) + SpA* (5 µg)
6. Buffer + AS01b (5 µg MPL and 5 µg QS-21)
7. Buffer + GLA-SE (1 µg GLA, 2% SE)
8. Buffer + Alhydrogel adjuvant (50 µl)
9. Buffer + Adju-Phos adjuvant (50 µl)
10. Buffer

Blood samples were taken prior to the start of the study and 2 weeks after the third immunization, as shown in FIG. 18A. Serum analyses were performed to evaluate serum immunoglobulin quantity and function. Vaccination with adjuvant or formulation buffer without vaccine antigens was used as a control.

Materials and Methods:

[0422] Antibody responses against LukAB and SpA measured by enzyme linked immunosorbent assay (ELISA): To measure IgG antibody levels against LukAB CC8 and LukAB CC45, 384-well Nunc plates (Thermo Fisher Scientific) were coated with 1.0 µg/ml LukAB CC8 or LukAB CC45 in PBS and incubated for 1h at 2-8°C. After washing with PBS + 0.05% Tween-20, plates were blocked with 2.5% skimmed milk, washed and serial 3-fold dilutions of serum prepared in diluent buffer (2.5% (w/v) skimmed milk powder in 1xPBS) starting at 1:90 dilution were added to the wells. Plates were incubated for 1 hour at room temperature, washed and anti-mouse IgG-HRP secondary antibody (Sigma Aldrich) diluted 1:2,000 was added. After incubation at room temperature for 1 hour, plates were developed with TMB substrate (Leinco Technologies). The reaction was stopped by adding 1M sulphuric acid. Absorbance was read at 450nm. EC₅₀ titers, defined as half maximal effective concentration, were calculated based on duplicate 12-step titration curves that were analyzed

with a 4-parameter logistic (PL) nonlinear regression model. Samples with an EC50 titer below 30 were censored to 30.

[0423] To measure antibodies against SpA*, 384-well maxisorp plates were coated with 0.25 µg/ml SpA* in PBS and incubated overnight at 2-8°C. Secondary antibody was a 1:2,000 dilution of anti-Mouse IgG-HRP in blocking buffer. The other steps were similar as described above for the measurement of anti-LukAB antibody responses.

[0424] **LukAB toxin neutralization assay.** Cyto-Tox-One kit (Promega) was used to measure the release of lactate dehydrogenase (LDH) from cells with a damaged membrane. THP-1 cells were centrifuged and resuspended with RPMI to a density of 2×10^6 cells/mL. Cells (50 µL) were added to the 96 well culture plates containing serial 3-fold dilutions of serum or a 3-fold serial dilution of a reference LukAB monoclonal antibody with a starting concentration of 2,500 ng/mL. LukAB toxin CC8 and CC45 was added to the test wells to a final concentration of 40 ng/mL or 20 ng/ml, respectively. Lysis solution (Promega) was added to the lysis control wells. The plates were incubated for 2 hours at 37°C in presence of 5% CO₂. The plates were centrifuged, 25 µL of the supernatant was transferred to a new plate and 25 µL CytoTox-ONE reagent (Promega) was added. Plates were incubated for 15 minutes at room temperature and stop solution (Promega) was added to the wells. Plates were read with the Biotek Synergy Neo 2 reader in monochromatic with an excitation wavelength of 560 and bandwidth of 5nm and an emission wavelength of 590 and bandwidth of 10nm. Gain is set at 120-130. IC₅₀ titers, representing the concentration at which 50% cytotoxicity was observed, were determined for all serum samples and the LukAB monoclonal reference antibody. Relative potency titers, representing the difference in IC₅₀ titers between serum samples and the reference monoclonal antibody were used as output value.

Results:

[0425] **Antibody responses induced against LukAB and SpA*.** The groups of mice mentioned above were immunized on three occasions, two weeks apart with the combination of LukAB RARPR-33 (5 µg) and SpA* (5 µg) with or without an adjuvant. As control, animals were immunized with an adjuvant and a formulation buffer or with a formulation buffer only, in both cases without antigens. See FIG. 18B.

[0426] Blood samples were taken according to FIG. 18A and sera was analyzed for antibody responses against LukAB sequence variants CC8 and CC45 and SpA* by ELISA. No LukAB or SpA*-specific pre-existing antibodies were detected in all groups before immunization (FIGs. 18C-E). In the animals immunized with adjuvant and/or with the formulation buffer only, antibody levels in the sera did not increase in time throughout the

course of the experiment (FIGs. 18C-E) indicating that the adjuvants by themselves do not induce a specific antibody response, and that antigen is required.

[0427] To evaluate whether an adjuvant could enhance the immunogenicity of LukAB RARPR-33 and SpA*, antibody IgG titers against these antigens were compared between
5 animals that have been immunized with LukAB RARPR-33 + SpA* with or without an adjuvant.

[0428] Immunization with LukAB RARPR-33, SpA* combined with AS01b resulted in higher geometric mean IgG titers for LukAB CC8 and CC45 and for SpA* (Geomean IgG LukAB CC8: 8079; Geomean IgG LukAB CC45: 5012; Geomean IgG SpA*: 31496) as
10 compared to the animals that were immunized with LukAB RARPR-33, SpA* without adjuvant (Geomean IgG LukAB CC8: 315; Geomean IgG LukAB CC45: 141; Geomean IgG SpA*: 282).

[0429] Immunization with LukAB RARPR-33, SpA* combined with GLA-SE also resulted in higher geometric mean IgG titers for LukAB CC8 and CC45 and for SpA*
15 (Geomean IgG LukAB CC8: 1401; Geomean IgG LukAB CC45: 3757; Geomean IgG SpA*: 9012) as compared to the animals that were immunized with LukAB RARPR-33, SpA* without adjuvant (Geomean IgG LukAB CC8: 315; Geomean IgG LukAB CC45: 141; Geomean IgG SpA*: 282). These results indicate that adjuvants containing a TLR4 agonists improve the immunogenicity of LukAB RARPR-33 and SpA*.

[0430] Immunization with LukAB RARPR-33, SpA* combined with Alhydrogel resulted in higher geometric mean IgG titer for LukAB CC8 and CC45 (Geomean IgG LukAB CC8: 595; Geomean IgG LukAB CC45: 263) as compared to the animals that were immunized
20 with LukAB RARPR-33, SpA* without adjuvant (Geomean IgG LukAB CC8: 315; Geomean IgG LukAB CC45: 141). For SpA*-specific antibody responses, the highest geometric mean
25 IgG titer was observed in the group of animals immunized with LukAB RARPR-33, SpA* combined with Alhydrogel (Geomean IgG SpA*: 93318).

[0431] Immunization with LukAB RARPR-33, SpA* combined with Adju-phos resulted in higher geometric mean titers for LukAB CC8 and CC45 (Geomean IgG LukAB CC8: 645; Geomean IgG LukAB CC45: 593) as compared to the animals that were immunized with
30 LukAB RARPR-33, SpA* without adjuvant (Geomean IgG LukAB CC8: 315; Geomean IgG LukAB CC45: 141). For SpA*, the geometric mean IgG titer was higher in the group of animals immunized with LukAB RARPR-33, SpA* combined with Adju-phos (Geomean IgG SpA*: 11614) as compared to the group that was immunized with LukAB RARPR-33, SpA* without adjuvant (Geomean IgG SpA*: 282).

[0432] These results indicate that alum-based adjuvants have a larger effect on Spa-specific antibody responses than on LukAB-specific antibody response. Combined, all adjuvants tested here, either containing a TLR4 agonists or Alum-based adjuvants, improved the immunogenicity of the combination vaccine of LukAB RARPR-33 and SpA*.

5 [0433] **Neutralization of cytotoxic activity of LukAB toxin.** The ability of the sera from immunized mice to protect THP-1 cells from cell death inflicted by a cytotoxic dose of LukAB CC8 and CC45 was assessed in a toxin neutralization. Only sera samples from animals immunized with LukAB RARPR-33 + SpA* with or without adjuvant isolated at day 42 (5 mice per group) were included, as in these groups LukAB CC8- and CC45- specific antibodies
10 were detected by ELISA (FIGs. 18C-D).

[0434] A reference monoclonal LukAB specific antibody was included in the assay. Differences in IC50 titers, representing the dilution at which 50% of the cytotoxicity is measured, between serum samples and the reference antibody were determined and plotted as relative potency titers (RP-titers).

15 [0435] For LukAB CC8, in the groups immunized with LukAB RARPR-33 + SpA* with adjuvant (AS01b: 1281; GLA-SE: 1502; Alhydrogel: 476; Adju-Phos: 425), higher GeoMean RP titers were measured in the sera after three immunizations as compared to the group immunized with LukAB RARPR-33 + SpA* without adjuvant (Geomean RP titer: 122) (FIG. 19A).

20 [0436] For LukAB CC45, in the groups immunized with LukAB RARPR-33 + SpA* with adjuvant (AS01b: 3392; GLA-SE: 3470; Alhydrogel: 365; Adju-Phos: 298), higher GeoMean RP titers were measured in the sera after three immunizations as compared to the group immunized with LukAB RARPR-33 + SpA* without adjuvant (Geomean RP titer: 131) (FIG. 19B).

25 [0437] These results, shown in FIGs. 19A-B, indicate that LukAB in the vaccine (RARPR-33) induces functional antibodies that block the cytotoxic activity of the LukAB toxin and that the addition of an adjuvant improves the functionality of the antibodies to neutralize LukAB toxicity.

[0438] **Conclusion:** The test whether different types of adjuvants could improve the
30 immunogenicity of the tested vaccine combination, consisting of LukAB RARPR-33 and SpA*, antibody titers and functionality were determined in sera of mice immunized with the vaccine combination together with alum-based adjuvants (Aluminium hydroxide or Aluminium phosphate) or adjuvants containing a TLR4 agonist (AS01b or GLA-SE). The addition of both types of adjuvants to the combination vaccine improved vaccine-specific antibody titers

compared to immunization without an adjuvant. In addition, in the presence of an adjuvant, LukAB-specific antibodies had a better LukAB toxin neutralizing capacity. These results show that the immunogenicity of the combination vaccine can be improved using different adjuvants.

5 REFERENCES

[0439] The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

1. Nielsen, O.L., et al., *A pig model of acute Staphylococcus aureus induced pyemia*. Acta Vet Scand, 2009. **51**: p. 14.
2. Johansen, L.K., et al., *A porcine model of acute, haematogenous, localized osteomyelitis due to Staphylococcus aureus: a pathomorphological study*. APMIS, 2011. **119**(2): p. 111-8.
3. Svedman, P., et al., *Staphylococcal wound infection in the pig: Part I. Course*. Ann Plast Surg, 1989. **23**(3): p. 212-8.
4. Luna, C.M., et al., *Animal models of ventilator-associated pneumonia*. Eur Respir J, 2009. **33**(1): p. 182-8.
5. Meurens, F., et al., *The pig: a model for human infectious diseases*. Trends in microbiology, 2012. **20**(1): p. 50-57.
6. Leroux-Roels et al., *Impact of adjuvants on CD4+ T cell and B cell responses to a protein antigen vaccine: Results from a phase II, randomized, multicenter trial*. Clinical Immunology 169 (2016) 16–27.

[0440] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the disclosure and these are therefore considered to be within the scope of the disclosure as defined in the claims which follow.

WHAT IS CLAIMED IS:

1. An immunogenic composition comprising:
 - (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
 - (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptidecomprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.
2. A combination of two or more immunogenic compositions, together comprising:
 - (i) a *Staphylococcus aureus* protein A (SpA) polypeptide, and
 - (ii) a *S. aureus* LukA variant polypeptide, said LukA variant polypeptidecomprising an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Lys83, Ser141, Val113, and Val193 of SEQ ID NO: 25.
3. The immunogenic composition of claim 1 or the combination of immunogenic compositions of claim 2, wherein the LukA variant polypeptide comprises an amino acid substitution at the amino acid residue corresponding to Glu323 of SEQ ID NO: 25.
4. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1-3, wherein said LukA variant polypeptide comprises amino acid substitutions at each amino acid residue corresponding to amino acid residues Lys83, Ser141, Val113, Val193, and Glu323 of SEQ ID NO: 25.
5. The immunogenic composition or the combination of immunogenic compositions of claim 4, wherein the amino acid substitutions comprise Lys83Met, Ser141Ala, Val113Ile, Val193Ile, and Glu323Ala.
6. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1-5, wherein said LukA variant polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 4.
7. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1-6, wherein said LukA variant polypeptide further comprises:

an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Tyr74, Asp140, Gly149, and Gly156 of SEQ ID NO: 25.

8. The immunogenic composition or the combination of immunogenic compositions of claim 7, wherein the amino acid substitutions comprise Tyr74Cys, Asp140Cys, Gly149Cys, and Gly156Cys.

9. The immunogenic composition or the combination of immunogenic compositions of claim 7 or claim 8, wherein said LukA variant polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 5 or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 6.

10. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1–9, wherein said variant LukA protein or polypeptide further comprises:

an amino acid substitution at the amino acid residue corresponding to amino acid residue Thr249 of SEQ ID NO: 25.

11. The immunogenic composition or the combination of immunogenic compositions of claim 10, wherein said LukA variant polypeptide comprises an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 7 or an amino acid sequence having at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 8.

12. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1–11, wherein the SpA polypeptide is a SpA variant polypeptide.

13. The immunogenic composition or the combination of immunogenic compositions of claim 12, wherein the SpA variant polypeptide has at least one amino acid substitution that disrupts Fc binding and at least a second amino acid substitution that disrupts VH3 binding.

14. The immunogenic composition or the combination of immunogenic compositions of claim 12 or 13, wherein the SpA variant polypeptide comprises a SpA D domain, said SpA D domain comprising an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO: 58.

15. The immunogenic composition or the combination of immunogenic compositions of claim 14, wherein the SpA variant polypeptide has an amino acid substitution at one or both of amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58.

16. The immunogenic composition or the combination of immunogenic compositions of claim 14 or claim 15, wherein the SpA variant polypeptide further comprises a SpA E, A, B, or C domain.

17. The immunogenic composition or the combination of immunogenic compositions of claim 16, wherein the SpA variant polypeptide comprises SpA E, A, B, and C domains and has an amino acid sequence having at least 90% identity to the amino acid sequence of SEQ ID NO:54.

18. The immunogenic composition or the combination of immunogenic compositions of claim 16 or 17, wherein each SpA E, A, B, and C domain has an amino acid substitution at one or both amino acid positions corresponding to amino acid positions 9 and 10 of SEQ ID NO: 58.

19. The immunogenic composition or the combination of immunogenic compositions of any one of claims 15–18, wherein the amino acid substitution at one or both amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 is a lysine residue for a glutamine residue.

