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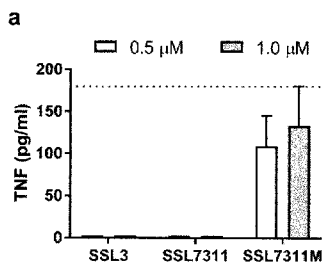
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(54) Title: MULTIVALENT VACCINE COMPOSITIONS

Figure 3



(57) Abstract: The present invention relates to multivalent *S. aureus* vaccine constructs, including multivalent polypeptide and polynucleotide constructs, compositions comprising such constructs including compositions such as pharmaceutical compositions comprising the constructs, methods of eliciting immune responses in a subject and methods of vaccinating a subject, related uses of such constructs, and uses of the constructs in the manufacture of medicaments for such purposes.

MULTIVALENT VACCINE COMPOSITIONS

TECHNICAL FIELD

The invention relates to multivalent *S. aureus* vaccine constructs, including multivalent polypeptide and polynucleotide constructs, compositions comprising such constructs including compositions such as pharmaceutical compositions comprising the constructs, methods of eliciting immune responses in a subject and methods of vaccinating a subject, related uses of such constructs, and uses of the constructs in the manufacture of medicaments for such purposes.

BACKGROUND OF THE INVENTION

The following includes information that may be useful in understanding the present invention. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not an admission that any of the information provided herein is prior art, or relevant, to the presently described or claimed inventions, or that any publication or document that is specifically or implicitly referenced is prior art. Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of the common general knowledge in the field.

Synthetic vaccines, such as synthetic peptide or polypeptide vaccines generally comprise a synthetic copy of at least an immunogenic part of a protein antigen or antigens, or more recently a nucleic acid capable of eliciting expression of such an immunogen in a cell into which it is introduced. These approaches to vaccine development have a number of advantages, including ease of synthesis, avoidance of potentially toxic biological by-products and ideally straightforward characterisation, particularly compared to vaccines comprising more complex mixtures, such as those generated from attenuated or inactivated whole viruses or bacteria.

A key issue in the development of a vaccine is ensuring appropriate immunological responses in target subjects, together with sufficient coverage of the target population to deliver meaningful health benefits to an individual, and to the population as a whole. Approaches to address this include attempting to overcome a potential lack of immunogenicity displayed by peptides and polypeptides as sole vaccine components by including adjuvants in the vaccine composition, selection of a protein thought to be essential to the pathogen as a potential target for identification and selection, or by the use of self-adjuvating vaccines which may have enhanced antigen uptake, presentation and dendritic cell maturation compared to simple co-formulation of the antigen with an external adjuvant.

A further issue in vaccine development, particularly for vaccines targeting pathogens capable of rapid mutagenesis and/or pathogens that are sufficiently widespread such that their effective population is sufficiently large so as to overcome comparatively low intrinsic rates of mutagenesis, is the loss of effective immunoprotection arising because of the evolution of variant pathogens that partially or entirely escape the immunological protection conferred by a vaccine directed to the original (progenitor) pathogen, or the pathogen that may be prevalent in one geographic region.

For example, there is a degree of geographical variance in the predominant *S. aureus* strains of concern, with different clonal complexes being prevalent in different regions, reflective of allelic variation across the globe.

In light of increasing prevalence of antibiotic resistance in various *Staphylococcus aureus* populations, and of contra-indications or other challenges with existing treatments and treatment agents, there is a substantial need for an effective vaccine against *Staphylococcus aureus*, particularly a vaccine capable of eliciting protective immunological responses in an individual subject, and/or a substantial portion of the human or animal populations, and/or to provide a degree of redundancy and/or future-proofing to mitigate against future vaccine escape via mutation.

The present invention seeks to go some way towards meeting one or more of these needs, and/or to at least provide the public with a useful choice. Other aims of the invention may become apparent from the following description which is given by way of example only.

10 SUMMARY OF THE INVENTION

In a first aspect the invention relates to an isolated, purified, recombinant, or synthesised multivalent polypeptide, the multivalent peptide comprising two or more Staphylococcal Superantigen-Like (SSL) virulence factors, wherein at least two of the Staphylococcal Superantigen-Like (SSL) virulence factors are independently selected from the group consisting of:

- 15 (a) an SSL virulence factor capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity;
- (b) a mutated SSL virulence factor that in the absence of the mutation is capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity;
- (c) an antigenic fragment of (a) or (b);
- 20 (d) an SSL virulence factor capable of binding to a complement molecule and/or of inhibiting complement activation;
- (e) a mutated SSL virulence factor that in the absence of the mutation is capable of binding to a complement molecule and/or of inhibiting complement activation;
- (f) an antigenic fragment of (d) or (e);
- 25 (g) an SSL virulence factor capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis;
- (h) a mutated SSL virulence factor that in the absence of the mutation is capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis;
- (i) an antigenic fragment of (g) or (h).

30 In one example, when present in the multivalent polypeptide the function or an activity of one or more of the SSL virulence factors is inhibited or ablated.

In one example, the antigenic fragment does not exhibit the or an activity of the SSL virulence factor of which it is a fragment.

In one example, the multivalent polypeptide is selected from any one of the following:

- 35 (a) the SSL virulence factor capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity is a Staphylococcal Superantigen-Like virulence factor 3 (SSL3); and/or

- (b) the SSL virulence factor capable of binding to a complement molecule and/or of inhibiting complement activation is a Staphylococcal Superantigen-Like virulence factor 7 (SSL7); and/or
- (c) the SSL virulence factor capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis is a Staphylococcal Superantigen-Like virulence factor 11 (SSL11); and/or
- (d) any combination of two or more of (a) to (c) above;
- (e) each of (a) to (c) above.

In another aspect the invention relates to a multivalent polypeptide, wherein the multivalent polypeptide comprises at least one Staphylococcal Superantigen-Like virulence factor 3 (SSL3) polypeptide, at least one Staphylococcal Superantigen-Like virulence factor 7 (SSL7) polypeptide, and at least one Staphylococcal Superantigen-Like virulence factor 11 (SSL11) polypeptide.

In one example, one or more of the at least one SSL3 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:

- (a) an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 3;
- (b) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3;
- (c) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3, wherein said at least 50 consecutive amino acids comprise a Toll-like receptor binding region;
- (d) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
- (e) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 3;
- (f) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24;
- (g) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24, wherein said at least 50 consecutive amino acids comprise a Toll-like receptor binding region;
- (h) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
- (i) an amino acid sequence set forth in any one of SEQ ID NO: 1 to 24;
- (j) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 13 to 24;
- (k) an amino acid sequence set forth in any one of SEQ ID NO: 13 to 24;

- (l) an amino acid sequence according to any one of (a) to (k) above wherein when present the Toll-like receptor binding region is inactive;
- (m) an amino acid sequence according to any one of (a) to (l) above wherein when present the sialic acid binding region is inactive;
- 5 (n) an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (m) above; or
- (o) any combination of any two or more of (a) to (n) above.

In one example, one or more of the at least one SSL7 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:

- 10 (a) an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 7;
- (b) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7;
- (c) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7, wherein said at least 50 consecutive amino acids comprise an immunoglobulin binding region;
- 15 (d) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7, wherein said at least 50 consecutive amino acids comprise a complement C5 binding region;
- 20 (e) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7;
- (f) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48;
- (g) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48, wherein said at least 50 consecutive amino acids comprise an immunoglobulin binding region;
- 25 (h) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48, wherein said at least 50 consecutive amino acids comprise a complement C5 binding region;
- 30 (i) an amino acid sequence set forth in any one of SEQ ID NO: 25 to 48;
- (j) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 37 to 48;
- (k) an amino acid sequence set forth in any one of SEQ ID NO: 37 to 48;
- 35 (l) an amino acid sequence according to any one of (a) to (k) above wherein when present the immunoglobulin binding region is inactive;

- (m) an amino acid sequence according to any one of (a) to (l) above wherein when present the complement binding region is inactive;
- (n) an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (m) above; or
- 5 (o) any combination of any two or more of (a) to (n) above.

In one example, one or more of the at least one SSL11 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:

- (a) an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 11;
- 10 (b) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 11;
- (c) at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 11, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
- 15 (d) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11;
- (e) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 49 to 72;
- (f) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in
20 any one of SEQ ID NO: 49 to 72, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
- (g) an amino acid sequence set forth in any one of SEQ ID NO: 49 to 72;
- (h) at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 61 to 72;
- 25 (i) an amino acid sequence set forth in any one of SEQ ID NO: 61 to 72;
- (j) an amino acid sequence according to any one of (a) to (i) above wherein when present the sialic acid binding region is inactive;
- (k) an amino acid sequence according to any one of (a) to (j) above wherein the SSL11 polypeptide does not exhibit chemotactic activity;
- 30 (l) an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (k) above; or
- (m) any combination of any two or more of (a) to (l) above.

In one example, the multivalent polypeptide comprises:

- (a) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal
35 Superantigen-Like virulence factor 3; and/or

- (b) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7; and/or
- (c) an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11.

5 In one example, the multivalent polypeptide comprises:

- (a) a Staphylococcal Superantigen-Like virulence factor 3 polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 3 and in which one or more of the Toll-like receptor binding activity, the Toll-like receptor inhibitory activity,
10 and/or the sialic acid binding activity is inactive; and/or
- (b) a Staphylococcal Superantigen-Like virulence factor 7 polypeptide comprising an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7 and in which one or more of the immunoglobulin binding activity, the complement binding activity, and/or the complement inhibiting activity is inactive;
15 and/or
- (c) a Staphylococcal Superantigen-Like virulence factor 11 polypeptide comprising an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11 and in which the sialic acid binding activity and/or the phagocyte chemotactic activity is inactive.

20 In one example, the multivalent polypeptide does not exhibit one or more of the biological activities selected from the group consisting of: Toll-like receptor binding activity, Toll-like receptor inhibitory activity, sialic acid binding activity, phagocyte chemotactic activity, immunoglobulin binding activity, complement binding activity, complement inhibiting activity, and any combination of two or more thereof.

25 In one example, each Staphylococcal Superantigen-Like virulence factor is from the same clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC1 clonal complex.

30 In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC5 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC8 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC15 clonal complex.

35 In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC22 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC30 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC45 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC93 clonal complex.

5 In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC97 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC121 clonal complex.

10 In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC398 clonal complex.

In one example, each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC88 clonal complex (of which JSNZ is an isolate).

In one example, at least two of the Staphylococcal Superantigen-Like virulence factors are separated from one another by an intervening amino acid sequence.

15 For example, the intervening amino acid sequence comprises a linker sequence or a proteolytic cleavage site.

In various examples, the linker sequence comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of: PGGGGSGGG [SEQ ID NO.: 157], PGVDAAA [SEQ ID NO.: 158], PGLQEFEL [SEQ ID NO.: 159], and GSVGGITKTG [SEQ ID NO.: 160].

20 In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence corresponding to at least about 50 contiguous amino acids from a sequence set forth in any one of SEQ ID NO.s: 73 to 144.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence corresponding to the sequence set forth in any one of SEQ ID NO.s: 73 to 144.

25 In one example, the multivalent polypeptide additionally comprises one or more amino acid sequences selected from the group consisting of:

- (a) an amino acid sequence comprising an intracellular translocation domain;
- (b) an amino acid sequence comprising a domain associated with improved proteolytic processing;
- 30 (c) an amino acid sequence comprising an adjuvant or a domain associated with enhanced immunological response or immunogenicity;
- (d) an amino acid sequence comprising a domain associated with enhanced epitope presentation or processing;
- (e) an amino acid sequence capable of inducing or increasing the activity of one or more
35 myeloid cells or one or more lymphoid cells;

- (f) an amino acid sequence capable of inducing or increasing the activity of one or more of a B-cell, a CD4+ cell, a CD8+ cell, or an antigen-presenting cell; and
- (g) any combination of two or more of any of (a) to (f) above.

5 In another aspect the invention relates to a polynucleotide construct encoding a multivalent polypeptide or polypeptide conjugate as contemplated herein.

In one example, said construct is a vaccine construct, such as an mRNA vaccine construct.

10 In another aspect the invention relates to an isolated, purified, recombinant, or synthesised polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence having at least about 90% or at least about 95% nucleic acid sequence identity to a nucleic acid sequence set forth in any one of SEQ ID NO.s: 145 to 156.

In another aspect the invention relates to a polynucleotide, construct, vector, cell-free system, or cell capable of expressing a multivalent polypeptide or polypeptide conjugate as contemplated herein, or comprising a polynucleotide as contemplated herein, or a polynucleotide encoding a multivalent polypeptide as contemplated herein.

15 In one example, the vector comprises, consists essentially of, or consists of a nucleotide sequence corresponding to the sequence of any one of SEQ ID NO.s: 145 to 156.

In another aspect the invention relates to a pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of one or more multivalent polypeptides as contemplated herein, together with one or more pharmaceutically acceptable carriers.

20 In another aspect the invention relates to a pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of a polynucleotide, construct or vector as contemplated herein, together with one or more pharmaceutically acceptable carriers.

In one example, the composition comprises two or more multivalent polypeptides as contemplated herein.

25 In one example, the composition comprises three or more multivalent polypeptides as contemplated herein.

In one example, the composition comprises four or more of the multivalent polypeptides as contemplated herein.

30 In one example, the composition comprises two or more polynucleotides, constructs, or vectors as contemplated herein or any combination thereof, together with one or more pharmaceutically acceptable carriers.

In one example, the pharmaceutical composition is an immunogenic composition.

For example, the composition is a vaccine.

35 In another aspect the invention relates to a method of treating or preventing a Staphylococcus infection, the method comprising administering to a subject in need thereof an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or a pharmaceutical composition as contemplated herein.

In another aspect the invention relates to a method of vaccinating a subject in need thereof, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or a pharmaceutical composition as contemplated herein.

5 In one example, the method is a method of vaccinating a subject against *Staphylococcus aureus*.

In another aspect the invention relates to the use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as contemplated herein for treating or preventing a *Staphylococcus* infection in a subject, for vaccinating a subject, and/or for eliciting an immune response in a subject.

10 In another aspect the invention relates to the use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition thereof as contemplated herein in the manufacture of a medicament for treating or preventing a *Staphylococcus* infection in a subject, for vaccinating a subject, and/or for eliciting an immune response in a subject.

15 In another aspect the invention relates to a method of eliciting in a subject in need thereof an immune response or conferring on a subject in need thereof a health benefit, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as contemplated herein.

In one example, the health benefit is decreased susceptibility to infection with *Staphylococcus aureus*, and/or reduced risk of symptoms associated with or sequelae of such infection.

20 In various examples, the immune response is or is characterised by an increase in the number or activity of one or more immunoglobulins capable of specifically binding to a *Staphylococcus* antigen.

In various examples, the immune response is or is characterised by an increase in the number or activity of one or more immune cells.

25 In various examples, the immune response is or is characterised by an increase in the number or activity of one or more cells selected from the group consisting of: any upregulated cell populations.

In one example, one or more of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO: 1 to 72.

30 In one example, at least one of the SSL polypeptides comprises, consists essentially of, or consists of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60. In one example, at least two of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60.

35 In one example, at least one of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72. In one example, at least two of the SSL polypeptides comprise,

consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72.

In one example, at least one of the SSL polypeptides comprises, consists essentially of, or consists of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60, and at least one of the SSL polypeptides comprises, consists essentially of, or consists of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72.

In one example, each of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 72. In one example, each of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60. In one example, each of the SSL polypeptides comprise, consist essentially of, or consist of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72.

In one example, the multivalent polypeptide comprises an amino acid sequence that corresponds to one of the amino acid sequences set forth in any one of SEQ ID NO.s: 1 to 72. In one example, the multivalent polypeptide comprises an amino acid sequence that corresponds to one of the amino acid sequences set forth in any one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60. In one example, the multivalent polypeptide comprises an amino acid sequence that corresponds to one of the amino acid sequences set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72. In one example, the multivalent polypeptide comprises an amino acid sequence that corresponds to one of the amino acid sequences set forth in any one of SEQ ID NO.s: 73 to 144.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising or consisting essentially of two or more of the amino acid sequences set forth in SEQ ID NO.s: 1 – 72, or an amino acid sequence having at least about 90% amino acid identity thereto. For example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising two or more of the amino acid sequences set forth in SEQ ID NO.s: 1 – 72.

In one example, the multivalent polypeptide comprises an amino acid sequence comprising one, two, three, four, five, six, or more of the amino acid sequences set forth in SEQ ID NO.s: 1 to 72.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 1 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 25 - 72.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 1 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 25 - 48.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 1 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 49 - 72.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 25 - 48, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 49 - 72.

5 In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 12 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 25 - 72.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 12 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 25 - 48.

10 In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 12 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 37 - 48.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 12 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 49 - 72.

15 In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 12 - 24, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 61 - 72.

20 In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 37 - 48, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 49 - 72.

In one example, the multivalent polypeptide comprises an amino acid sequence corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 37 - 48, and one or more of the amino acid sequences set forth in SEQ ID NO.s: 61 - 72.

25 In one example, the multivalent polypeptide comprises one or more amino acid sequences corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 1 - 24, one or more amino acid sequences corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 25 - 48, and one or more amino acid sequences corresponding to one of the amino acid sequences set forth in SEQ ID NO.s: 49 - 72.

30 In one example, the multivalent polypeptide comprises an amino acid sequence comprising at least one of the amino acid sequences set forth in SEQ ID NO.s: 1 - 12 or 13 - 24, at least one of the amino acid sequences set forth in SEQ ID NO.s: 25 - 36 or 37 - 48, and at least one of the amino acid sequences set forth in SEQ ID NO.s: 49 - 60 or 61 - 72. For example, the multivalent polypeptide comprises an amino acid sequence comprising one of the amino acid sequences set forth in SEQ ID
35 NO.s: 13 - 24, one of the amino acid sequences set forth in SEQ ID NO.s: 37 - 48, and one of the amino acid sequences set forth in SEQ ID NO.s: 61 - 72.

In one aspect, the invention relates to a multivalent vaccine construct comprising or encoding a multivalent polySSL polypeptide as herein contemplated.

In various examples, the multivalent vaccine construct comprises or encodes a polySSL construct depicted in Table 1 herein. In one example, the multivalent vaccine construct comprises or encodes a polypeptide sequence corresponding to any one of SEQ ID NO.s: 73 to 144.

5 In various examples, the one or more SSL polypeptides present in the multivalent polypeptide or encoded by the multivalent polynucleotide construct correspond to one or more of the polypeptides set out in Table 1.

In various examples, the multivalent vaccine construct comprises or encodes two or more of the polypeptides presented in Table 1, at least one of said polypeptides being selected from the group consisting of SEQ ID NO.s: 13 to 24.

10 In various examples, the multivalent vaccine construct comprises or encodes two or more of the polypeptides presented in Table 1, at least one of said polypeptides being selected from the group consisting of SEQ ID NO.s: 37 to 48.

15 In various examples, the multivalent vaccine construct comprises or encodes two or more of the polypeptides presented in Table 1, at least one of said polypeptides being selected from the group consisting of SEQ ID NO.s: 61 to 72.

In one example, the multivalent polypeptide comprises SSL polypeptides that collectively are capable of binding to or predicted to bind to an immunoglobulin capable of specifically binding to *S. aureus* or a secreted protein or secreted factor therefrom.

20 In one example, the multivalent polypeptide comprises an SSL3 polypeptide having reduced or no TLR2 binding. In one example, the multivalent polypeptide comprises an SSL3 polypeptide wherein the TLR2 binding domain of said SSL3 polypeptide comprises one or more amino acid substitutions that reduces or ablates TLR2 binding. In various examples, said SSL3 polypeptide comprising one or more amino acid substitutions that reduces or ablates TLR2 binding is an SSL3 polypeptide consisting essentially of or consisting of the amino acid sequence of any one of SEQ ID NO.s: 13 to 24.

25 In one example, the multivalent polypeptide comprises an SSL3 polypeptide having reduced or no sialic acid binding. In one example, the multivalent polypeptide comprises an SSL3 polypeptide wherein the sialic acid binding domain of said SSL3 polypeptide comprises one or more amino acid substitutions that reduces or ablates sialic acid binding. In various examples, said SSL3 polypeptide comprising one or more amino acid substitutions that reduces or ablates sialic acid binding is an SSL3
30 polypeptide consisting essentially of or consisting of the amino acid sequence of any one of SEQ ID NO.s: 13 to 24.

In one example, the multivalent polypeptide comprises an SSL7 polypeptide having reduced or no IgA binding. In one example, the multivalent polypeptide comprises an SSL7 polypeptide wherein the IgA binding domain of said SSL7 polypeptide comprises one or more amino acid substitutions that
35 reduces or ablates IgA binding. In various examples, said SSL7 polypeptide comprising one or more amino acid substitutions that reduces or ablates IgA binding is an SSL7 polypeptide consisting essentially of or consisting of the amino acid sequence of any one of SEQ ID NO.s: 37 to 48.

In one example, the multivalent polypeptide comprises an SSL7 polypeptide having reduced or no C5 binding. In one example, the multivalent polypeptide comprises an SSL7 polypeptide wherein

the C5 binding domain of said SSL7 polypeptide comprises one or more amino acid substitutions that reduces or ablates C5 binding. In various examples, said SSL7 polypeptide comprising one or more amino acid substitutions that reduces or ablates C5 binding is an SSL7 polypeptide consisting essentially of or consisting of the amino acid sequence of any one of SEQ ID NO.s: 37 to 48.

5 In one example, the multivalent polypeptide comprises an SSL11 polypeptide having reduced or no sialic acid binding. In one example, the multivalent polypeptide comprises an SSL11 polypeptide wherein the sialic acid binding domain of said SSL11 polypeptide comprises one or more amino acid substitutions that reduces or ablates sialic acid binding. In various examples, said SSL11 polypeptide comprising one or more amino acid substitutions that reduces or ablates sialic acid binding is an
10 SSL11 polypeptide consisting essentially of or consisting of the amino acid sequence of any one of SEQ ID NO.s: 61 to 72.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL3 amino acid sequence, an SSL7 amino acid sequence, and an SSL11 amino acid
15 sequence.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL3 amino acid sequence, an SSL11 amino acid sequence, and an SSL7 amino acid sequence.