20. The immunogenic composition or the combination of immunogenic compositions of any one of claims 12–19, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

21. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1–20, wherein said composition further comprises a *S. aureus* Leukocidin B (LukB) polypeptide or variant thereof.

22. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein the LukB polypeptide is a LukB polypeptide of SEQ ID NO: 15 or a LukB polypeptide of SEQ ID NO: 16.

23. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein the LukB polypeptide is a LukB variant polypeptide.

24. The immunogenic composition or the combination of immunogenic compositions of claim 23, wherein the LukB variant polypeptide comprises an amino acid
5 sequence having at least 85% sequence similarity to the amino acid sequence of SEQ ID NO:15 or an amino acid sequence having at least 85% sequence similarity to the amino acid sequence of SEQ ID NO: 16.

25. The immunogenic composition or the combination of immunogenic compositions of claim 24, wherein the LukB variant polypeptide comprises an amino acid
10 substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 15 and SEQ ID NO: 16.

26. The immunogenic composition or the combination of immunogenic compositions of claim 25, wherein the amino acid substitution is a valine to leucine substitution.

27. The immunogenic composition or the combination of immunogenic
15 compositions of any one of claims 23–26, wherein said LukB variant polypeptide comprises an amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu109, Thr121, and Arg154 of SEQ ID NO: 15.

28. The immunogenic composition or the combination of immunogenic compositions of any one of claims 23–26, wherein said LukB variant polypeptide comprises an
20 amino acid substitution at one or more amino acid residues corresponding to amino acid residues Glu45, Glu110, Thr122, and Arg155 of SEQ ID NO: 16.

29. The immunogenic composition or the combination of immunogenic compositions of any one of claim 23–26, wherein the LukB variant polypeptide comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected
25 from SEQ ID NOs: 17-22.

30. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 4 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 4 and a LukB polypeptide comprising the amino acid

sequence of SEQ ID NO: 16, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 16.

31. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 3 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 15, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 15.

32. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein said composition comprises a LukA variant polypeptide comprising the amino acid sequence SEQ ID NO: 3 or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 3 and a LukB polypeptide comprising the amino acid sequence of SEQ ID NO: 18, or an amino acid sequence having at least 90% sequence identity to SEQ ID NO: 18.

33. The immunogenic composition or the combination of immunogenic compositions of any one of claims 30–32, wherein the SpA polypeptide is a SpA variant polypeptide.

34. The immunogenic composition or the combination of immunogenic compositions of claim 33, wherein the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has (i) a lysine substitution at the glutamine residues corresponding to positions 9 and 10 of SEQ ID NO: 58 and (ii) a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

35. The immunogenic composition or the combination of immunogenic compositions of claim 21, wherein (i) the SpA variant polypeptide comprises at least one SpA A, B, C, D, or E domain, and wherein the at least one domain has lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58; (ii) the LukA variant polypeptide comprises a CC8 LukA variant polypeptide comprising a methionine substitution at the amino acid position corresponding to position 80 of SEQ ID NO: 1, an alanine substitution at the amino acid position corresponding to position 138 of SEQ ID NO: 1, an isoleucine substitutions at the amino acid positions corresponding to positions 110 and 190 of

SEQ ID NO:1, and an alanine substitution at the amino acid position corresponding to position 320 of SEQ ID NO: 1; and (iii) the LukB polypeptide is a CC45 LukB variant polypeptide comprising a leucine substitution at the amino acid position corresponding to position 53 of SEQ ID NO: 16.

5 36. The immunogenic composition or the combination of immunogenic compositions of claim 35, wherein the SpA variant polypeptide comprises consecutively SpA E, D, A, B, and C domains, each domain having the lysine substitutions at the amino acid positions corresponding to positions 9 and 10 of SEQ ID NO: 58 and a glutamate substitution at the amino acid position corresponding to position 33 of SEQ ID NO: 58.

10 37. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1 to 34, further comprising an adjuvant.

 38. The immunogenic composition or the combination of immunogenic compositions of claim 37, wherein the adjuvant is a stable oil-in-water emulsion,

15 39. The immunogenic composition or the combination of immunogenic compositions of claim 37, wherein the adjuvant comprises a saponin.

 40. The immunogenic composition or the combination of immunogenic compositions of claim 39, wherein the saponin is QS21.

 41. The immunogenic composition or the combination of immunogenic compositions of claim 37, wherein the adjuvant comprises a TLR4 agonist.

20 42. The immunogenic composition or the combination of immunogenic compositions of claim 41, wherein the TLR4 agonist is lipid A or an analog or derivative thereof.

 43. The immunogenic composition or the combination of immunogenic compositions of claim 41, wherein the TLR4 agonist is glycopyranosyl lipid adjuvant (GLA).

25 . The immunogenic composition or the combination of immunogenic compositions of claim 41, wherein the adjuvant comprises a TLR-4 agonist in combination with a stable oil-in-water emulsion.

 45. The immunogenic composition or the combination of immunogenic compositions of claim 43, wherein the adjuvant comprises GLA-SE.

46. The immunogenic composition or the combination of immunogenic compositions of claim 41, wherein the adjuvant comprises a TLR-4 agonist in combination with a saponin.

47. The immunogenic composition or the combination of immunogenic
5 compositions of claim 43, wherein the adjuvant comprises GLA-LSQ.

48. An immunogenic composition or a combination of immunogenic compositions, wherein said compositions comprise one or more nucleic acid molecules encoding the *Staphylococcus aureus* protein A (SpA) polypeptide or variant thereof, the LukA variant polypeptide, and the LukB polypeptide or variant thereof of the immunogenic compositions of
10 any one of claims 1–36.

49. The immunogenic composition or the combination of immunogenic compositions of claim 48, wherein the one or more nucleic acid molecules are contained in one or more vectors.

50. The immunogenic composition or the combination of immunogenic
15 compositions of claim 48 or 49, wherein said compositions comprise a host cell, wherein said host cell comprises said one or more nucleic acid molecules or said one or more vectors.

51. A method for treating or preventing a *Staphylococcus* infection in a subject in need thereof, the method comprising:

administering to the subject in need thereof an effective amount of the
20 immunogenic composition or the combination of immunogenic compositions of any one of claims 1 to 50.

52. A method for eliciting an immune response to a *Staphylococcus* bacterium in a subject in need thereof, the method comprising:

administering to the subject in need thereof an effective amount of the
25 immunogenic composition or the combination of immunogenic compositions of any one of claims 1 to 50.

53. A method for decolonization or preventing colonization or recolonization of a *Staphylococcus* bacterium in a subject in need thereof, the method comprising:

administering to the subject in need thereof an effective amount of the immunogenic composition or the combination of immunogenic compositions of any one of claims 1 to 50.

54. The immunogenic composition or the combination of immunogenic compositions of any one of claims 1–50 for use in a method of generating an immune response against *S. aureus* in a subject.

55. The immunogenic composition or the combination of immunogenic compositions of any one of embodiments 1–50 for use as a medicament.

Luka Sequence Alignment

SEQ	ID	NO:1	---	HKDSQDQNKKEHV	DKSQDQDKRNV	TN	KDKNST	APDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	57	CC8																						
SEQ	ID	NO:2	--	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTV	SEYD	KETNI	58	CC45																							
SEQ	ID	NO:27	NS	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTV	SEYD	KETNI	60	HMERF0772_044(TCH60)																							
SEQ	ID	NO:36	NS	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTV	SEYD	KETNI	60	SAR2108(MRSA252)																							
SEQ	ID	NO:34	NS	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTV	SEYD	KETNI	60	SALG_02329(A9635)																							
SEQ	ID	NO:35	NS	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTT	EV	YDEK	TNI	60	SAPIG2061(ST398)																						
SEQ	ID	NO:37	NS	ANKDSQDQTKKEH	VDKAQOKEKRN	VNDK	DNTP	PGDD	IGKNGK	VT	KRTT	EV	YDEK	TNI	60	SALG_01930(DI39)																						
SEQ	ID	NO:26	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	APDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	NEWMAN																						
SEQ	ID	NO:32	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	VPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	SAB1876C(RF122)																						
SEQ	ID	NO:38	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	VPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	SAV2005(Mu50)																						
SEQ	ID	NO:31	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	VPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	SAB1813(N315)																						
SEQ	ID	NO:33	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	APDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	SACOL2006																						
SEQ	ID	NO:29	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	APDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	USA300(TCH959)																						
SEQ	ID	NO:28	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	VPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	TCH130																						
SEQ	ID	NO:30	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	VPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	MW1942(MW2)																						
SEQ	ID	NO:25	NS	AHKDSQDQNKKEH	VDKSQDQDKRNV	TN	KDKNST	XPDD	IGKNGK	IT	KRTT	EV	YDEK	TNI	60	Majority																						
SEQ	ID	NO:1	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	KKE	NS	NW	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	117				
SEQ	ID	NO:2	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	R	N	E	T	N	A	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	118
SEQ	ID	NO:27	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	R	N	E	T	N	A	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	120
SEQ	ID	NO:36	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	R	N	E	T	N	A	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	120
SEQ	ID	NO:34	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	R	N	E	T	N	A	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	120
SEQ	ID	NO:35	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	K	E	E	N	N	S	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	S	N	120
SEQ	ID	NO:37	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	K	E	E	N	N	S	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	120
SEQ	ID	NO:26	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS	I	HS	NL	K	FESH	K	E	E	N	N	S	L	K	Y	P	S	E	H	V	D	F	Q	V	K	R	N	120	
SEQ	ID	NO:32	LQ	NLQDFD	FIDDP	TY	DK	NVLL	VVK	QGS																												

Fig. 1

FIG. 1 (cont.)

LukA Sequence Alignment (Cont.)

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SEQ_ID_NO:1      SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNRPGIHYAPPILKKNKDGQRLIVTYE 297
SEQ_ID_NO:2      SGNPEFITYISNEKTNKTRFEVYTRNQDILKNKPGIHYGQPILEQNKGQRFIVVYE 298
SEQ_ID_NO:27     SGNPEFITYISNEKSNEKTRFEVYTRNQDILKNKPGIHYGQPILEQNKGQRFIVVYE 300
SEQ_ID_NO:36     SGNPEFITYISNEKSNEKTRFEVYTRNQDILKNKPGIHYGQPILEQNKGQRFIVVYE 300
SEQ_ID_NO:34     SGNPEFITYISNEKTNKTRFEVYTRNQDILKNKPGIHYGQPILEQNKGQRFIVVYE 300
SEQ_ID_NO:35     SGNPEFITYLSNEKSNEKTQFEVYTRNQDVLKNKPGIHYAPPILKKNKDGQRLIVTYE 300
SEQ_ID_NO:37     SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNKPGIHYAPPILKKNKDGQRLIVTYE 300
SEQ_ID_NO:26     SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNRPGIHYAPPILKKNKDGQRLIVTYE 299
SEQ_ID_NO:32     SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNRPGIHYAPPILKKNKDGQRLIVTYE 300
SEQ_ID_NO:38     SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNRPGIHYAPSIKKNKDGQRLIVTYE 300
SEQ_ID_NO:31     SGNPEFITYLSNEKSNEKTQFEVYTRNQDILKNRPGIHYAPPILKKNKDGQRLIVTYE 300
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SEQ_ID_NO:35     VDWKNKTVKVVDKYSDDNKPYKEG 324
SEQ_ID_NO:37     VDWKNKTVKVVDKYSDDNKPYKEG 324
SEQ_ID_NO:26     VDWKNKTVKVVDKYSDDNKPYKEG 323
SEQ_ID_NO:32     VDWKNKTVKVVDKYSDDNKPYKEG 324
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SEQ_ID_NO:33     VDWKNKTVKVVDKYSDDNKPYKEG 324
SEQ_ID_NO:29     VDWKNKTVKVVDKYTDN-KSFREG 322
SEQ_ID_NO:28     VDWKNKTVKVVDKYSQDN-KSFREG 323
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FIG. 1 (Cont.)

LukB Sequence Alignment

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FIG. 2

LukB Sequence Alignment (Cont.)