20 In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL7 amino acid sequence, an SSL3 amino acid sequence, and an SSL11 amino acid sequence.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an
25 amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL7 amino acid sequence, an SSL11 amino acid sequence, and an SSL3 amino acid sequence.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL11 amino acid sequence, an SSL3 amino acid sequence, and an SSL7 amino acid
30 sequence.

In one example, the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus an SSL11 amino acid sequence, an SSL7 amino acid sequence, and an SSL3 amino acid
35 sequence.

In one example, the multivalent polypeptide comprises an amino acid sequence encoding an SSL3 polypeptide, an amino acid sequence encoding an SSL7 polypeptide, and an amino acid sequenced encoding an SSL11 polypeptide, wherein either the amino acid sequence encoding the
40 N-terminus of the multivalent polypeptide.

In one example, the multivalent polypeptide comprises an amino acid sequence encoding an SSL3 polypeptide, an amino acid sequence encoding an SSL7 polypeptide, and an amino acid sequenced encoding an SSL11 polypeptide, wherein the amino acid sequence encoding the SSL3 polypeptide is most proximal to the N-terminus of the multivalent polypeptide.

5 In one example, the multivalent polypeptide comprises an amino acid sequence encoding an SSL3 polypeptide, an amino acid sequence encoding an SSL7 polypeptide, and an amino acid sequenced encoding an SSL11 polypeptide, wherein the amino acid sequence encoding the SSL11 polypeptide is most proximal to the N-terminus of the multivalent polypeptide.

10 In various examples, the multivalent vaccine construct comprises or encodes one or more amino acid sequences capable of inducing or increasing the activity of one or more immune cells.

In one example, the amino acid sequence capable of inducing or increasing the activity of one or more immune cells is capable of inducing or increasing the specific activity of a B-cell.

15 In one example, the one or more amino acid sequences capable of inducing or increasing the activity of one or more immune cells is an amino acid sequence capable of inducing or increasing the activity of one or more of a CD4⁺ cell, a CD8⁺ cell, or an antigen-presenting cell.

In one example, the one or more amino acid sequences capable of inducing or increasing the activity of one or more immune cells is an amino acid sequence capable of inducing or increasing the specific activity of a CD8⁺ cell.

In one example, the polypeptide is selected from the group consisting of:

- 20 (a) a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 144; or
- (b) a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence corresponding to at least 50 contiguous amino acids from an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 144; or
- 25 (c) a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence corresponding to at least 50 contiguous amino acids from an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 144 and comprising at least about two SSL amino acid sequences; or
- 30 (d) a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence corresponding to at least 50 contiguous amino acids from an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 144 and comprising at least one SSL amino acid sequence selected from the group consisting of an amino acid sequence comprising, consisting essentially of, or consisting of an amino acid sequence set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, and/or 61 to 72; or
- 35 (e) a polypeptide encoded by a polynucleotide sequence having at least about 90% nucleic acid sequence identity to at least 50 contiguous nucleotides from a sequence set forth in any one of SEQ ID NO.s: 145 to 156;
- (f) any combination of any two or more of (a) to (g) above.

In a particularly contemplated example, the multivalent vaccine construct is a polynucleotide encoding a multivalent polypeptide as disclosed above.

In another aspect, the present invention relates to an isolated, purified, recombinant, or synthesised polynucleotide comprising at least about 70%, at least 75%, at least 80%, at least 85%,
5 at least about 90%, at least about 95%, or at least about 99% nucleic acid sequence identity to the nucleic acid sequence set forth in any one of SEQ ID NO.s: 145 to 156.

In another aspect, the present invention relates to an isolated, purified, recombinant, or synthesised polynucleotide comprising at least about 70%, at least 75%, at least 80%, at least 85%,
10 at least about 90%, at least about 95%, or at least about 99% nucleic acid sequence identity to a nucleic acid sequence encoding one of the amino acid sequences set forth in any one of SEQ ID NO.s: 13 to 24, 37 to 48, 61 to 72, or 73 to 144.

In another aspect, the present invention relates to a polynucleotide construct, vector, cell-free system, or cell capable of expressing a multivalent polypeptide or polypeptide conjugate as described herein, or comprising a polynucleotide as described herein, or a polynucleotide encoding a multivalent
15 polypeptide or polypeptide conjugate as described herein.

In one example, the vector comprises, consists essentially of, or consists of a nucleotide sequence corresponding to the sequence of any one of SEQ ID NO.s: 145 to 156.

In one example, the construct or vector is a construct or vector exemplified herein in the Examples, such as a construct or vector encoding a multivalent SSL polypeptide as exemplified in the
20 Examples herein.

In one example, the construct or vector comprises at least about 100 contiguous nucleotides of the nucleotide sequence set forth in any one of SEQ ID NO.s: 145 to 156.

In one example, the construct or vector comprises at least about 100 contiguous nucleotides of a nucleotide sequence encoding a multivalent SSL polypeptide exemplified in the Examples herein. For
25 example, the construct or vector comprises at least about 100 contiguous nucleotides encoding at least about 33 contiguous amino acids from an amino acid sequence of a multivalent SSL polypeptide exemplified in the Examples herein. For example, the construct or vector comprises at least about 100 contiguous nucleotides encoding at least about 33 contiguous amino acids from an amino acid sequence of a multivalent SSL polypeptide of any one of SEQ ID NO.s: 73 to 144.

In another aspect, the present invention relates to a pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of one or more multivalent polypeptides,
30 and/or one or more polymer particles as described herein, or a pharmaceutically acceptable salt or solvate of any thereof, or any combination of two or more thereof, together with one or more pharmaceutically acceptable carriers.

In another aspect, the present invention relates to a pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of one or more multivalent
35 polynucleotide constructs as described herein or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers.

In another aspect, the present invention relates to a pharmaceutical composition comprising, consisting essentially of, or consisting of one or more of the group consisting of: one or more multivalent polypeptides as described herein, one or more multivalent polypeptide conjugates as described herein, one or more polymer particles as described herein, one or more multivalent polynucleotide constructs as described herein, or one or more pharmaceutically acceptable salts or solvates of any thereof, together with one or more pharmaceutically acceptable carriers.

In one example, the pharmaceutical composition comprises or consists essentially of one or more multivalent vaccine constructs which encode or comprise at least three different SSL polypeptides. For example, the pharmaceutical composition comprises or consists essentially of multivalent vaccine constructs which collectively encode or comprise at least ten different SSL polypeptides or SSL polypeptide variants, or which collectively encode or comprise at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, or more than 40 different SSL polypeptides or SSL polypeptide variants.

In one example, the pharmaceutical composition comprises two or more multivalent vaccine constructs which collectively encode or comprise at least three different SSL polypeptides, such as at least three of the SSL polypeptides specifically disclosed herein.

In one example, the pharmaceutical composition comprises two or more multivalent constructs, wherein each multivalent construct individually comprises or encodes two, three, four, five, six, seven, or more than seven SSL polypeptides, and wherein collectively the constructs present in the composition comprise or encode at least six different SSL polypeptides. In certain examples, collectively the two or more multivalent constructs present in the pharmaceutical composition encode or comprise at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, at least 26, at least 27, at least 28, at least 29, at least 30, at least 31, at least 32, at least 33, at least 34, at least 35, at least 36, at least 37, at least 38, at least 39, at least 40, or more than 40 different SSL polypeptides.

In various examples, one or more of the SSL polypeptides of the pharmaceutical composition comprises, consists essentially of, or consists of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 42, such as an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 8 to 14, 22 to 28, or 36 to 42.

Specifically contemplated are pharmaceutical compositions comprising or encoding at least 15 or more different SSL polypeptides, such as at least 15 or more different SSL polypeptides each comprising, consisting essentially of, or consisting of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 42, such as an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 8 to 14, 22 to 28, or 36 to 42.

Specifically contemplated are pharmaceutical compositions comprising or encoding at least one polySSL polypeptide, such as at least one polySSL polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence corresponding to an amino acid sequence set forth in any one of SEQ ID NO.s: 43 to 49.

5 In one example, when the multivalent construct or the pharmaceutical composition comprises or encodes an epitope capable of binding to or predicted to bind to an SSL-specific immunoglobulin, the multivalent construct or pharmaceutical composition comprises or encodes two or more epitopes capable of binding to or predicted to bind to that SSL-specific immunoglobulin.

10 In one example, the multivalent construct or the pharmaceutical composition comprises or encodes at least two epitopes capable of binding to or predicted to bind to a selected *S. aureus*-specific immunoglobulin, such as an SSL-specific immunoglobulin.

In one example, when the multivalent construct or the pharmaceutical composition comprises or encodes an epitope capable of binding to or predicted to bind to a specific HLA class 1 variant, the multivalent construct or pharmaceutical composition comprises or encodes two or more epitopes capable of binding to or predicted to bind to that HLA class 1 variant.

15 In one example, the multivalent construct or the pharmaceutical composition comprises or encodes at least two epitopes capable of binding to or predicted to bind to a selected HLA class 1 variant.

In one example, the pharmaceutical composition is an immunogenic composition.

20 In one example, the pharmaceutical composition is a vaccine.

In various examples, the pharmaceutical composition comprises one or more adjuvants. For example, the pharmaceutical composition comprises one or more TLR agonists, such as but not limited to one or more TLR2 agonists and/or one or more TLR7 agonists.

25 In one example, the pharmaceutical composition comprises one or more adjuvants, wherein the one or more adjuvants is selected from the group consisting of: potassium aluminium sulfate (Alum); squalene, including oil-in-water emulsions of squalene such as MF59; and AddaVax™.

30 In one example, the pharmaceutical composition comprises one or more adjuvants, wherein the one or more adjuvants comprise one or more Pam (S-[2,3-bis(palmitoyloxy)propyl]) moieties. For example, the adjuvant comprises, consists essentially of, or consists of any one or more of the group consisting of: Pam1, Pam1Cys, Pam1CSK4, PEG-Pam1, PEG-Pam1Cys, PEG-Pam1CSK4, Pam2, Pam2Cys, Pam2CSK4, PEG-Pam2, PEG-Pam2Cys, PEG-Pam2CSK4, Pam3, Pam3Cys, Pam3CSK4, PEG-Pam3, PEG-Pam3Cys, and PEG-Pam3CSK4.

35 In particularly contemplated examples, the pharmaceutical composition comprises one or more adjuvants selected from the group consisting of Pam2CSK4 and Pam3CSK4. For example, the pharmaceutical composition comprises Pam2CSK4. In another example, the pharmaceutical composition comprises Pam3CSK4. In another example, the pharmaceutical composition comprises Pam2CSK4 and Pam3CSK4.

In another aspect, the present invention relates to a method of vaccinating a subject in need thereof, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as described herein.

5 In another aspect, the present invention relates to a method of vaccinating a subject in need thereof, the method comprising administering to the subject a pharmaceutical composition as described herein.

In one example, the method is a method of vaccinating a subject against *S. aureus*.

10 In another aspect, the present invention relates to the use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as described herein for eliciting an immune response in a subject.

In another aspect, the present invention relates to the use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition thereof as described herein in the manufacture of a medicament for eliciting an immune response in a subject.

15 In another aspect, the present invention relates to a method of eliciting in a subject in need thereof an immune response or conferring on a subject in need thereof a health benefit, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as described herein.

In one example, the immune response is or is characterised by an increase in the number or activity of one or more immune cells.

20 In one example, the immune response is or is characterised by an increase in the number or activity of one or more myeloid cells. For example, the immune response is or is characterised by an increase in the number or activity of one or more cells selected from the group consisting of: granulocytes; myeloid-derived suppressor cells; dendritic cells; macrophages; and monocytes.

25 In one example, the immune response is or is characterised by an increase in the number or activity of one or more lymphoid cells. For example, the immune response is or is characterised by an increase in the number or activity of one or more cells selected from the group consisting of: T cells, such as cytotoxic T cells or helper T cells; NKT cells, NK cells, B cells, and Plasma cells.

30 In one example, the immune response is or is characterised by an increase in the number or activity of one or more cells selected from the group consisting of: B-cells, T-helper cells, dendritic cells, neutrophils, macrophages, and monocytes.

In one example, the immune response is or is characterised by an increase in the number or activity of one or more T-helper cell effector cells, such as T Effector Memory Cells (TEM), including CD62L-CD44hi TEM cells.

35 In one example, the immune response is or is characterised by an increase in the number or activity of one or more cells selected from the group consisting of: CD8+ cells, CD8+ interferon gamma secreting T cells, CD4+ cells, CD4+ interferon gamma secreting T cells, and IL-17A+ T cells.

In one example, the health benefit is decreased susceptibility to infection with *S. aureus*, and/or reduced risk of symptoms associated with or sequelae of such infection.

In another aspect, the present invention relates to the use of a polypeptide or polypeptide conjugate as described herein for vaccinating a subject or a pharmaceutically acceptable salt or solvate thereof.

Other aims, aspects, features and advantages of the present invention will become apparent from the following description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred examples of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

The invention is exemplified in the following non limiting examples and with reference to the accompanying figures.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts a schema of a representative multivalent polypeptide construct (Figure 1, top) and a representative multivalent polynucleotide construct (Figure 1, bottom) as described herein.

Figure 2 presents three graphs showing that the removal of *ssl* genes from *S. aureus* JSNZ results in a reduced ability to respond to immune attack compared to wildtype bacteria, as described herein in Example 1. (A) Survival of wild-type JSNZ bacteria, the *S. aureus*Δ*ssl* strain and the *S. aureus*Δ*ssl* strain re-complemented with selected *ssl* genes in the presence of human whole blood after 20h; (B) Attenuated development of disease after intraperitoneal infection of CD1 mice with 10⁸ CFU of wildtype or *S. aureus*Δ*ssl* bacteria shown by assessing weight loss relative to baseline over 5 days; or (C) tissue CFU at day 5. Results in (A) are representative of independent experiments with two different blood donors, shown as mean+standard deviation of replicate samples and statistically significant differences relative to the JSNZΔ*SSL* strain determined using a Kruskal Wallis test with Dunn's multiple comparisons test. The remaining data are combined from two independent experiments, each with n=5 mice. Body weights (B) are displayed as mean +standard deviation, differences over time were compared by running multiple t tests; (C) shows individual data points and the median, with significant differences identified by Mann-Whitney test.

Figure 3 presents four graphs showing that the polySSL polypeptide SSL7311 has all the functional activities of the individual components, whereas the SSL7311M protein has attenuated activity, as described herein in Example 2. (A) THP-1 cells were incubated with SSL3, SSL3711 or SSL7311M protein and 2 µg/mL LTA overnight. TNF levels in the supernatants were quantified by ELISA. The dotted line indicates the quantity of TNF made in the presence of LTA alone. (B) SSL7, SSL3711 or SSL7311M protein was pre-incubated with human serum and cleavage of complement C5 determined by measuring deposition of C5b-9 onto heat-killed *S. aureus*. The upper dotted line shows maximum C5b-9 formation in the presence of serum only, and the lower dotted line shows background levels produced in the presence of heat-inactivated serum. (C) Serum proteins bound to SSL7, SSL7M, SSL7311 or SSL7311M coupled to sepharose beads, or a negative control of sepharose beads alone, were analysed for the presence of human IgA by SDS-PAGE and Western blot. (D) White Blood Cells (WBC) bound to SSL11, SSL11M, SSL7311 or SSL7311M coupled to sepharose beads, or a negative control of sepharose beads alone (PBS), were analysed for the presence of human PSGL-1 by SDS-PAGE and Western blot. (A) and (B)

show mean $\pm$ standard deviation from a minimum of two independent experiments, whereas (C) and (D) are representative from independent experiments.

Figure 4 presents three graphs showing that Vaccination with the PolySSL7311 vaccine in adjuvant stimulates humoral and cellular responses to SSLs 3, 7 and 11. Mice were vaccinated subcutaneously on days 0, 14 and 28 with PBS control or 10 μ g of SSL7311 protein delivered in AdjuPhos or AddaVax and blood collected on days 11, 25, 39. (A) Serum IgG responses to the individual SSLs were measured by ELISA. (B) Splenocytes were stimulated with individual SSL proteins for 72h, proliferation quantified by addition of tritiated thymidine for the final 6h and results presented as stimulation indexes (SI). Development of IgG responses to each SSL over time were compared using a Friedman test with Dunn's multiple comparison test applied. Data from two independent experiments each containing n=6 mice per treatment group displayed as box and whisker plots (min – max) or individual data points with a horizontal line indicating the median value. Differences in proliferation responses were assessed using a Kruskal-Wallis test with Dunn's Multiple Comparisons test applied. Data are combined from two independent studies, each containing n=3 mice per treatment group. The horizontal line is a median value. Asterisks denote a significant increase in specific IgG or SI relative to PBS controls (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

Figure 5 presents four graphs showing that Treatment with the PolySSL7311 vaccine reduces *S. aureus* disease. Mice were vaccinated subcutaneously on days 0, 14 and 28 with PBS control or 10 μ g of PolySSL7311 vaccine delivered in AdjuPhos or AddaVax, challenged with $\sim 5 \times 10^7$ CFU *S. aureus* JSNZ on day 42 and tissues harvested on day 46. Data are combined from four independent studies, each containing n=6 mice per treatment group. The horizontal line is a median value. Body weights are shown as mean $\pm$ standard deviation. Differences between treatment groups were assessed using a Mann-Whitney test. Asterisks denote a significant difference between an adjuvant only controls and the PolySSL7311 vaccinated control group for each adjuvant (* p < 0.05; **** p < 0.0001).

Figure 6 presents four graphs showing that a reduction in *S. aureus* disease requires the PolySSL7311 vaccine. Mice were vaccinated subcutaneously on days 0, 14 and 28 with PBS control; 3.3 μ g each of SSL7, 3 and 11 (SSLs); or 10 μ g of PolySSL7311 protein (vaccine) delivered in Alum or MF59, challenged with $\sim 5 \times 10^7$ CFU *S. aureus* JSNZ on day 42 and tissues harvested on day 46. Data are combined from two independent studies, each containing n=6 mice per treatment group. Body weights are shown as mean $\pm$ standard deviation. The horizontal line is a median value. Differences between treatment groups were assessed using a Kruskal-Wallis test with Dunn's test multiple comparisons test applied.

Figure 7 presents four graphs showing that vaccination with the PolySSL7311 vaccine in adjuvant stimulates increased specific IgG responses and enhanced anti-SSL7 neutralising antibodies. Mice were vaccinated subcutaneously on days 0, 14 and 28 with PBS control; 3.3 μ g each of SSL7, 3 and 11; or 10 μ g of PolySSL7311 protein delivered in AdjuPhos or AddaVax and serum antibody responses assessed on day 39. (A) IgG endpoint titres to each SSL in the groups vaccinated with individual SSLs or the PolySSL7311 vaccine, compared using a Mann-Whitney test. (B) Development of antibodies able to inhibit wildtype SSL protein function. Inhibition of SSL3 function was determined by measuring restoration of TNF production by

THP-1 cells stimulated with LTA in the presence of SSL3 (SSL3-TNF); inhibition of SSL7 binding to human IgA (SSL7-IgA); prevention of SSL7 from forming a complex with complement C5 to stop activation of complement, measured by detecting C5b-9 deposition on whole *S. aureus* (SSL7-C5b-9). Results are shown as a percentage of TNF production, IgA binding or C5b9 deposition relative to positive controls. Statistically significant differences between all were determined using a Kruskal-Wallis test with Dunn's multiple comparisons test applied. Asterisks denote * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$. Data from two independent experiments each containing $n=6$ mice per treatment group displayed as box and whisker plots (min – max).

Figure 8 presents five graphs showing the impact of protein order on serum anti-SSL IgG titres (A) and protection (B) in Kidney (B, left graph) and Liver (B, right graph), as described herein in Example 2.

Figure 9 presents a graph showing immunization with the SSL polypeptide vaccine stimulates a humoral immune response to all components and attenuates *S. aureus* infection, as described herein in Example 3.

Figure 10 depicts the geographical distribution of the prevalent MRSA clones. Figure adapted from Monarco M. et al. (2017) Curr Top Microbiol Immunol 409:21-29.

Figure 11 presents a photo of 1µg of six different purified multivalent polypeptides (also referred to herein as polySSLs) separated by 12.5% SDS-PAGE. The six recombinant polySSLs were constructed as herein described to cover the globally prevalent MRSA clonal complexes (CC1, CC5, CC8, CC22, CC30 and ST93) causing staphylococcal disease in humans. These polySSLs were expressed in *E. coli* and purified by affinity, ion exchange, and size exclusion chromatography to yield proteins of high purity, as can readily be seen.

Figure 12 presents 9 graphs showing that combinations of recombinant polySSLs in a vaccine can protect against strains that are not covered by that vaccine as described in example 4. Vaccination with either polySSL JSNZ, or combinations of polySSL from CC5 and CC22 (PolySSL CC5/CC22), or CC8 and CC30 (PolySSL CC8/CC30) can induce cross-reactive SSL-specific antibodies in mice infected with *S. aureus* strain JSNZ, CC5, CC8, CC22, or CC30.

Figure 13 presents 2 heatmap images depicting that combination vaccines can generate neutralizing antibodies against SSL variants not included in the vaccine as described in example 4. Shown are heatmaps of the percentage neutralization of SSL3 variants inhibiting TLR2 activation (Figure 13 A) and neutralization of SSL7 variants binding to IgA (Figure 13 B) by antisera from mice vaccinated with either polySSL JSNZ, or PolySSL CC5/CC22, or polySSL CC8/CC30.

Figure 14 presents 8 graphs showing that immunising with only one or a combination of two polySSLs provides cross-protection to a range of *S. aureus* strains as described in example 4.

Figure 15 depicts an amino acid alignment of the SSL3 amino acid sequence from each of the 7 clonal complexes. Amino acids comprising the TLR2 binding region are highlighted and identified by surmounted "+", and amino acids comprising the sialic acid binding site are highlighted and identified by surmounted "%".

Figure 16 depicts an amino acid alignment of the SSL7 amino acid sequence from each of the 7 clonal complexes. Amino acids comprising the IgA binding region are highlighted and

identified by surmounted "+", and amino acids comprising the C5 binding region are highlighted and identified by surmounted "%".

Figure 17 depicts an amino acid alignment of the SSL11 amino acid sequence from each of the 7 clonal complexes. Amino acids comprising the sialic acid binding site are highlighted and identified by surmounted "+".