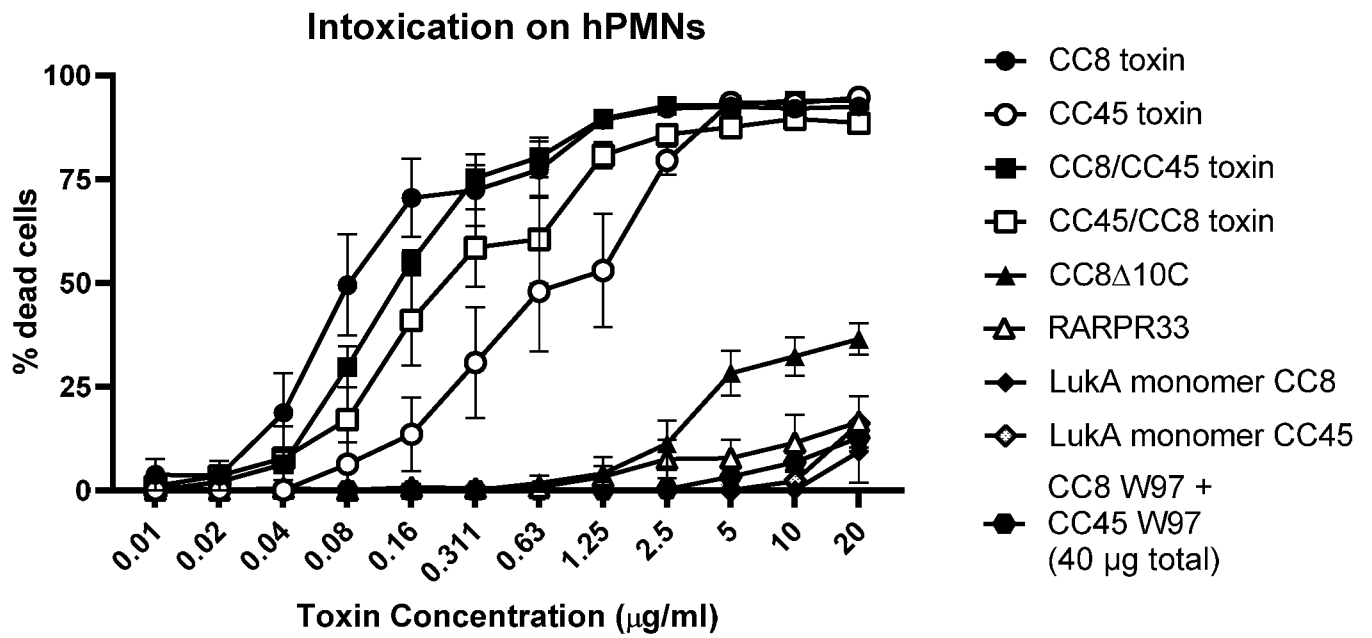
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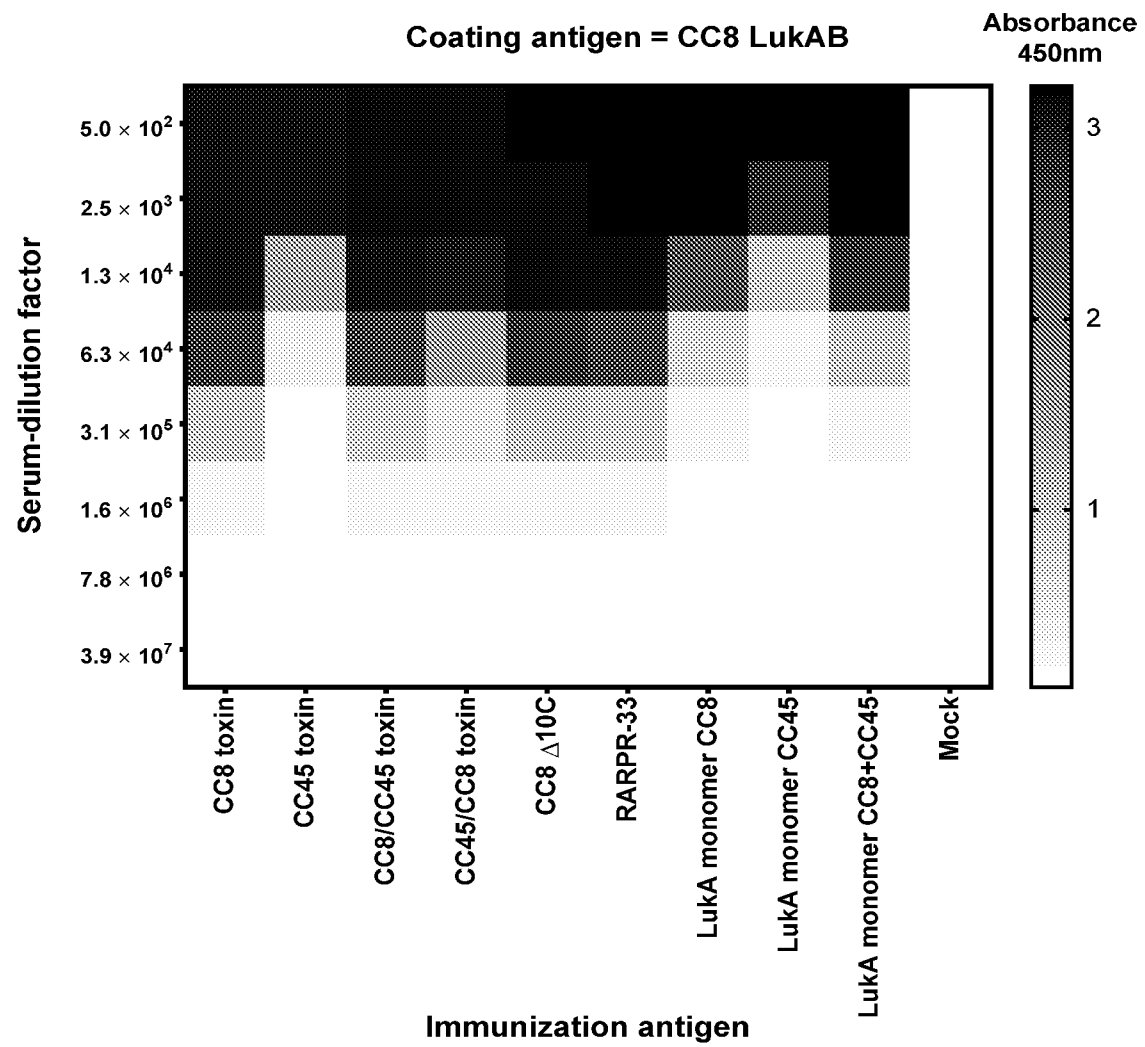
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FIG. 2 (Cont.)

FIG. 2 (Cont.)

**FIG. 3**

**FIG. 4A**

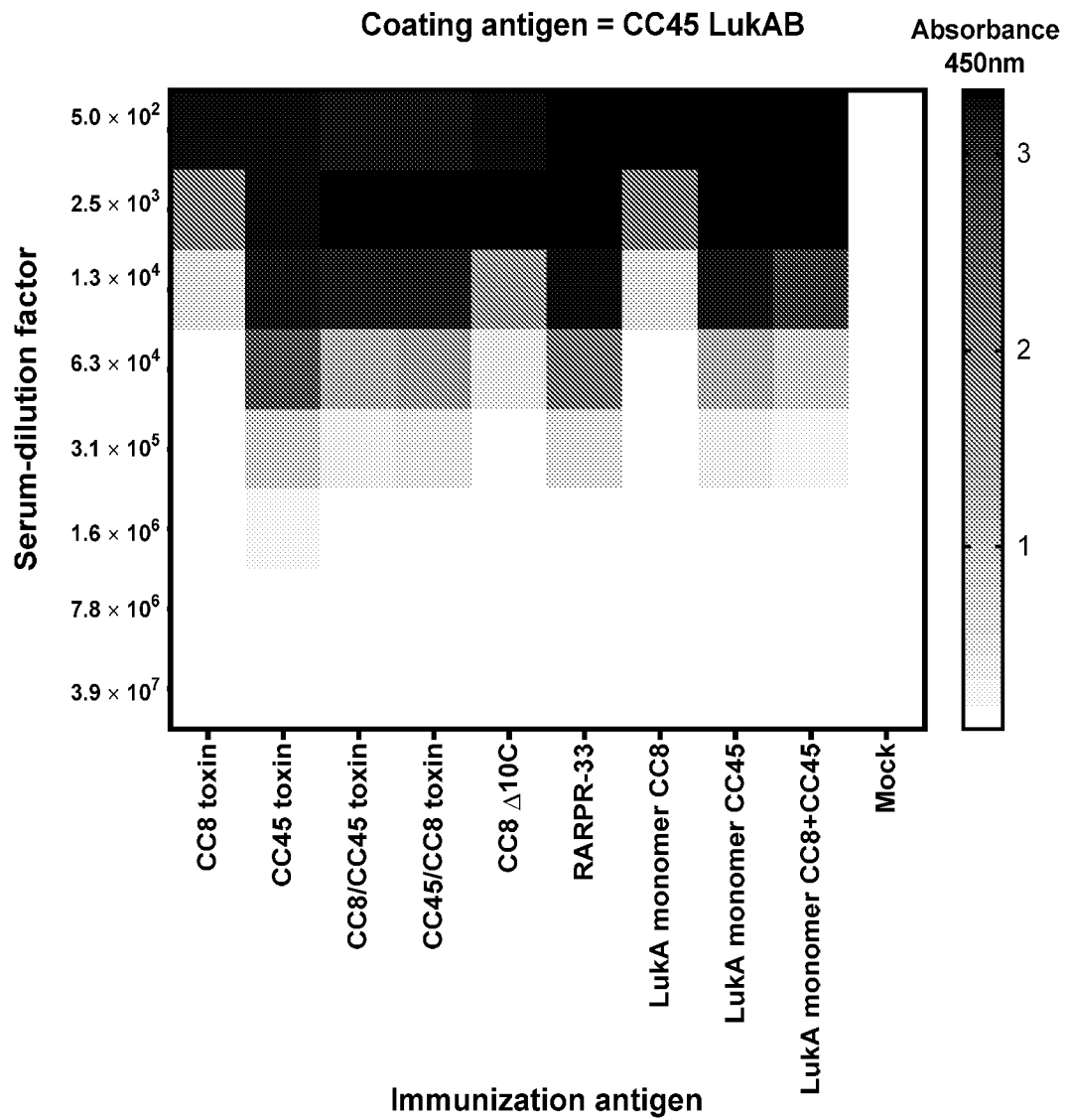
**FIG. 4B**



FIG. 5

Neutralization Assay: % Dead Human PMNs (Intoxication with LD ₅₀ of LukAB variants + 2% serum from immunized mice); n = 4 donors												
LukAB Immunization		LukAB Variant										
Antigen	CC8	CC30	CC45	CC1	CC5	CC398	CC76	ST188	ST109	ST10	ST22A	
CC8	8.86	19.09	11.91	2.07	3.14	2.35	3.94	3.11	2.57	2.46	33.02	
CC45	79.63	1.50	1.05	81.08	80.10	50.46	67.85	81.92	75.50	38.83	2.60	
CC8/CC45	4.11	1.46	0.00	0.38	0.00	0.96	1.73	0.00	0.00	0.00	0.76	
CC45/CC8	8.80	1.84	1.41	0.13	0.22	0.54	0.54	2.94	0.95	0.99	1.73	
CC8 E323A/CC45	2.90	1.37	0.66	1.65	0.23	0.00	4.37	0.06	4.26	0.86	3.91	
CC45 E323A/CC8	7.92	1.28	0.00	0.00	0.56	0.00	9.29	2.91	2.30	2.22	2.86	
CC8 E323A/CC45 B5	37.18	51.31	53.86	40.06	39.43	74.44	49.27	23.54	47.20	66.99	35.62	
CC45 E323A/CC8 B5	19.57	10.70	47.46	54.36	44.74	64.77	71.72	82.49	75.41	49.76	13.32	
RARPR 13	23.49	6.31	3.94	42.95	29.57	1.87	4.58	32.08	16.15	8.42	7.25	
RARPR 15	9.22	4.02	4.35	4.02	6.08	4.60	7.66	7.07	6.82	7.15	8.81	
RARPR 17	36.11	8.90	4.35	58.37	64.23	4.56	5.00	52.92	40.46	7.22	8.88	
RARPR 19	42.11	10.05	10.23	40.04	49.00	9.71	9.49	44.67	17.04	9.39	9.81	
SM1W97	9.59	87.32	89.66	2.63	2.90	22.13	16.21	4.85	6.57	5.60	87.36	
Mock	89.81	81.28	86.39	87.04	89.89	87.03	92.31	90.54	96.08	91.77	92.30	

CC45 B5 and CC8 B5 are LukB deletion variants

*CC8 LukA W97 monomer expressed in HEK293 cells

Neutralization Assay: % Dead Human PMNs (Intoxication with LD ₅₀ of LukAB variants + 2% serum from immunized mice); n = 4 donors												
LukAB Immunization		LukAB Variant										
Antigen	CC8	CC30	CC45	CC1	CC5	CC398	CC76	ST188	ST109	ST10	ST22A	
CC8 delta10C	25.46	16.21	17.06	30.25	18.63	27.37	62.25	17.97	23.44	21.63	18.86	
RARPR 30	26.73	18.82	19.72	35.26	19.17	18.00	16.62	27.99	29.64	16.48	19.95	
RARPR 31	20.61	75.41	79.18	19.06	11.23	42.24	47.72	14.23	23.67	11.37	82.07	
RARPR 32	15.70	30.25	30.64	4.69	6.10	6.86	5.11	9.97	5.86	6.17	43.44	
RARPR 33	17.33	9.43	10.14	8.95	12.32	11.81	14.58	12.55	11.55	9.08	10.46	
RARPR 34	15.77	11.63	12.92	10.47	9.79	5.96	7.19	10.11	5.42	9.00	19.29	
CC8 W97	12.75	78.67	79.90	3.78	4.49	35.56	39.56	9.09	10.88	13.01	85.04	
CC45 W97	86.48	26.11	23.68	87.12	88.52	81.79	88.11	85.80	86.48	84.08	40.99	
RARPR 31 + CC45 SW97	20.49	27.42	35.17	49.31	18.69	53.84	69.31	23.93	35.80	36.26	44.91	
Mock	89.05	89.85	89.56	87.67	87.61	93.38	89.26	87.11	90.31	87.72	88.05	

FIG. 6A

Neutralization Assay: % Dead Human PMNs (Intoxication with LD₅₀ of LukAB variants + 1% serum from immunized mice); n = 4 donors

LukAB Immunization Antigen	LukAB Variant										
	CC8	CC30	CC45	CC1	CC5	CC398	CC75	ST188	ST109	ST10	ST22A
CC8	6.31	79.12	78.00	4.06	2.72	2.12	23.47	2.31	1.45	2.06	89.13
CC45	85.75	8.41	5.43	89.18	86.90	52.07	86.32	52.99	51.13	81.55	1.64
CC8/CC45	2.95	2.99	2.55	0.00	0.00	0.00	13.65	0.92	0.00	0.00	19.64
CC45/CC8	12.02	2.54	0.00	69.77	19.76	38.40	30.76	77.31	36.67	48.34	1.02
CC8 E323A/CC45	4.92	5.40	5.15	3.89	1.29	4.67	44.20	0.03	0.50	1.49	16.39
CC45 E323A/CC8	14.19	0.00	0.00	65.49	33.06	67.53	82.11	74.34	29.92	59.20	0.00
CC8 E323A/CC45 B5	44.80	85.26	72.72	88.32	62.49	85.71	98.69	61.54	76.01	81.73	85.04
CC45 E323A/CC8 B5	56.69	58.06	63.51	86.04	73.53	81.75	87.05	86.17	88.14	80.33	72.51
RARPR 13	77.93	4.99	3.09	84.41	80.70	12.54	15.60	80.10	71.92	15.54	0.37
RARPR 15	27.35	2.99	0.67	43.23	50.38	0.93	6.47	33.91	17.17	1.51	1.63
RARPR 17	87.67	5.78	1.96	86.75	85.31	55.72	46.87	86.40	87.35	46.46	3.17
RARPR 19	87.20	6.79	2.53	90.10	89.81	36.00	51.36	87.24	82.62	26.12	5.66
SM1W97	3.48	93.23	89.90	25.96	0.00	73.66	63.47	0.70	6.14	3.10	94.47
Mock	90.53	94.26	93.47	93.61	93.03	93.21	93.34	91.75	92.03	92.35	90.72

Neutralization Assay: % Dead Human PMNs (Intoxication with LD₅₀ of LukAB variants + 1% serum from immunized mice); n = 4 donors

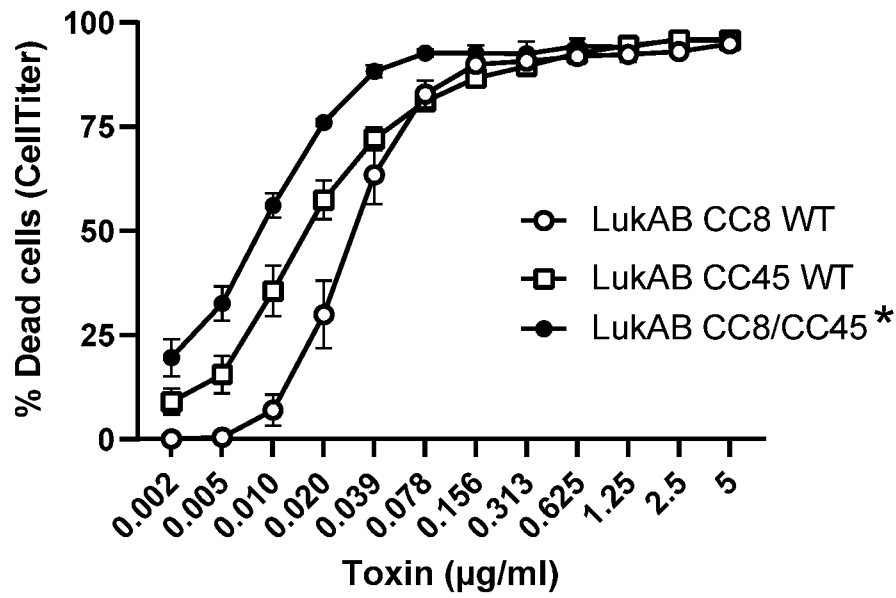
LukAB Immunization Antigen	LukAB Variant										
	CC8	CC30	CC45	CC1	CC5	CC398	CC75	ST188	ST109	ST10	ST22A
CC8 delta10C	21.27	48.38	34.07	71.43	15.91	59.63	79.56	41.56	68.51	56.94	41.67
RARPR 30	28.95	16.90	21.26	77.12	35.04	33.77	26.01	61.04	71.78	32.93	14.38
RARPR 31	21.83	86.11	87.37	64.22	38.40	73.38	75.84	27.79	67.07	33.51	89.54
RARPR 32	9.35	65.16	67.49	4.22	4.52	2.79	12.24	4.71	2.24	2.80	76.25
RARPR 33	17.16	15.09	17.73	14.45	8.25	19.25	15.80	5.89	11.21	6.59	34.68
RARPR 34	22.52	42.51	44.83	6.80	5.30	6.77	15.77	5.97	4.55	6.97	51.43
CC8 W97	11.18	86.60	87.22	32.08	5.73	62.26	64.70	12.38	36.39	22.36	86.48
CC45 W97	86.20	49.88	54.36	86.55	86.81	87.97	87.96	88.78	86.29	86.15	63.54
RARPR 31 + CC45 SW97	37.40	57.23	64.18	85.41	53.18	74.04	81.87	55.68	79.97	67.11	73.77
Mock	95.48	91.80	91.50	95.81	94.80	91.87	94.48	94.48	93.85	94.51	94.79

FIG. 6B

Neutralization Assay: % Dead Human PMNs (Intoxication with LD ₅₀ of LukAB variants + 0.5% serum from immunized mice); n = 4 donors													
LukAB Immunization Antigen	LukAB Variant												
	CC8	CC30	CC45	CC1	CC5	CC398	CC75	ST188	ST109	ST10	ST22A		
CC8	4.72	91.30	89.33	14.96	4.66	39.86	84.43	3.02	3.41	1.99	90.83		
CC45	89.11	13.32	9.54	91.24	91.47	87.94	91.77	85.99	87.64	87.15	26.57		
CC8/CC45	4.20	65.59	74.67	62.14	10.31	77.09	84.91	0.29	18.82	32.31	84.21		
CC45/CC8	71.54	72.15	37.01	89.70	80.64	86.07	85.81	88.62	89.05	88.11	70.59		
CC8 E323A/CC45	15.35	49.18	68.28	86.87	26.59	72.20	86.68	17.24	64.65	24.31	83.01		
CC45 E323A/CC8	78.55	51.02	12.91	89.15	85.25	86.12	90.10	86.75	86.46	88.42	37.10		
CC8 E323A/CC45 B5	84.73	92.88	89.97	90.77	83.44	90.97	93.02	86.62	89.11	89.31	91.77		
CC45 E323A/CC8 B5	89.10	88.13	90.30	89.04	90.22	88.34	91.53	90.08	88.93	91.35	91.77		
RARPR 13	92.34	1.48	1.63	91.95	91.88	55.81	62.35	91.87	88.36	80.69	6.74		
RARPR 15	90.27	3.55	0.00	92.63	88.06	58.82	82.81	91.54	89.37	57.13	1.32		
RARPR 17	92.25	1.35	0.48	92.38	92.90	91.11	89.94	92.48	92.80	87.28	1.26		
RARPR 19	92.63	3.62	2.11	90.72	88.79	84.15	87.78	91.37	93.34	85.11	4.16		
SM1W97	19.13	89.96	92.05	90.59	38.57	87.10	89.10	29.16	81.63	61.72	92.22		
Mock	93.93	91.94	90.60	92.04	94.88	93.06	91.54	93.06	91.73	93.38	95.19		

Neutralization Assay: % Dead Human PMNs (Intoxication with LD ₅₀ of LukAB variants + 0.5% serum from immunized mice); n = 4 donors													
LukAB Immunization Antigen	LukAB Variant												
	CC8	CC30	CC45	CC1	CC5	CC398	CC75	ST188	ST109	ST10	ST22A		
CC8 delta10C	19.20	65.77	66.31	86.52	42.23	76.84	85.46	79.13	84.89	76.20	75.28		
RARPR 30	46.84	15.56	14.27	88.08	68.72	68.45	52.63	84.36	88.95	65.90	16.73		
RARPR 31	34.37	87.92	86.47	86.14	59.72	85.14	86.23	67.96	87.03	70.30	87.93		
RARPR 32	9.45	83.44	82.39	11.75	2.85	8.76	50.03	2.16	2.19	4.66	87.02		
RARPR 33	10.98	54.83	51.44	47.75	4.47	51.89	51.85	7.31	43.18	24.47	76.12		
RARPR 34	13.46	72.28	75.09	10.15	5.99	21.47	54.74	4.39	9.09	5.88	84.63		
CC8 W97	72.27	80.37	87.69	18.45	80.44	81.25	52.60	58.74	54.93	78.52	84.32		
CC45 W97	87.00	72.09	74.38	88.00	87.58	90.84	86.58	85.34	86.90	90.92	82.63		
RARPR 31 + CC45 SW97	65.05	79.43	81.40	90.56	84.01	88.00	87.48	83.63	89.68	81.75	85.32		
Mock	93.59	88.06	90.18	94.58	94.90	90.17	93.48	94.27	94.28	92.16	91.91		

FIG. 6C



*LukAB CC8/CC45 tested in 4 donors only

FIG. 7A

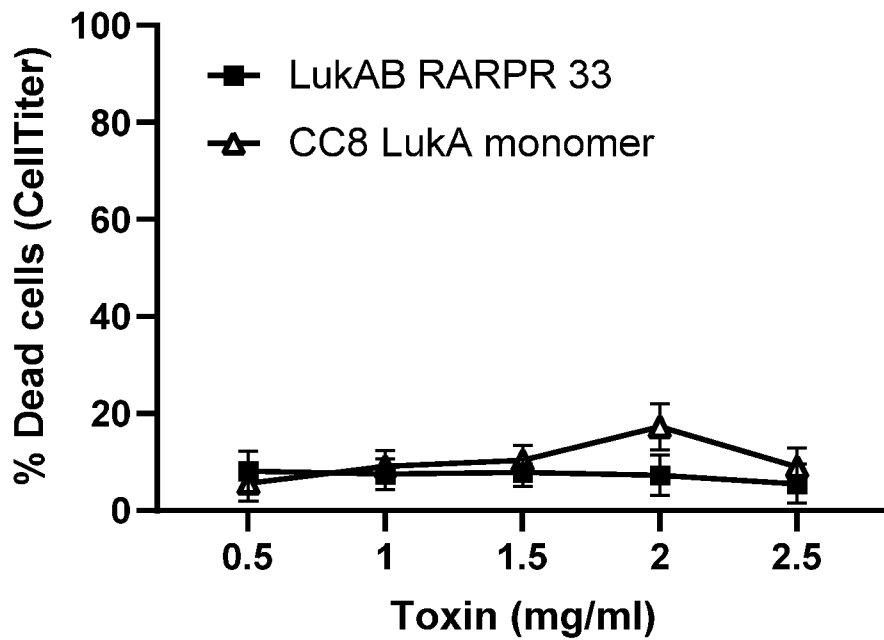
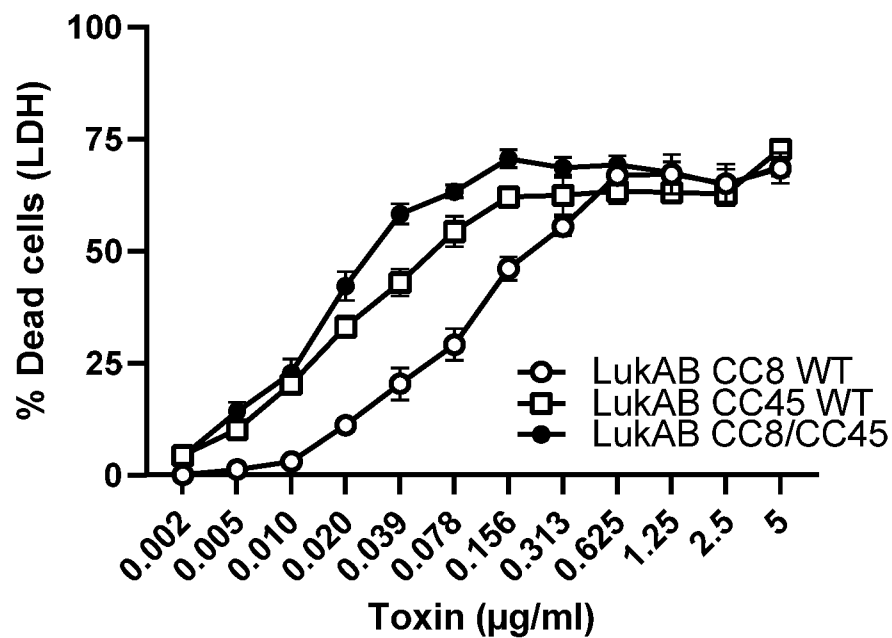
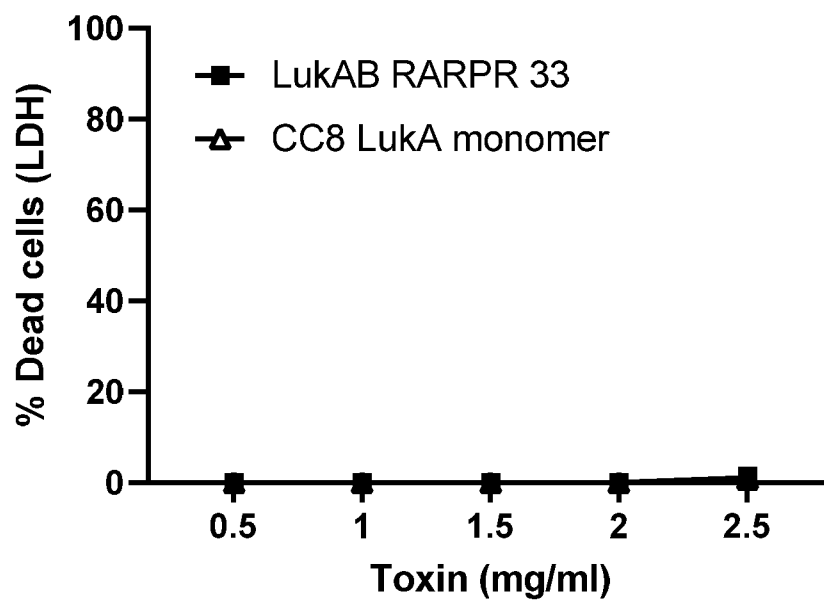
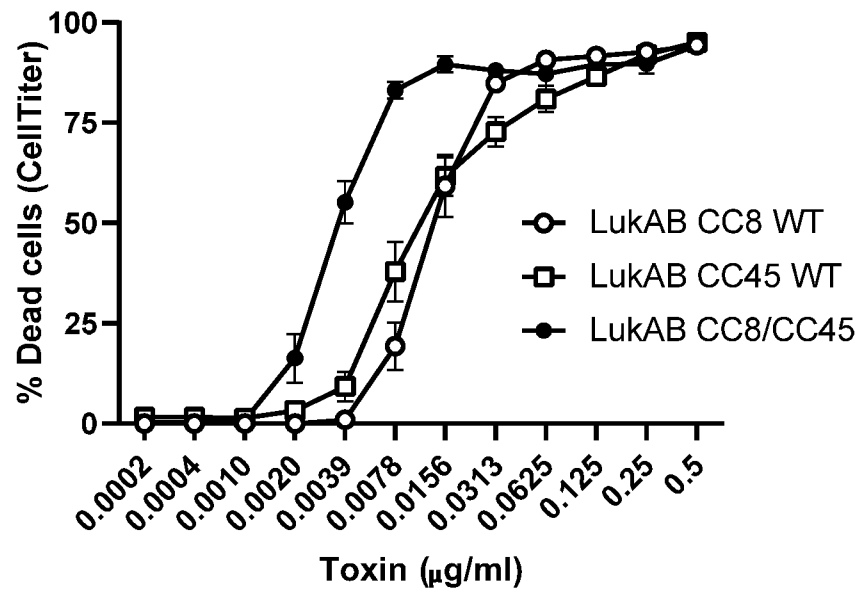
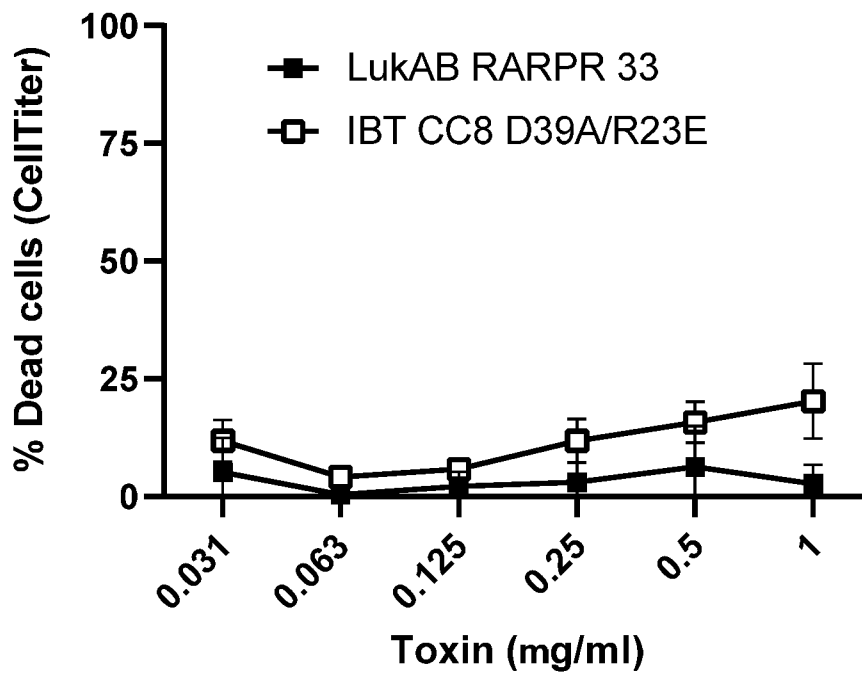
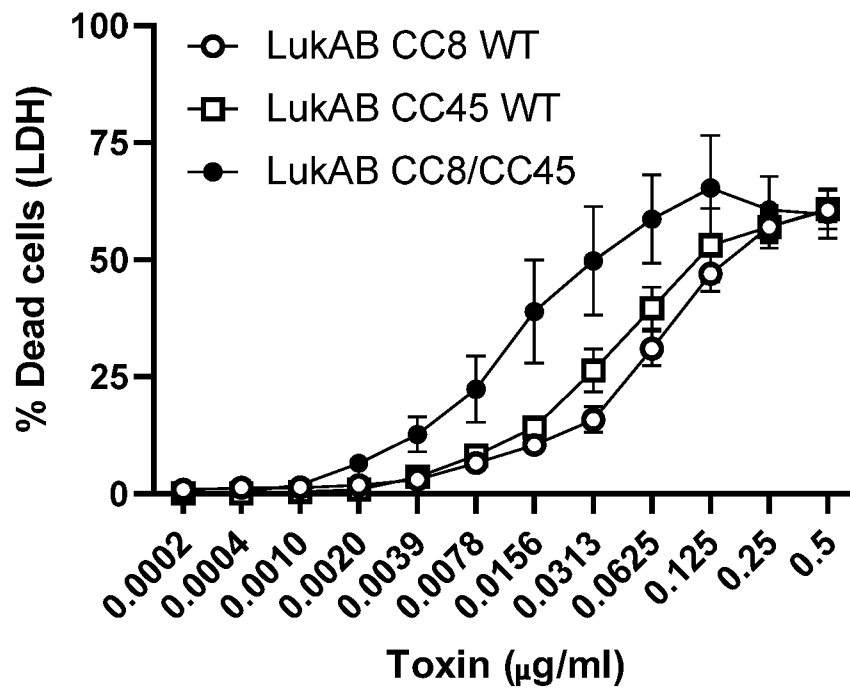
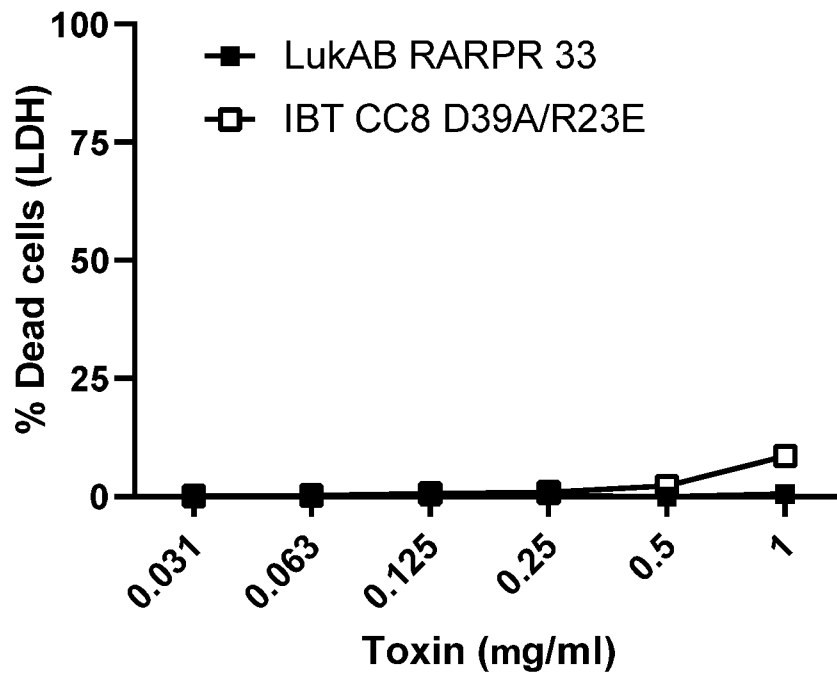