DETAILED DESCRIPTION

The present invention relates to multivalent *S. aureus* vaccine constructs, including multivalent polypeptide and polynucleotide constructs, compositions including pharmaceutical compositions comprising such constructs, and methods of using such constructs and compositions, wherein the multivalent constructs comprise or encode multiple *S. aureus* Staphylococcal Superantigen-Like (SSL) polypeptides. Such constructs, compositions and methods are suitable for use in eliciting an immunological response in a subject in need thereof, such as vaccinating a subject in need thereof against *S. aureus*.

The Staphylococcal Superantigen-Like (SSL) virulence factors are a family of secreted proteins produced by all *S. aureus* that act to dampen immune signalling and impair phagocytic function. SSL polypeptides contemplated for use herein will in certain examples comprise a complete SSL virulence factor, but will in other examples comprise mutated variants or fragments of the naturally occurring SSL virulence factor, such as mutated variants or fragments that do not exhibit one or more of the activities associated with the naturally occurring or wild-type SSL virulence factor. Representative examples of mutated variants of SSL polypeptides are presented herein in the Examples, in Table 1, and in the accompanying sequence ID listing.

In certain examples, such as representative examples of the multivalent polypeptides exemplified herein, the multivalent polypeptide or vaccine construct comprises SSLs with non-redundant roles in immune evasion. For example, Staphylococcal Superantigen-Like virulence factor 3 (SSL3) has been reported to be a potent inhibitor of Toll-like receptor 2 (TLR2) activity. Staphylococcal Superantigen-Like virulence factor 7 (SSL7) has been reported to inhibit complement activation, using human IgA as a scaffold to concentrate its potent anti-complement activity at the mucosa. Staphylococcal Superantigen-Like virulence factor 11 (SSL11) has been reported to be a powerful inhibitor of neutrophil chemotaxis.

Specifically contemplated vaccine compositions comprising multiple multivalent SSL polypeptides have been developed to cover these globally prevalent-clonal complexes (CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, and CC88 (a representative isolate of which is JSNZ exemplified herein)) causing staphylococcal disease in humans and livestock.

Various aspects of the invention are described in further detail in the following subsections. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which this invention belongs. In case of conflict, the present specification, including definitions, will control. Although methods and materials similar or equivalent to those described herein can be used in the practice of the invention, examples of suitable methods and materials are described below. The materials, methods, and examples described herein are illustrative only and are not intended to be limiting.

Selected definitions

It is intended that reference to a range of numbers disclosed herein (for example, 1 to 10) also incorporates reference to all rational numbers within that range (for example, 1, 1.1, 2, 3, 3.9, 4, 5, 6, 6.5, 7, 8, 9 and 10) and also any range of rational numbers within that range (for example, 2 to 8, 1.5 to 5.5 and 3.1 to 4.7). These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner.

Those skilled in the art will appreciate the meaning of various terms of degree used herein. For example, as used herein in the context of referring to an amount (e.g., "about 9%"), the term "about" represents an amount close to and including the stated amount that still performs a desired function or achieves a desired result, e.g. "about 9%" can include 9% and amounts close to 9% that still perform a desired function or achieve a desired result. For example, the term "about" can refer to an amount that is within less than 10% of, within less than 5% of, within less than 1% of, within less than 0.1% of, or within less than 0.01% of the stated amount. It is also intended that where the term "about" is used, for example with reference to a figure, concentration, amount, integer or value, the exact figure, concentration, amount, integer or value is also specifically contemplated.

The term "and/or" can mean "and" or "or".

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises", and the terms "including", "include" and "includes" are to be interpreted in the same manner.

The term "consisting essentially of" when used in this specification refers to the features stated and allows for the presence of other features that do not materially alter the basic characteristics of the features specified.

The term "consisting of" as used herein means the specified materials or steps of the claimed invention, excluding any element, step, or ingredient not specified in the claim.

The term "expression construct" refers to a genetic construct that includes elements that permit transcribing the polynucleotide molecule of interest, and, optionally, translating the transcript into a polypeptide. An expression construct typically comprises in a 5' to 3' direction:

- (1) a promoter, functional in the host cell into which the construct will be introduced,
- (2) the polynucleotide to be expressed, and
- (3) a terminator functional in the host cell into which the construct will be introduced.

Expression constructs, such as those for expression of an mRNA vaccine as herein contemplated, will generally also comprise at least one untranslated region (UTR), such as a 5' UTR, a 3' UTR, and/or a polyA tail. Other elements, such as IRES or elements for self replication may also be present.

Expression constructs of the invention are inserted into a replicable vector for cloning or for expression, or are incorporated into the host genome.

The term "polynucleotide(s)" as used herein, means a single or double-stranded deoxyribonucleotide or ribonucleotide polymer of any length, and include as non-limiting examples, coding and non-coding sequences of a gene, sense and antisense sequences, exons, introns, genomic DNA, cDNA, pre-mRNA, mRNA, circRNA, saRNA, rRNA, siRNA, miRNA, tRNA, ribozymes, recombinant polynucleotides, isolated and purified naturally occurring DNA or RNA sequences, synthetic RNA and

DNA sequences, nucleic acid probes, primers, fragments, genetic constructs, vectors and modified polynucleotides. Reference to nucleic acids, nucleic acid molecules, nucleotide sequences and polynucleotide sequences is to be similarly understood. It will be appreciated that a wide variety of synthetic and/or non-naturally occurring nucleotide analogues are available, such that polynucleotides comprising one or more of said synthetic or non-naturally occurring nucleotide analogues can be prepared. The use of such polynucleotides in the methods and compositions described herein is likewise contemplated.

As used herein "purified" does not require absolute purity; rather, it is intended as a relative term where the material in question is more pure than in the environment it was in previously. In practice the material has typically, for example, been subjected to fractionation to remove various other components, and the resultant material has substantially retained its desired biological activity or activities. The term "substantially purified" refers to materials that are at least about 60% free, preferably at least about 75% free, and most preferably at least about 90% free, at least about 95% free, at least about 98% free, or more, from other components with which they may be associated, such as during manufacture.

The term "vector" as used herein refers to a polynucleotide molecule, usually but not limited to a double stranded DNA, which is amenable to use in molecular biological techniques, for example to modify, manipulate, replicate, amplify, or transport a polynucleotide molecule. In certain examples, a vector is used to transport a polynucleotide molecule, such as but not limited to a genetic construct, for example an expression construct, into a host cell or organism. In certain examples the vector is capable of replication and/or maintenance in more than one host system.

Staphylococcus aureus

The present invention relates in part to methods for treating or preventing a *Staphylococcus aureus* bacterial infection in a subject. *S. aureus* is a commensal gram-positive spherical bacterium, and is one of the most important bacterial pathogens in humans and many other animals. *S. aureus* readily acquires antibiotic resistance, and indeed Methicillin Resistant *S. aureus* (MRSA) is a particular concern, as existing antibiotic treatments have little or no efficacy, and the development of new antibiotic agents and/or treatment regimens is slow.

S. aureus is the causative pathogen of many soft tissue and skin infections in humans and animals, is a major livestock pathogen and can cause infections in companion animals, and is responsible for significant morbidity and mortality worldwide.

S. aureus can cause a spectrum of diseases, ranging from local infections such as boils through to life-threatening conditions including bacteraemia and toxic shock syndrome. There is a heightened risk of contracting bacterial infections such as *S. aureus* in the nosocomial setting (Swartz MN., 1994), particularly when undergoing surgical procedures or with extended use of indwelling catheters (Safdar N, Maki DG., 2002).

S. aureus is a complex pathogen, with a suite of virulence factors capable of subverting many aspects of host immunity (Lu T, DeLeo FR., 2016). To date, despite many attempts including a number of clinical trials, there remains no clinically approved, effective vaccine for *S. aureus*.

As exemplified herein in the Examples, the applicants have prepared multivalent polypeptide constructs and vaccine compositions comprising same to provide robust immunological benefit to subjects and comprehensive coverage of the global population, coupled in certain examples with

redundancy (in terms of binding and presentation) and future proofing to mitigate against vaccine escape, for example by mutation, and the corresponding loss of vaccine efficacy at a population level.

Various representative examples of the multivalent polySSL polypeptide constructs contemplated herein are exemplified in the Examples herein. In one example, a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 87 having as its SSL repertoire SSL7, SSL3, and SSL11 from CC8 has been assessed to establish immune response and protection against the CC8 clonal complex.

In various other examples, six polySSL polypeptides each comprising an amino acid sequence set forth in one of SEQ ID NO.s: 139 to 144 and having as their SSL repertoire different arrangements of SSL3, SSL7, and SSL11 from JSNZ have been assessed to establish immune response and protection against the CC88 clonal complex, of which JSNZ is a representative example strain.

In still further examples, six further polySSL polypeptides each comprising an amino acid sequence set forth in one of SEQ ID NO.s: 76, 82, 88, 100, 106, and 118 and having as their SSL repertoire SSL7, SSL11, and SSL3 from clonal complexes 1, 5, 8, 22, 30, and 93, respectively, have been assessed to establish immune response and protection against CC1, CC5, CC8, CC22, CC30, and CC93, respectively.

In yet further examples, combinations of polySSL polypeptide constructs have been assessed in the Examples herein. For example, combinations of a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 142 (targeting clonal complex CC88 (exemplified herein with reference to the example strain JSNZ)), a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 82 (targeting clonal complex CC5), a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 100 (targeting clonal complex CC22), a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 88 (targeting clonal complex CC8), and a polySSL polypeptide comprising the amino acid sequence set forth in SEQ ID NO.: 106 (targeting clonal complex CC30), are exemplified herein in the Examples.

Multivalent constructs

The multivalent constructs described herein comprising or encoding multiple SSL polypeptides (also referred to herein as polySSLs) are suitable for eliciting one or more immunological responses in a subject to which they are administered. In particularly contemplated examples, the immunological response is immunity, for example, immunity to *S. aureus*.

Humoral immunity is primarily mediated by B-cells, which produce antibodies in response to the presence of a pathogen, and T follicular helper cells, a subset of T helper cells which when activated produce cytokines and other factors which stimulate B-cell proliferation, class switching, and antibody production.

An important component of the humoral immune response is mediated by secreted antibodies produced by B cells. The secreted antibodies bind to antigens presented on the surface of invading pathogens, essentially flagging those pathogens for destruction, or bind to the active site of pathogen proteins to neutralize their activity.

Presentation of pathogenic antigens bound to MHC Class II molecules, such as the SSL polypeptides described and exemplified herein, activates a CD4⁺ T-cell response. Upon binding of the naive cell to the antigen-MHC II complex, CD4⁺ cells undergo differentiation and secrete a number of interleukins which may include IL-9, IFN- γ , IL-17, and IL-21, resulting in target B-cell proliferation and activation.

Methods to assess and monitor the onset or progression of an immune response comprising a humoral response in a subject are well known in the art. Convenient exemplary methods include those in which the presence of or the level of one or more pathogen-specific antibodies is assessed, and representative examples of such methods are provided herein in the Examples. Various antibody binding assays, including ELISA, skin-prick tests and the like, are routinely performed to identify and/or monitor antibody presence and titre. Similarly, cell-based methods to assess or monitor the onset and progression of an immune response comprising a humoral response are amenable to use in the present invention, and may include cell proliferation or activation assays, including assays targeted at identifying activation or expansion of one or more populations of immune cells, such as B-lymphocytes or Th2 cells.