FIG. 7B

**FIG. 7C****FIG. 7D**

**FIG. 8A****FIG. 8B**

**FIG. 8C****FIG. 8D**

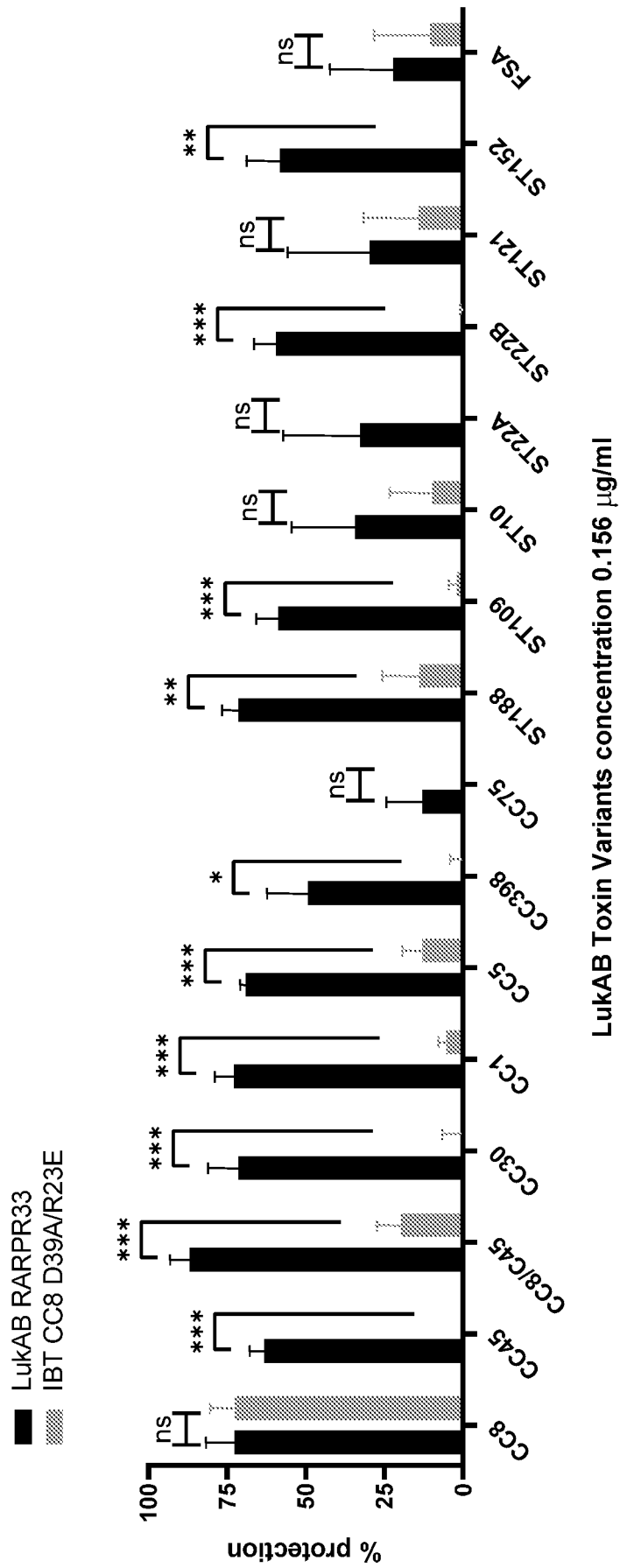
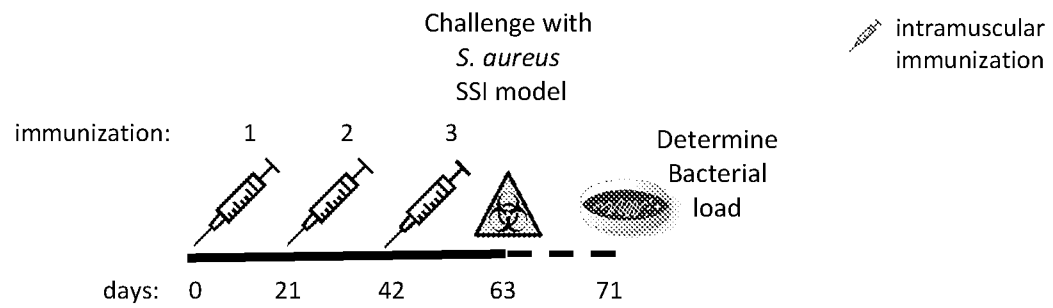
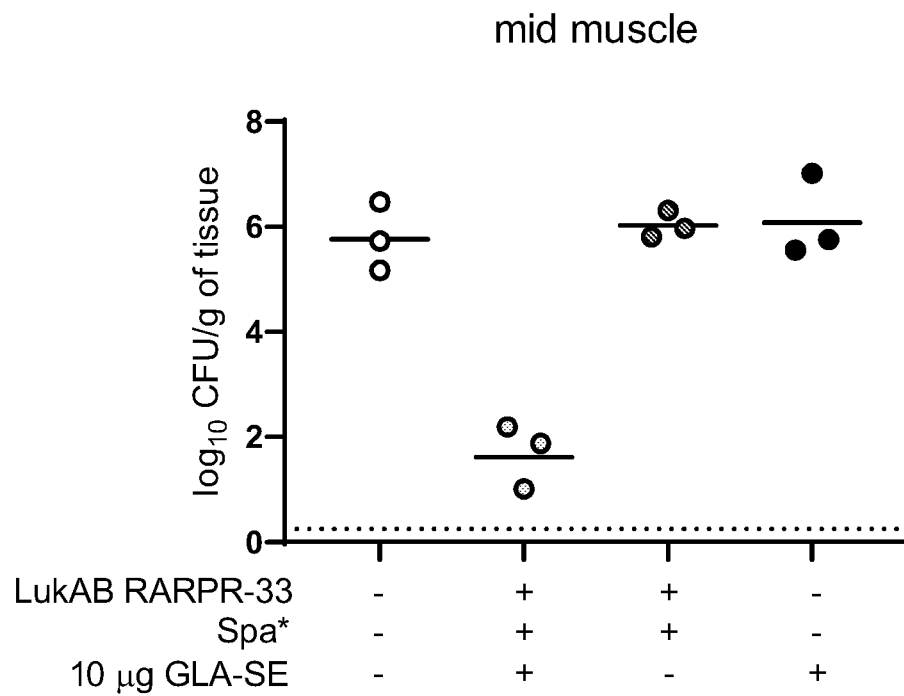


FIG. 9

**FIG. 10****FIG. 11A**

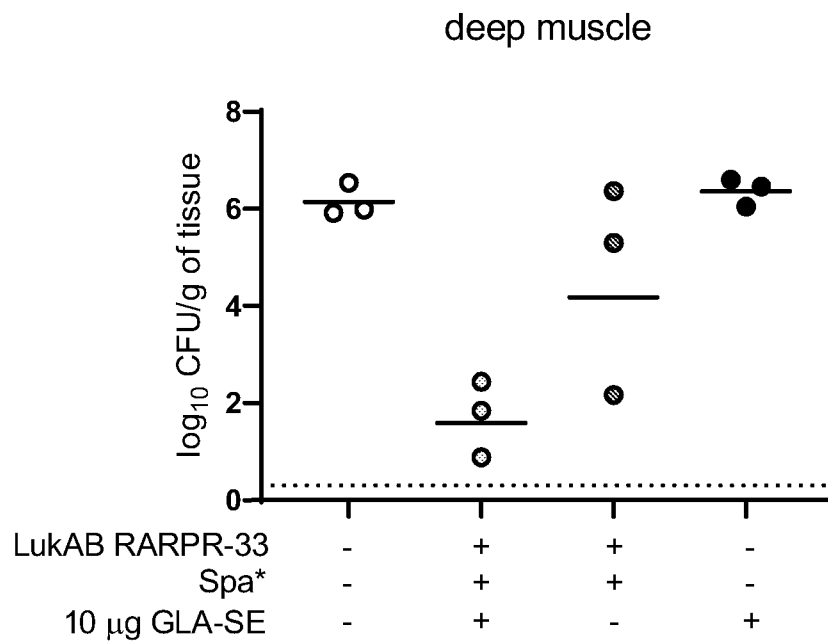
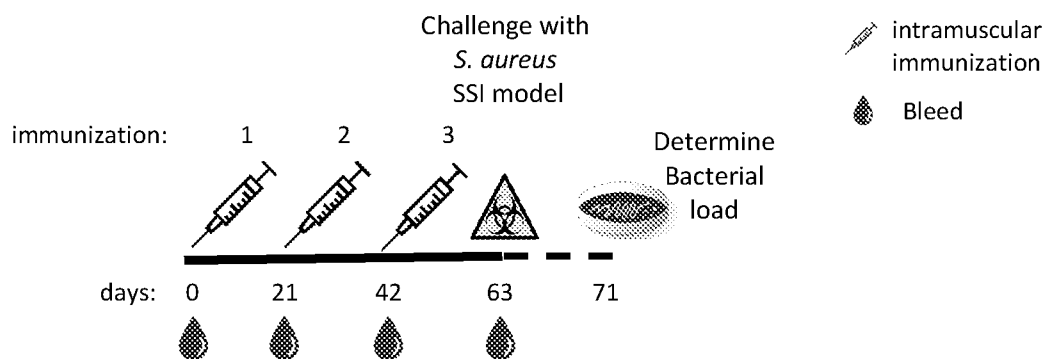
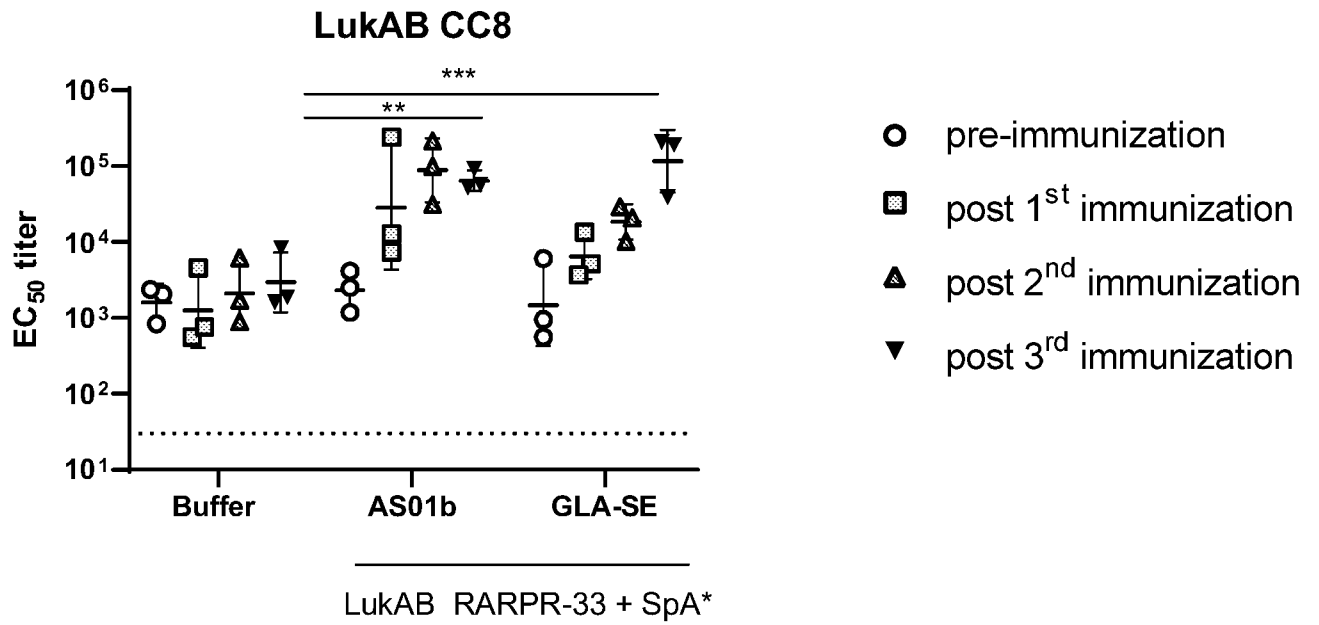
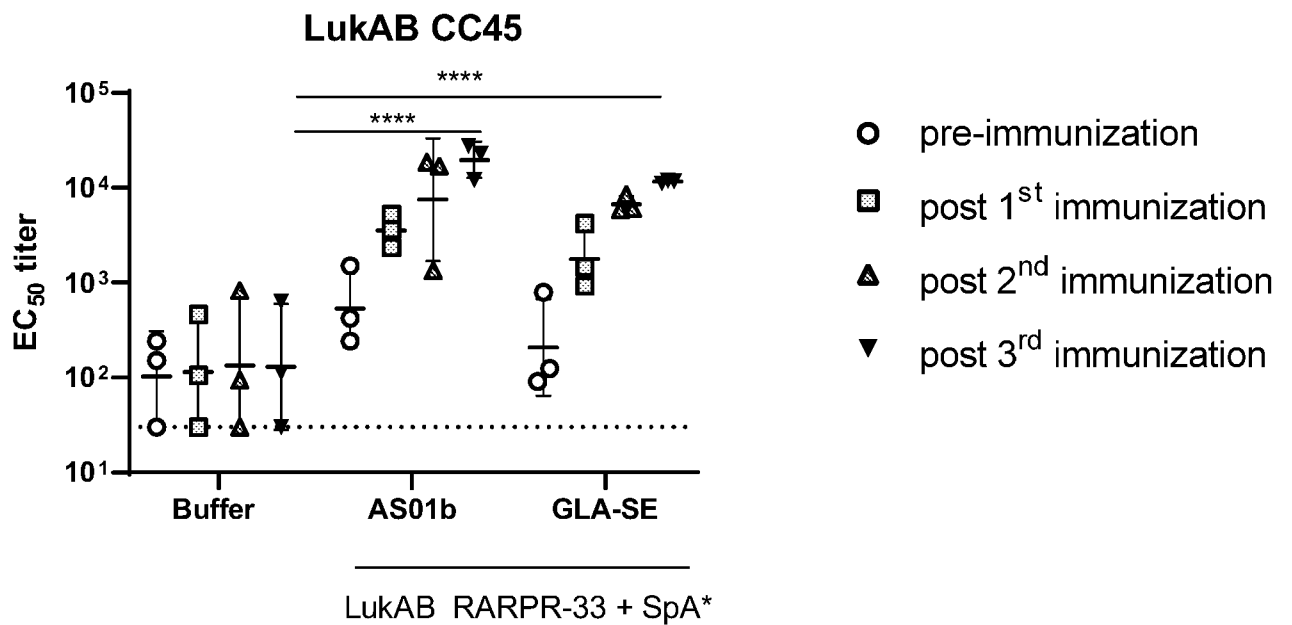


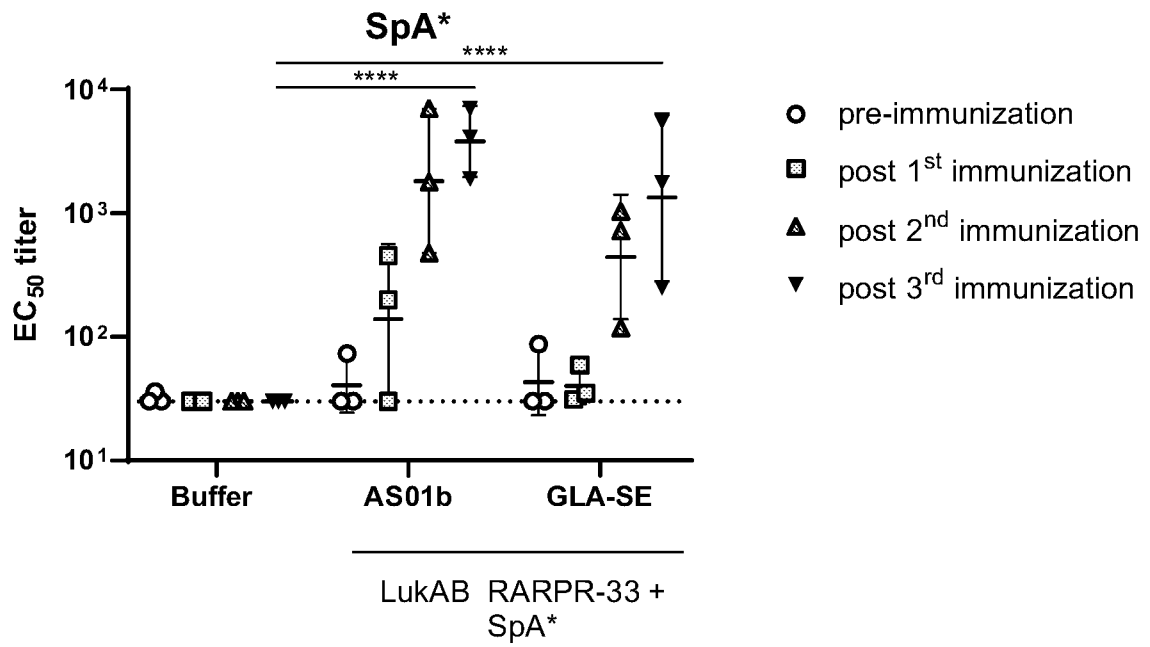
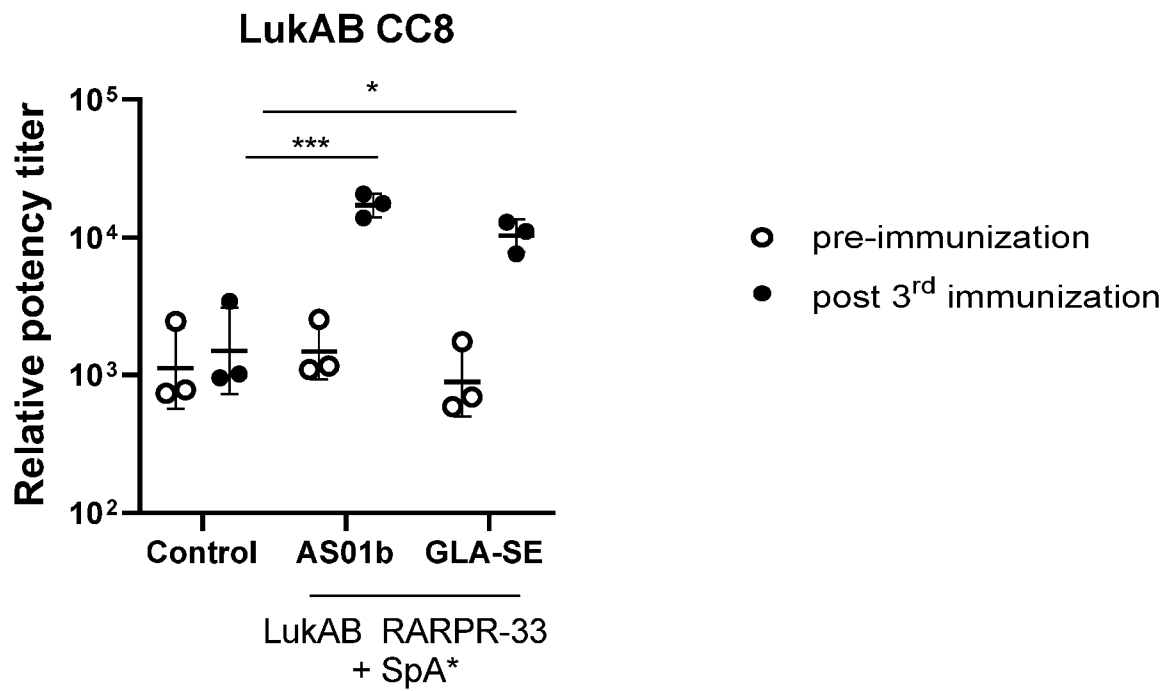
FIG. 11B

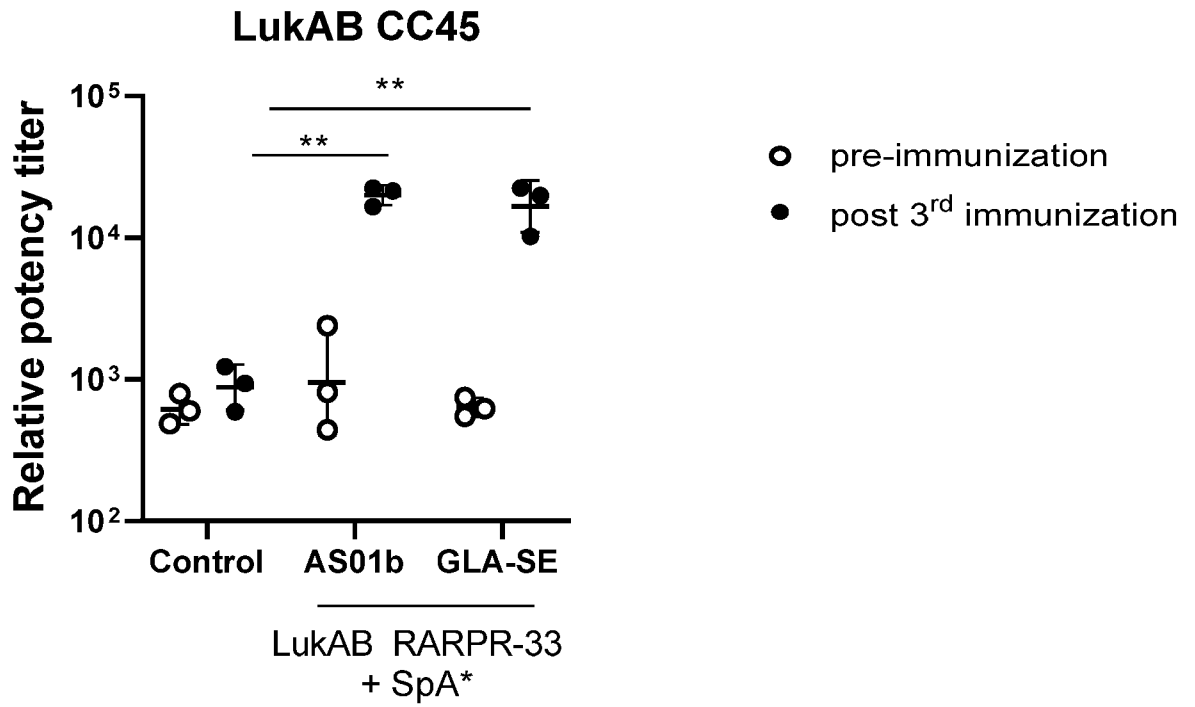
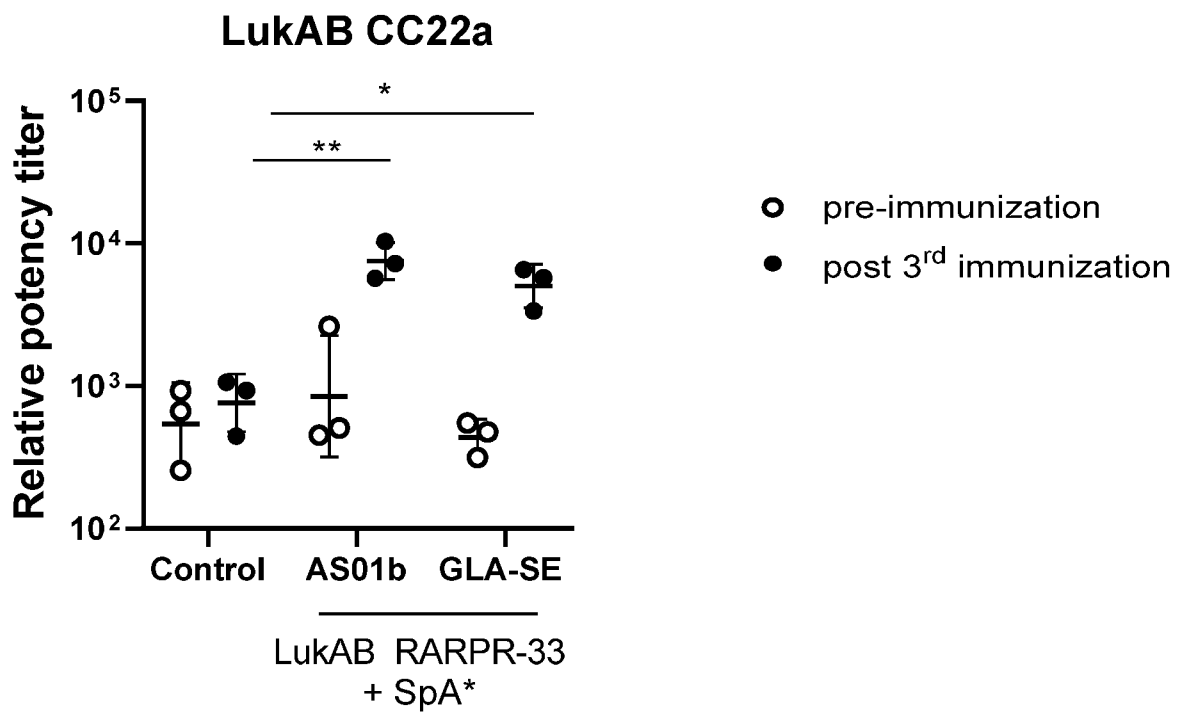