Cell-mediated immunity is primarily mediated by T-lymphocytes. Protein antigens are processed into peptides by and expressed on the surface of antigen presenting cells (APCs) such as macrophages, B-lymphocytes, and dendritic cells, bound to either major histocompatibility MHC Class I or MHC Class II molecules. Presentation of pathogenic antigen coupled to MHC Class I activates a cytotoxic (CD8+) T-cell response. Upon binding of the T-cell to the antigen-MHC I complex, CD8+ cells secrete perforin and other mediators, resulting in target cell death. Presentation of pathogenic antigen coupled to MHC Class II activates a T-helper cell response and the Th phenotype shaped by the accompanying cytokine profile provided by the APC. Enhanced phagocyte function through the induction of Th1 and Th17 cellular immunity has been reported to be important for combatting *S. aureus* infection.

Methods to assess and monitor the onset or progression of an immune response comprising a cell-mediated response in a subject are well known in the art. Convenient exemplary methods include those in which the presence of or the level of one or more cytokines associated with a cell-mediated response is assessed. Similarly, cell-based methods to assess or monitor the onset and progression of an immune response comprising a cell-mediated response are amenable to use in the present invention, and may include cell proliferation or activation assays, including assays targeted at identifying activation or expansion of one or more populations of immune cells, such as cytotoxic T-lymphocytes.

In certain examples, the constructs, compositions, and methods contemplated herein elicit a humoral response. In certain example both a humoral response and a cell-mediated immune response are elicited.

In one example, the multivalent construct contemplated herein comprises or encodes at least one SSL3 polypeptide, at least one SSL7 polypeptide, and at least one SSL11.

In various examples, the SSL3 polypeptide is an SSL3 polypeptide variant from any clonal complexes, such as a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

In various examples, the SSL7 polypeptide is an SSL7 polypeptide variant from any clonal complexes, such as a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

In various examples, the SSL11 polypeptide is an SSL11 polypeptide variant from any clonal complexes, such as a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

In various examples, the SSL3 polypeptide is an SSL3 polypeptide variant from any one of the *Staphylococcal* clonal complexes, such as a mutated SSL3 polypeptide derived from an SSL3 polypeptide from a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

5 In various examples, the SSL7 polypeptide is an SSL7 polypeptide variant from any one of the *Staphylococcal* clonal complexes, such as a mutated SSL7 polypeptide derived from an SSL7 polypeptide from a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

10 In various examples, the SSL11 polypeptide is an SSL11 polypeptide variant from any one of the *Staphylococcal* clonal complexes, such as a mutated SSL11 polypeptide derived from an SSL11 polypeptide from a clonal complex selected from the group consisting of CC1, CC5, CC8, CC15, CC22, CC30, CC45, CC93, CC97, CC121, CC398, CC88, and ST93.

Representative polypeptides particularly contemplated for use in the multivalent constructs are set forth herein in Table 1, and presented in the accompanying Sequence ID listing as SEQ ID NO.s: 1
15 to 72. It will be appreciated that, given the structure of the multivalent constructs described herein, the component polypeptides can be configured in various arrangements and in varying number. Representative polySSL constructs particularly contemplated for use herein are also set forth in Table 1 below, with their amino acid sequences presented in the accompanying Sequence ID listing as SEQ ID NO.s: 73 to 144.

20 **Table 1. SSLs and PolySSL construct sequences**

Polypeptide	clonal complex	Sequence ID
SSL3	CC1	1
SSL3	CC5	2
SSL3	CC8	3
SSL3	CC15	4
SSL3	CC22	5
SSL3	CC30	6
SSL3	CC45	7
SSL3	CC93	8
SSL3	CC97	9
SSL3	CC121	10
SSL3	CC398	11
SSL3	CC88 (e.g., JSNZ)	12
SSL3m	CC1	13
SSL3m	CC5	14
SSL3m	CC8	15
SSL3m	CC15	16
SSL3m	CC22	17
SSL3m	CC30	18
SSL3m	CC45	19
SSL3m	CC93	20
SSL3m	CC97	21
SSL3m	CC121	22
SSL3m	CC398	23
SSL3m	CC88 (e.g., JSNZ)	24
SSL7	CC1	25
SSL7	CC5	26
SSL7	CC8	27
SSL7	CC15	28
SSL7	CC22	29
SSL7	CC30	30
SSL7	CC45	31
SSL7	CC93	32
SSL7	CC97	33
SSL7	CC121	34
SSL7	CC398	35

SSL7	CC88 (e.g., JSNZ)	36
SSL7m	CC1	37
SSL7m	CC5	38
SSL7m	CC8	39
SSL7m	CC15	40
SSL7m	CC22	41
SSL7m	CC30	42
SSL7m	CC45	43
SSL7m	CC93	44
SSL7m	CC97	45
SSL7m	CC121	46
SSL7m	CC398	47
SSL7m	CC88 (e.g., JSNZ)	48
SSL11	CC1	49
SSL11	CC5	50
SSL11	CC8	51
SSL11	CC15	52
SSL11	CC22	53
SSL11	CC30	54
SSL11	CC45	55
SSL11	CC93	56
SSL11	CC97	57
SSL11	CC121	58
SSL11	CC398	59
SSL11	CC88 (e.g., JSNZ)	60
SSL11m	CC1	61
SSL11m	CC5	62
SSL11m	CC8	63
SSL11m	CC15	64
SSL11m	CC22	65
SSL11m	CC30	66
SSL11m	CC45	67
SSL11m	CC93	68
SSL11m	CC97	69
SSL11m	CC121	70
SSL11m	CC398	71
SSL11m	CC88 (e.g., JSNZ)	72
PolySSL 3711	CC1	73
PolySSL 3117	CC1	74
PolySSL 7311	CC1	75
PolySSL 7113	CC1	76
PolySSL 1137	CC1	77
PolySSL 1173	CC1	78
PolySSL 3711	CC5	79
PolySSL 3117	CC5	80
PolySSL 7311	CC5	81
PolySSL 7113	CC5	82
PolySSL 1137	CC5	83
PolySSL 1173	CC5	84
PolySSL 3711	CC8	85
PolySSL 3117	CC8	86
PolySSL 7311	CC8	87
PolySSL 7113	CC8	88
PolySSL 1137	CC8	89
PolySSL 1173	CC8	90
PolySSL 3711	CC15	91
PolySSL 3117	CC15	92
PolySSL 7311	CC15	93
PolySSL 7113	CC15	94
PolySSL 1137	CC15	95
PolySSL 1173	CC15	96
PolySSL 3711	CC22	97
PolySSL 3117	CC22	98
PolySSL 7311	CC22	99
PolySSL 7113	CC22	100
PolySSL 1137	CC22	101
PolySSL 1173	CC22	102
PolySSL 3711	CC30	103

PolySSL 3117	CC30	104
PolySSL 7311	CC30	105
PolySSL 7113	CC30	106
PolySSL 1137	CC30	107
PolySSL 1173	CC30	108
PolySSL 3711	CC45	109
PolySSL 3117	CC45	110
PolySSL 7311	CC45	111
PolySSL 7113	CC45	112
PolySSL 1137	CC45	113
PolySSL 1173	CC45	114
PolySSL 3711	CC93	115
PolySSL 3117	CC93	116
PolySSL 7311	CC93	117
PolySSL 7113	CC93	118
PolySSL 1137	CC93	119
PolySSL 1173	CC93	120
PolySSL 3711	CC97	121
PolySSL 3117	CC97	122
PolySSL 7311	CC97	123
PolySSL 7113	CC97	124
PolySSL 1137	CC97	125
PolySSL 1173	CC97	126
PolySSL 3711	CC121	127
PolySSL 3117	CC121	128
PolySSL 7311	CC121	128
PolySSL 7113	CC121	130
PolySSL 1137	CC121	131
PolySSL 1173	CC121	132
PolySSL 3711	CC398	133
PolySSL 3117	C398	134
PolySSL 7311	C398	135
PolySSL 7113	C398	136
PolySSL 1137	C398	137
PolySSL 1173	C398	138
PolySSL 3711	CC88 (e.g., JSNZ)	139
PolySSL 3117	CC88 (e.g., JSNZ)	140
PolySSL 7311	CC88 (e.g., JSNZ)	141
PolySSL 7113	CC88 (e.g., JSNZ)	142
PolySSL 1137	CC88 (e.g., JSNZ)	143
PolySSL 1173	CC88 (e.g., JSNZ)	144

SSL polypeptides denoted with an 'm' suffix are mutated SSL polypeptides, and comprise at least one amino acid substitution to inactivate one or more functions mediated by the respective wild-type SSL, as described herein in the Examples.

Polypeptides

- 5 As will be appreciated from this disclosure, polypeptide constructs comprising multiple functionalities, including functionalities selected from the group consisting of multiple SSL polypeptides, multiple epitopes, spacer elements, proteolytic cleavage sites, and other functionalities, are provided herein. Representative polypeptides, such as the polypeptides comprising, consisting essentially of, or consisting of the amino acid sequences depicted in SEQ ID NO.s: 73 to 144, or
- 10 functional elements and/or domains present in the constructs and polypeptides discussed herein, such as the polypeptides discussed herein including those comprising, consisting essentially of, or consisting of the amino acid sequences presented in, for example, SEQ ID NO.s: 1 to 24, 25 to 48, or 49 to 72, are suitable for use in the preparation of the multivalent constructs and compositions contemplated herein. Particularly contemplated are constructs and compositions comprising one or
- 15 more polypeptides comprising, consisting essentially of, or consisting of the amino acid sequence of one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72 or a sequence having at least about 90% amino acid sequence identity with one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72 and comprising one or

more amino acid substitutions present in one of SEQ ID NO.s: 13 to 24, 37 to 48, or 61 to 72 when compared to the corresponding wild type sequence presented in one of SEQ ID NO.s: 1 to 12, 25 to 36, or 49 to 60.

In various examples, one or more of the polypeptides described above comprises a fusion polypeptide, such as a polypeptide conjugate as exemplified herein. For example, a fusion polypeptide as contemplated herein will in certain examples comprise one or more functional domains derived from, comprising or consisting of one of the sequences presented herein, such as the multivalent vaccine construct presented in, for example, any one of SEQ ID NO.s: 1 to 24, 25 to 48, or 49 to 72, fused to another amino acid sequence to provide a fusion polypeptide or polypeptide conjugate.

Those skilled in the art will recognise, on reading this description, that these proteins can be considered representative examples of certain examples of the multivalent vaccine constructs suitable for use as contemplated herein. As such, various uses of and for these polypeptides, particularly in vaccinating a subject in need thereof against *S. aureus*, are contemplated.

Polypeptides suitable for use herein include naturally-occurring proteins and peptides, and derivatives thereof including proteins and peptides having one or more amino acid variations from a naturally-occurring protein or peptide. Those skilled in the art will recognise on reading this disclosure, however, that the amino acid sequence of certain elements of the multivalent constructs contemplated herein, such as the one or more SSL polypeptides described and exemplified herein, are highly specific and variations from the depicted sequences will typically impact the efficacy or immunogenicity of such elements. Indeed, as discussed and exemplified herein, mutated variants of representative SSL polypeptides in which selected amino acids, such as certain amino acids involved in one or more SSL functions, have been substituted exhibit altered activity.

The term "amino acid" refers to natural amino acids, non-natural amino acids, and amino acid analogues. Unless otherwise indicated, the term "amino acid" includes both D and L stereoisomers if the respective structure allows such stereoisomeric forms.

Natural amino acids include alanine (Ala or A), arginine (Arg or R), asparagine (Asn or N), aspartic acid (Asp or D), cysteine (Cys or C), glutamine (Gln or Q), glutamic acid (Glu or E), glycine (Gly or G), histidine (His or H), isoleucine (Ile or I), leucine (Leu or L), Lysine (Lys or K), methionine (Met or M), phenylalanine (Phe or F), proline (Pro or P), serine (Ser or S), threonine (Thr or T), tryptophan (Trp or W), tyrosine (Tyr or Y) and valine (Val or V).

Non-natural amino acids include, but are not limited to, azetidinecarboxylic acid, 2-aminoadipic acid, 3-aminoadipic acid, beta-alanine, naphthylalanine ("naph"), aminopropionic acid, 2-aminobutyric acid, 4-aminobutyric acid, 6-aminocaproic acid, 2-aminoheptanoic acid, 2-aminoisobutyric acid, 3-aminoisobutyric acid, 2-aminopimelic acid, tertiary-butylglycine ("tBuG"), 2,4-diaminoisobutyric acid, desmosine, 2,2'-diaminopimelic acid, 2,3-diaminopropionic acid, N-ethyl glycine, N-ethylasparagine, homoproline ("hPro" or "homoP"), hydroxylysine, allo-hydroxylysine, 3-hydroxyproline ("3Hyp"), 4-hydroxyproline ("4Hyp"), isodesmosine, allo-isoleucine, N-methylalanine ("MeAla" or "Nime"), N-alkylglycine ("NAG") including N-methylglycine, N-methylisoleucine, N-alkylpentylglycine ("NAPG") including N-methylpentylglycine, N-methylvaline, naphthylalanine, norvaline ("Norval"), norleucine ("Norleu"), octylglycine ("OctG"), ornithine ("Orn"), pentylglycine ("pG" or "PGly"), pipecolic acid, thioproline ("ThioP" or "tPro"), homoLysine ("hLys"), and homoArginine ("hArg").

The term "amino acid analogue" refers to a natural or non-natural amino acid where one or more of the C-terminal carboxy group, the N-terminal amino group and side-chain functional group

has been chemically blocked, reversibly or irreversibly, or otherwise modified to another functional group. For example, aspartic acid-(beta-methyl ester) is an amino acid analogue of aspartic acid; N-ethylglycine is an amino acid analogue of glycine; or alanine carboxamide is an amino acid analogue of alanine. Other amino acid analogues include methionine sulfoxide, methionine sulfone, S-(carboxymethyl)-cysteine, S-(carboxymethyl) cysteine sulfoxide and S-(carboxymethyl)-cysteine sulfone.

A "fragment" of a polypeptide is a subsequence of the polypeptide, typically one that performs a function that is required for activity, such as enzymatic or binding activity, and/or provides a three dimensional structure of the polypeptide or a part thereof, such as an epitope. It will be appreciated that a fragment of a polypeptide may possess or elicit a different function or functions from that possessed or exhibited by the full-length polypeptide from which it is derived.

As used herein, the term "peptide" refers a short polymer of amino acids linked together by peptide bonds. While it will be recognised that the names associated with various classes of amino acid polymers (e.g., peptides, proteins, polypeptides, etc.) are somewhat arbitrary, peptides are generally of about 50 amino acids or less in length. A peptide can comprise natural amino acids, non-natural amino acids, amino acid analogues, and/or modified amino acids. A peptide can be a subsequence of naturally occurring protein or a non-natural, including a synthetic, sequence.

As used herein, the term "synthetic peptide" encompasses a peptide having a distinct amino acid sequence from those found in natural peptides and/or proteins. A "synthetic peptide," as used herein, can be produced or synthesized by any suitable method (e.g., recombinant expression, chemical synthesis, enzymatic synthesis, etc.), and can include any chemical modification to a parent peptide, and may include, but is not limited to such methods as truncations, deletions, cyclization or non-peptidic synthetic or semi-synthetic derivatives that retain the same biological function(s) as the starting peptide.. Methods of protein synthesis, such as solid state synthesis, are well known in the art.

The terms "peptide mimetic" or "peptidomimetic" refer to a peptide-like molecule that emulates a sequence derived from a protein or peptide. A peptide mimetic or peptidomimetic can contain amino acids and/or non-amino acid components. Examples of peptidomimetics include chemically modified peptides, peptoids (side groups are appended to the nitrogen atom of the peptide backbone, rather than to the α -carbons), β -peptides (amino group bonded to the β carbon rather than the α -carbon), etc. Chemical modification includes one or more modifications at amino acid side groups, α -carbon atoms, terminal amine group, or terminal carboxy group. A chemical modification can be adding chemical moieties, creating new bonds, or removing chemical moieties. Modifications at amino acid side groups include, without limitation, acylation of lysine ϵ -amino groups, N-alkylation of arginine, histidine, or lysine, alkylation of glutamic or aspartic carboxylic acid groups, lactam formation via cyclization of lysine ϵ -amino groups with glutamic or aspartic acid side group carboxyl groups, hydrocarbon "stapling" (e.g., to stabilize alpha-helix conformations), and deamidation of glutamine or asparagine. Modifications of the terminal amine group include, without limitation, the desamino, N-lower alkyl, N-di-lower alkyl, constrained alkyls (e.g. branched, cyclic, fused, adamantyl) and N-acyl modifications. Modifications of the terminal carboxy group include, without limitation, the amide, lower alkyl amide, constrained alkyls (e.g. branched, cyclic, fused, adamantyl) alkyl, dialkyl amide, and lower alkyl ester modifications. Lower alkyl is C1-C4 alkyl. Furthermore, one or more side groups, or

terminal groups, can be protected by protective groups known to the ordinarily skilled peptide chemist. The α -carbon of an amino acid can be mono- or dimethylated.

It will be appreciated that any one of the proteins or peptides described herein in certain examples comprises one or more non-naturally occurring amino acids, one or more amino acid analogues, or is or comprises a synthetic peptide or polypeptide or a peptide mimetic. Similarly, it will be appreciated that any one of the proteins or peptides described herein will in certain examples be the starting point for one or more modifications, synthetic methods, or protein engineering methods to develop a peptide analogue having a desired biological activity – for example, a qualitatively similar bioactivity as the parent protein or peptide, but an effect of a quantitatively different magnitude, or indeed a different bioactivity from that elicited by the parent protein or peptide.

The term “fusion polypeptide”, as used herein, refers to a polypeptide comprising two or more amino acid sequences, for example two or more polypeptide domains, fused through respective amino and carboxyl residues by a peptide linkage to form a single continuous polypeptide. It should be understood that the two or more amino acid sequences can either be directly fused or indirectly fused through their respective amino and carboxyl termini through a linker or spacer or an additional polypeptide.

The term “polypeptide”, as used herein, encompasses amino acid chains of any length but preferably at least 10 amino acids, including full-length proteins, in which amino acid residues are linked by covalent peptide bonds. Polypeptides described herein are purified natural products, or are produced partially or wholly using recombinant or synthetic techniques. The term may refer to a polypeptide, an aggregate of a polypeptide such as a dimer or other multimer, a fusion polypeptide, a polypeptide variant, or derivative thereof.

It will be understood that, for the particular polypeptides and proteins contemplated herein, natural variations can exist between individual strains or species. These variations may be demonstrated by (an) amino acid difference(s) in the overall sequence or by deletions, substitutions, insertions, inversions or additions of (an) amino acid(s) in said sequence. Amino acid substitutions which do not essentially alter biological and/or immunological activities, are well known. Amino acid replacements between related amino acids or replacements which have occurred frequently in evolution are, inter alia, Ser/Ala, Ser/Gly, Asp/Gly, Asp/Asn, Ile/Val. Other amino acid substitutions include Asp/Glu, Thr/Ser, Ala/Gly, Ala/Thr, Ser/Asn, Ala/Val, Thr/Phe, Ala/Pro, Lys/Arg, Leu/Ile, Leu/Val and Ala/Glu. Based on this information, methods for rapid and sensitive protein comparison and determining the functional similarity between homologous proteins were developed. Such amino acid substitutions of the exemplary examples described herein, as well as variations having deletions and/or insertions are within the scope of the invention as long as the resulting proteins retain useful biological activity and/or immune reactivity. This explains why one or more proteins described herein, when isolated from different strains, field isolates, or species, may have identity levels well below 100%, while still representing the same protein with the same immunological characteristics and/or biological function. Those variations in the amino acid sequence of a certain protein described herein that still provide a protein having useful biological activity related to that of a protein specifically identified herein are considered functional equivalents. For example, those variations in the amino acid sequence of a certain protein described herein that still provide a protein capable of reacting with an antibody specific to a protein specifically identified herein are considered as immunologically

functional equivalents of the proteins identified herein, and as such do not essentially influence the immunogenicity of the protein.

When a protein is used for example for diagnostic or therapeutic purposes, for example for reacting with antibodies, or for mediating a biological effect, for example one or more of the biological functions associated with the native protein or part thereof *in vivo*, while it can be expedient to do so it is not necessary to use the whole protein. It is also possible to use a polypeptide fragment of that protein (as such or coupled to a carrier or as a component in a fusion polypeptide, for example) or a polypeptide fragment derived from that protein or a related amino acid sequence that is capable of eliciting a desired biological effect, such as an immune response against that protein or of being recognised by an antibody specific to that protein, of mediating a cell-signalling effect, or the like. Such a polypeptide fragment may be referred to with reference to the function it possesses, such as the function it shares with the full-length protein from which it was derived. For example, a polypeptide fragment having an immunological effect is in certain examples referred to herein as an epitope, and other fragments having an immunological effect may be referred to as an immunogenic fragment, where an "immunogenic fragment" is understood to be a fragment of the full-length protein that retains its capability to induce an immune response in a vertebrate host or be recognised by an antibody specific to the parent protein. Similarly, a polypeptide fragment retaining or possessing one or more biological effects elicited by the full-length protein from which it was derived, or possessing a related or different biological effect, is referred to herein as a "bioactive fragment" or a "bioactive polypeptide fragment". Likewise, a polypeptide having a biological effect, such as a polypeptide capable of stimulating a biological response in a cell or eliciting a therapeutic effect, may be referred to herein as a "bioactive fragment" or a "bioactive polypeptide fragment", or grammatical equivalents thereof.

A variety of techniques is available to identify such polypeptide fragments, as well as DNA fragments encoding such fragments. For example, in the case of immunogenic fragments, such fragments may comprise one or more determinants or epitopes. Well-established empirical and *in silico* methods for the detection of epitopes exist and are well known to those skilled in the art. For example, computer algorithms are able to designate specific protein fragments as the immunologically important epitopes on the basis of their sequential and/or structural agreement with epitopes that are known. The determination of these regions is typically based on a combination of the hydrophilicity criteria and secondary structural features. An immunogenic fragment usually has a minimal length of 6, more commonly 8 amino acids, preferably more than 8, such as 9, 10, 12, 15 or even 20 or more amino acids. The nucleic acid sequences encoding such a fragment therefore have a length of at least 18, more commonly 24 and preferably 27, 30, 36, 45 or even 60 nucleic acids.