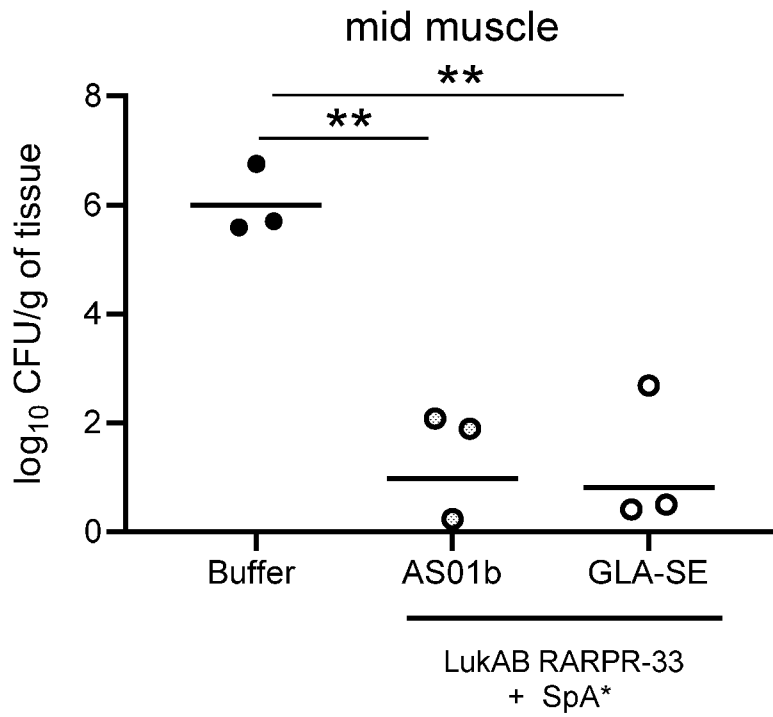
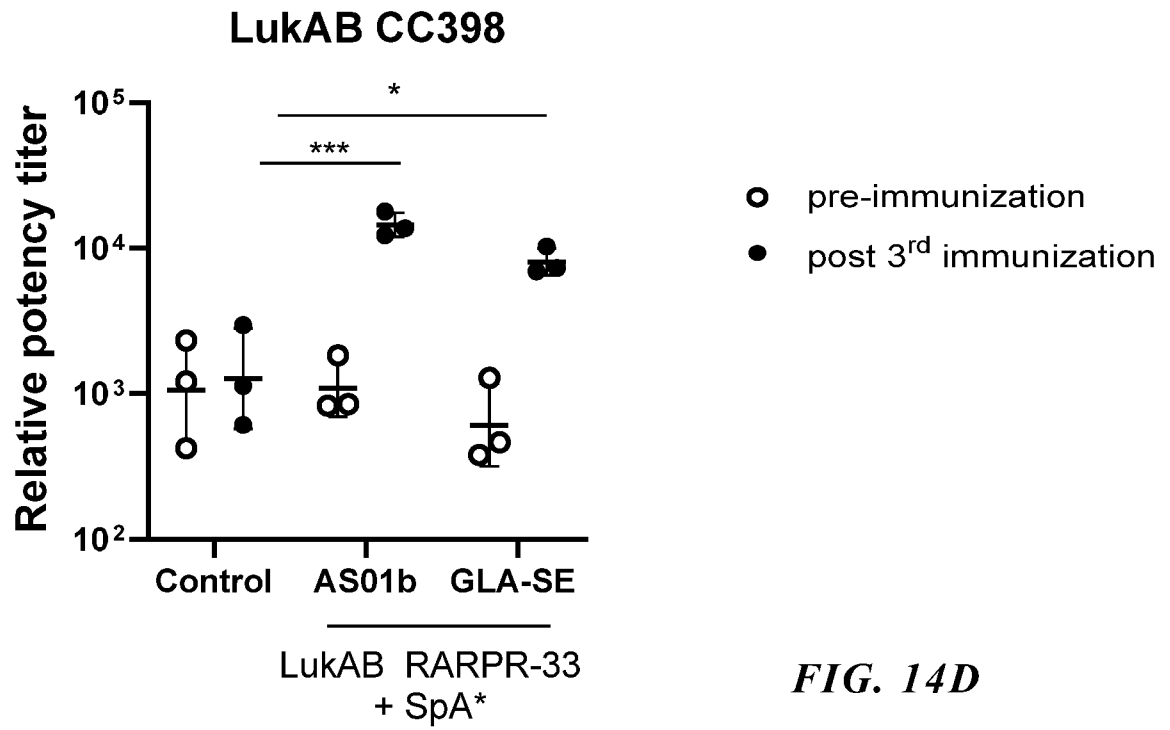
Group	Antigen 1	Dose (µg)	Antigen 2	Dose (µg)	Adjuvant	Dose (µg)	Volume (µl)
1. N=3	control	-	-	-	-	-	500
2. N=3	RARPR-33	100	SpA*	100	AS01b (MPL, QS-21)	25/25	500
3. N=3	RARPR-33	100	SpA*	100	GLA-SE (2% SE)	10	500

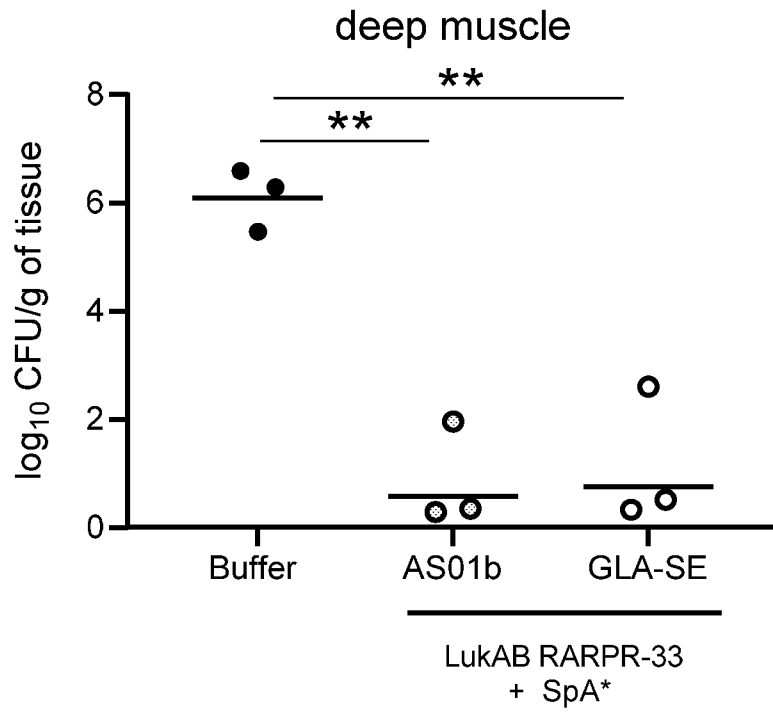
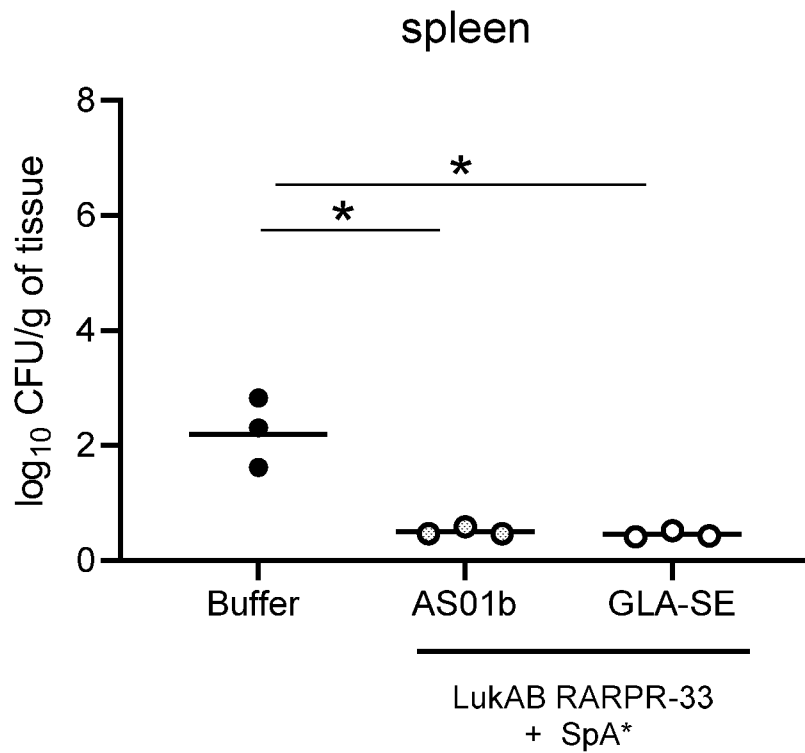
FIG. 12

**FIG. 13A****FIG. 13B**

**FIG. 13C****FIG. 14A**

**FIG. 14B****FIG. 14C**



*FIG. 15B**FIG. 15C*

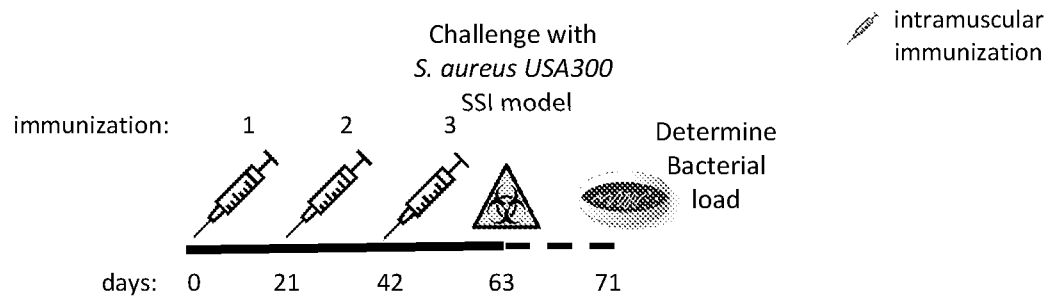


FIG. 16A

Group	Antigen 1	Dose (μg)	Antigen 2	Dose (μg)
1. N=3	Buffer	-	-	-
2. N=3	LukAB RARPR-33	100	SpA*	100

FIG. 16B

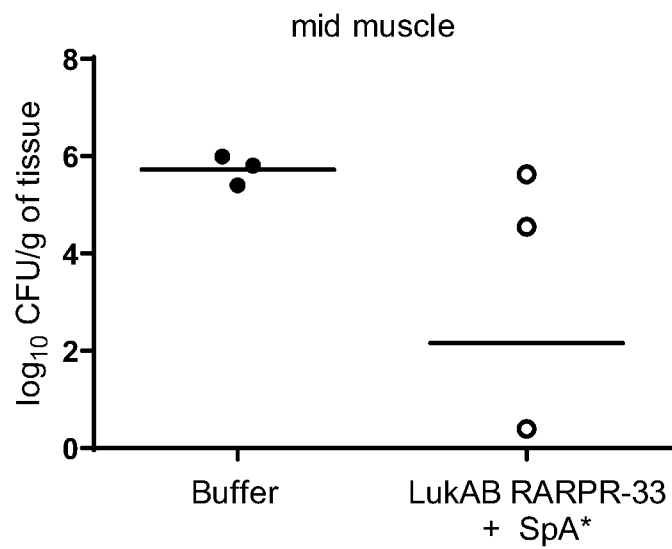


FIG. 16C

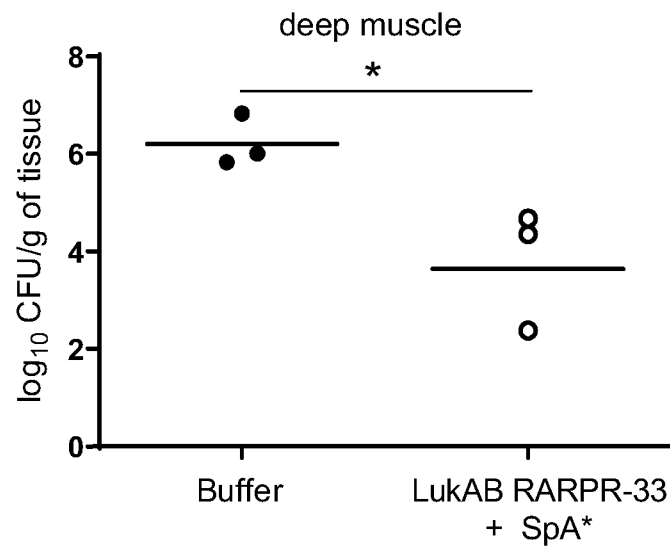


FIG. 16D

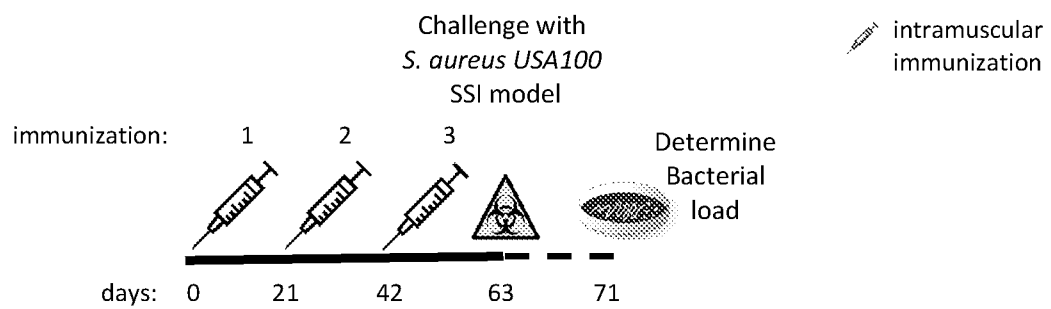
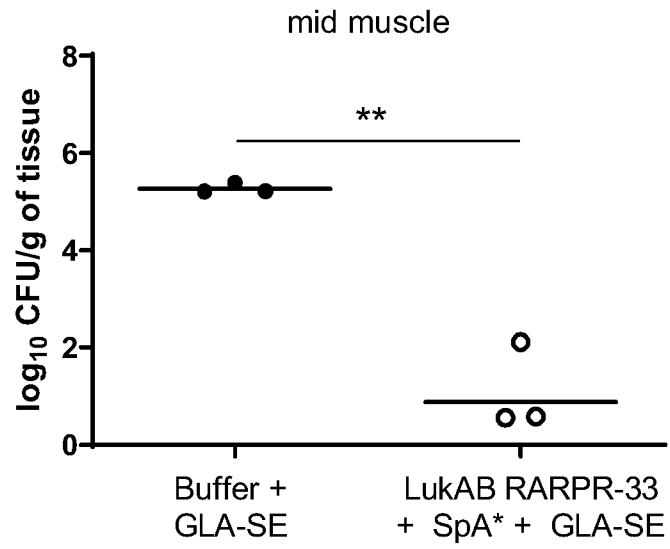
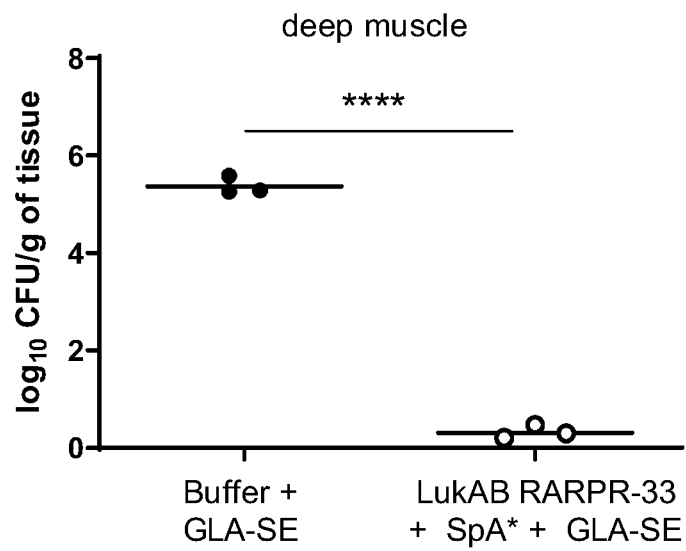
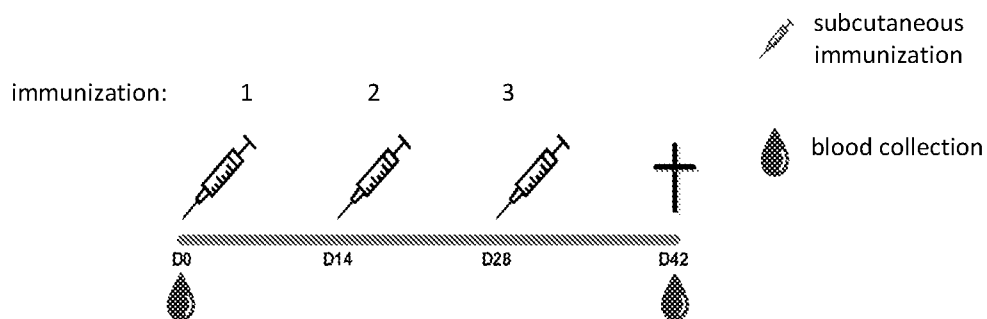


FIG. 17A

Group	Antigen 1	Dose (μg)	Antigen 2	Dose (μg)	Adjuvant	Dose (μg)
1. N=3	Buffer	-	-	-	GLA-SE	10 μg GLA 2% SE
2. N=3	RARPR-33	100	Spa*	100	GLA-SE	10 μg GLA 2% SE

FIG. 17B

*FIG. 17C**FIG. 17D*

**FIG. 18A**

Group	Antigen 1	Dose (µg)	Antigen 2	Dose (µg)	Adjuvant	Dose or volume	# Animals
1.	LukAB His-RARPR-33	5	SpA*	5	AS01b	5 µg MPL, 5 µg QS-21	10
2.	LukAB His-RARPR-33	5	SpA*	5	GLA-SE	1 µg GLA (2%SE)	9
3.	LukAB His-RARPR-33	5	SpA*	5	Alhydrogel	50 µl	10
4.	LukAB His-RARPR-33	5	SpA*	5	Adju-Phos	50 µl	10
5.	LukAB His-RARPR-33	5	SpA*	5	-	-	10
6.	Buffer	-	-	-	AS01b	5 µg MPL, 5 µg QS-21	5
7.	Buffer	-	-	-	GLA-SE	1 µg GLA (2%SE)	5
8.	Buffer	-	-	-	Alhydrogel	50 µl	5
9.	Buffer	-	-	-	Adju-Phos	50 µl	5
10.	Buffer	-	-	-	-	-	5

FIG. 18B

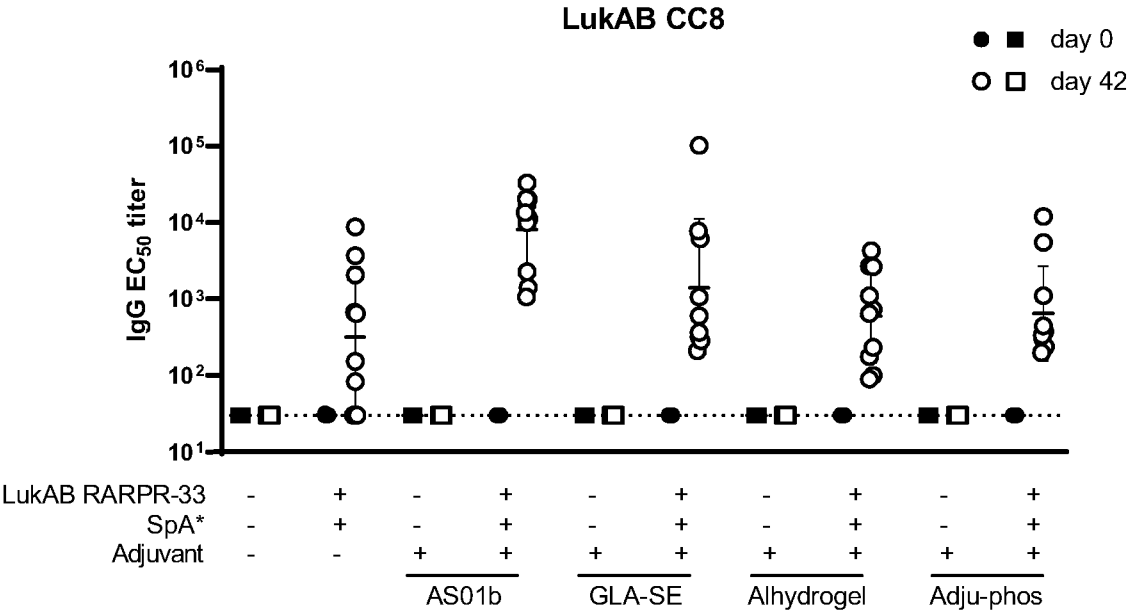


FIG. 18C

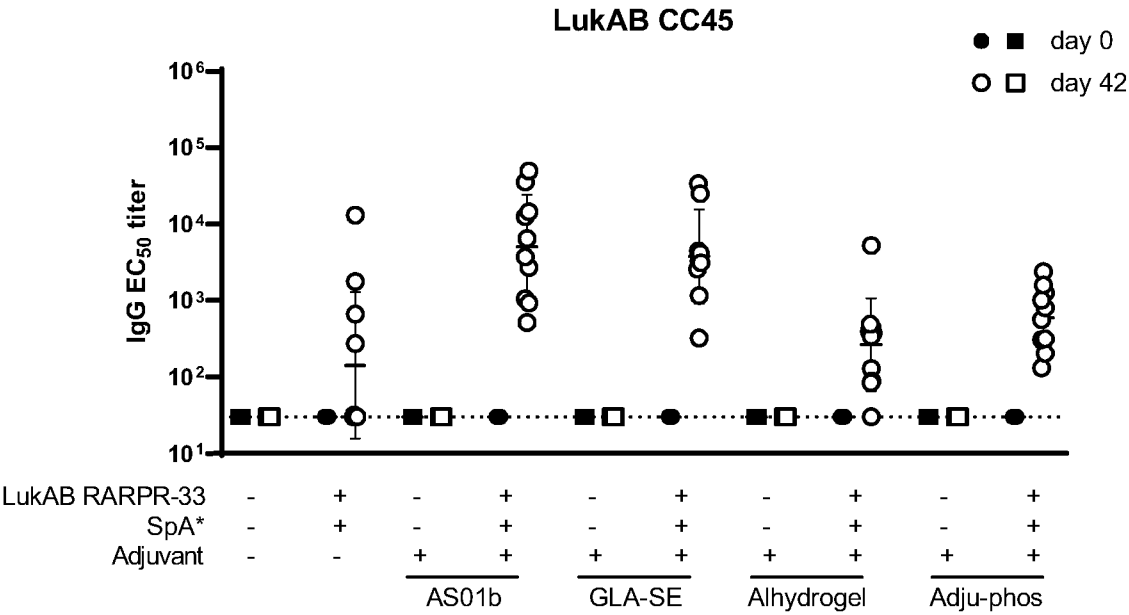


FIG. 18D

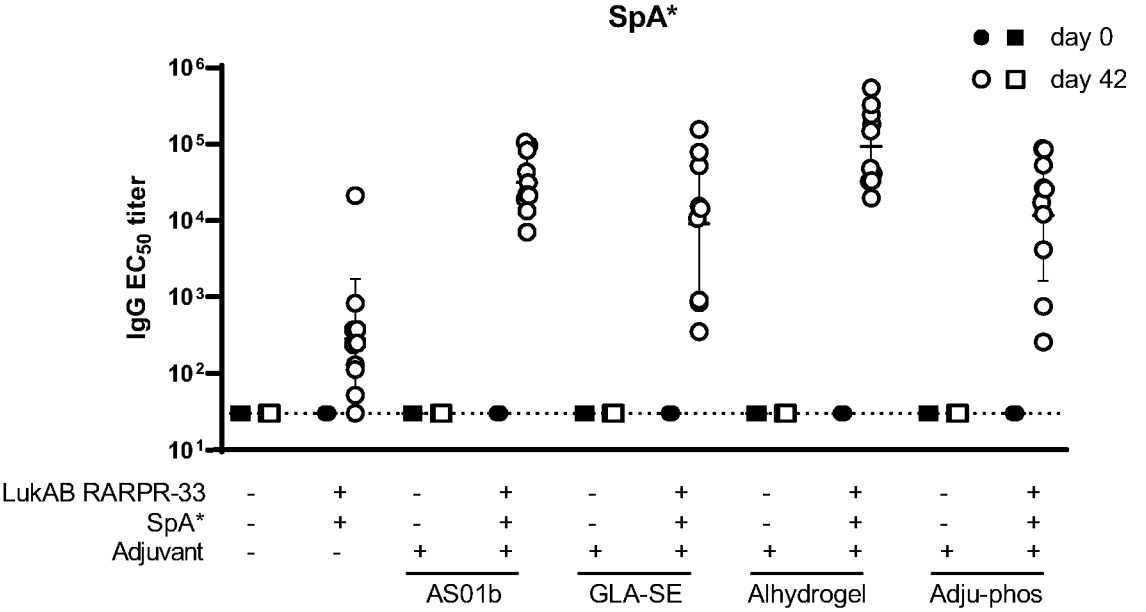
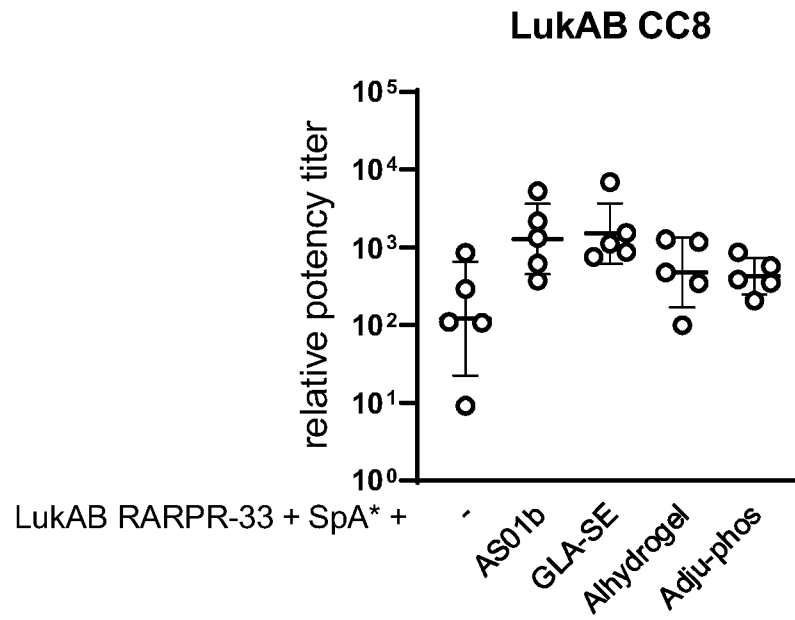
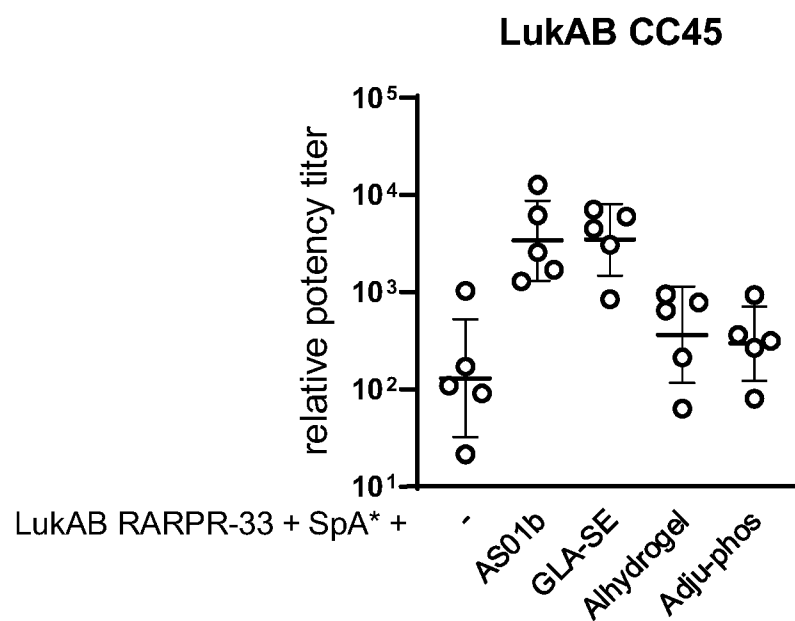


FIG. 18E

**FIG. 19A****FIG. 19B**