Similarly, those skilled in the art will be aware of methods to identify bioactive fragments using various assays targeted at identifying or detecting a particular biological response. Representative methods suitable for use in the identification or detection of bioactive fragments contemplated herein are presented below, including in the Examples.

The term "variant" with reference to polypeptides encompasses naturally occurring, recombinantly, and synthetically produced polypeptides, including those comprising one or more non-natural amino acids, one or more amino acid analogues, and peptide mimetics. Variant polypeptide sequences preferably exhibit at least 50%, more preferably at least 51%, at least 52%, at least 53%, at least 54%, at least 55%, at least 56%, at least 57%, at least 58%, at least 59%, at least 60%, at

least 61%, at least 62%, at least 63%, at least 64%, at least 65%, at least 66%, at least 67%, at least 68%, at least 69%, at least 70%, at least 71%, at least 72%, at least 73%, at least 74%, at least 75%, at least 76%, at least %, at least 77%, at least 78%, at least 79%, at least 80%, at least 81%, at least 82%, at least 83%, at least 84%, at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, 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% identity to a sequences of the present invention. Identity is found over a comparison window of at least 20 amino acid positions, preferably at least 50 amino acid positions, at least 100 amino acid positions, or over the entire length of a polypeptide of the invention.

Polypeptide sequence identity can be determined in the following manner. The subject polypeptide sequence is compared to a candidate polypeptide sequence using BLASTP (from the BLAST suite of programs, version 2.2.10 [Oct 2004]) in bl2seq, which is publicly available from NCBI (<ftp://ftp.ncbi.nih.gov/blast/>). The default parameters of bl2seq are utilized except that filtering of low complexity regions should be turned off.

Polypeptide sequence identity may also be calculated over the entire length of the overlap between a candidate and subject polynucleotide sequences using global sequence alignment programs. EMBOSS-needle (available at <http://www.ebi.ac.uk/emboss/align/>) and GAP (Huang, X. (1994) On Global Sequence Alignment. Computer Applications in the Biosciences 10, 227-235.) as discussed above are also suitable global sequence alignment programs for calculating polypeptide sequence identity.

Polypeptide variants contemplated herein also encompass those which exhibit a similarity to one or more of the specifically identified sequences that is likely to preserve the functional equivalence of those sequences and which could not reasonably be expected to have occurred by random chance. Such sequence similarity with respect to polypeptides can be determined using the publicly available bl2seq program from the BLAST suite of programs (version 2.2.10 [Oct 2004]) from NCBI (<ftp://ftp.ncbi.nih.gov/blast/>). The similarity of polypeptide sequences can be examined using the following unix command line parameters:

bl2seq -i peptideseq1 -j peptideseq2 -F F -p blastp

Variant polypeptide sequences preferably exhibit an E value of less than 1×10^{-10} , more preferably less than 1×10^{-20} , less than 1×10^{-30} , less than 1×10^{-40} , less than 1×10^{-50} , less than 1×10^{-60} , less than 1×10^{-70} , less than 1×10^{-80} , less than 1×10^{-90} , less than 1×10^{-100} , less than 1×10^{-110} , less than 1×10^{-120} or less than 1×10^{-123} when compared with any one of the specifically identified sequences.

The parameter -F F turns off filtering of low complexity sections. The parameter -p selects the appropriate algorithm for the pair of sequences. This program finds regions of similarity between the sequences and for each such region reports an "E value" which is the expected number of times one could expect to see such a match by chance in a database of a fixed reference size containing random sequences. For small E values, much less than one, this is approximately the probability of such a random match.

Conservative substitutions of one or several amino acids of a described polypeptide sequence without significantly altering its biological activity are also included in the invention. A skilled artisan will be aware of methods for making phenotypically silent amino acid substitutions (see, e.g., Bowie et al., 1990, Science 247, 1306).

A polypeptide variant contemplated herein also encompasses that which is produced from the nucleic acid encoding a polypeptide but differs from the wild-type polypeptide in that it is processed differently such that it has an altered amino acid sequence. For example, in one example a variant is produced by an alternative splicing pattern of the primary RNA transcript to that which produces a wild-type polypeptide.

Polynucleotides

As will be appreciated from this disclosure, the multivalent constructs contemplated herein include polynucleotide constructs which encode a multivalent polypeptide itself comprising multiple functionalities, including multiple epitopes, spacer elements, proteolytic cleavage sites, and other functionalities, as provided herein. It will be appreciated that such multivalent polynucleotides are useful not only in the synthesis of purified polypeptide constructs, but directly as therapeutic agents themselves. Accordingly, specifically contemplated herein is the use of the multivalent polynucleotides in, for example, expression vectors comprising the multivalent polynucleotide constructs disclosed herein, in hosts other than the target subject of therapy, such as prokaryotic or eukaryotic expression hosts as exemplified herein. Such use enables the production of multivalent polypeptide constructs and conjugates as described herein, whereby these polypeptide constructs and conjugates are the therapeutic agent. Also specifically contemplated herein is the use of the multivalent polynucleotides in, for example, expression vectors comprising the multivalent polynucleotide constructs disclosed herein, in a host that is the target subject of therapy – for example, a human to be immunised against *S. aureus*.

In certain examples, the multivalent polynucleotide is codon optimised for expression, for example, codon optimised for expression in the host to which it is to be administered.

Such polynucleotide constructs include DNA or RNA vaccine constructs which, following administration to a cell or subject support the expression of the encoded multivalent polypeptide construct in the cell or subject thereby effecting the targeted biological response(s), such as an immunological response in the cell or subject. Again, DNA or RNA vaccine constructs as contemplated herein will usefully be codon optimised for expression in the subject to be vaccinated.

Representative full length polynucleotide constructs, such as the polynucleotides comprising, consisting essentially of, or consisting of the nucleotide sequences depicted in any one of SEQ ID NO.s: 145 to 156, and functional elements and/or domains encoded therein, such as the nucleotide sequences encoding the SSL sequences discussed herein such as those comprising the amino acid sequences presented in, for example, any one of SEQ ID NO.s: 1 to 72, or 73 to 144, are suitable for use in the preparation of the multivalent constructs and compositions contemplated herein.

Such multivalent polynucleotide constructs are amenable to formulation and administration in vaccine compositions and via administration methods as is well understood in the art. Examples including vaccine compositions comprising naked or encapsulated polynucleotides such as mRNA or DNA vaccines, compositions in which the payload polynucleotide sequence is administered via a viral vector, such as adenoviral vectors, adeno-associated viral vectors, pox virus vectors, or virus-like particles are all well known and are suitable for use with the multivalent polynucleotide constructs contemplated herein.

Multivalent polynucleotide constructs for use as vaccines, such as as a DNA or mRNA vaccine, are specifically contemplated. As discussed above, the polynucleotide construct, and particularly the nucleotide sequence of such vaccine constructs, will typically be optimised for its intended use,

including for example by codon or other optimisation to enhance stability and expression in a host species when introduced thereto, for example as an mRNA or DNA vaccine.

Methods for producing multivalent constructs

5 In one aspect the invention relates to a method for producing at least one multivalent construct or conjugate thereof, the method comprising modifying or transforming a host cell to comprise at least one polynucleotide, vector, or expression construct as herein described, or to express one or more polypeptides or polynucleotides as herein described.

10 In one example the host cell is produced by modifying or transforming a cell to comprise at least one polynucleotide or construct as herein described.

In various examples, the host cell is modified or transformed to comprise at least one polynucleotide selected from the group consisting of SEQ ID NO.s: 145 to 156.

15 In various examples, the host cell is modified or transformed to comprise at least one polynucleotide encoding a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence set forth in any one of SEQ ID NO.s: 1 to 72, or 73 to 144.

20 In certain examples, including those in which the multivalent vaccine construct or conjugate thereof to be administered comprises one or more polynucleotides, for example the vaccine construct is a DNA or RNA (such as an mRNA) vaccine, the method of synthesis will provide for the scalable synthesis of such constructs. Cell-based synthetic methods are specifically contemplated, such as plant cell-based synthetic methods including those directed to the preparation of plant-derived virus-like particle vaccines. In vitro synthetic methods, such as in vitro transcription (IVT) are likewise specifically contemplated.

25 In other examples, the method for producing at least one multivalent construct or conjugate thereof comprises contacting at least one polynucleotide, vector, or expression construct as herein described with an in vitro protein expression system to express one or more polypeptides or polynucleotides as herein described. Cell-free synthetic systems are well known in the art, and are amenable to the large-scale production of both polypeptide-based and polynucleotide vaccine constructs.

30 Methods of synthesising polypeptides, for example using solid phase peptide synthesis, are also suitable for producing the multivalent polypeptide constructs contemplated herein. The basic principle for solid phase peptide synthesis (SPPS) is a stepwise addition of amino acids to a growing polypeptide chain anchored via a linker molecule to a solid phase support, typically a resin particle, which allows for cleavage and purification once the polypeptide chain is complete. Briefly, a solid phase resin support and a starting amino acid are attached to one another via a linker molecule. Such resin-linker-acid matrices are commercially available.

35 The amino acid to be coupled to the resin is protected at its N-terminus by a chemical protecting group. The amino acid may also have a side-chain protecting group. Such protecting groups prevent undesired or deleterious reactions from taking place during the process of forming the new peptide bond between the carboxyl group of the amino acid to be coupled and the unprotected N-amino group of the peptide chain attached to the resin.

40 The amino acid to be coupled is reacted with the unprotected N-amino group of the N-terminal amino acid of the peptide chain, increasing the chain length of the peptide chain by one amino acid. The carboxyl group of the amino acid to be coupled may be activated with a suitable chemical

activating agent to promote reaction with the N α -amino group of the peptide chain. The N α -protecting group of N-terminal amino acid of the peptide chain is then removed in preparation for coupling with the next amino acid residue. This technique consists of many repetitive steps making automation attractive whenever possible. Those skilled in the art will appreciate that peptides may be coupled to the N α -amino group of the solid phase bound amino acid or peptide instead of an individual amino acid, for example where a convergent peptide synthesis is desired. When the desired sequence of amino acids is achieved, the peptide is cleaved from the solid phase support at the linker molecule.

Likewise, synthetic methods for the in vitro production of polynucleotide constructs, such as RNA constructs via the use of reverse transcriptases, are well known in the art and are amenable to use as herein described.

Multivalent polypeptide and polynucleotide conjugates

In certain examples, the multivalent constructs are prepared as a conjugate, for example, a conjugate comprising the multivalent construct conjugated to a polymer particle or polymer particle-forming protein.

Such conjugates are suitable for use in the methods and uses contemplated herein. Accordingly, methods of vaccinating or eliciting an immunological response in a subject comprising administering multivalent construct conjugates comprising a polymer particle and/or a polymer particle forming protein, or the use of a multivalent construct conjugate comprising a polymer particle and/or a polymer particle forming protein in the preparation of a medicament for immunising a subject against *S. aureus*, or of a composition for eliciting an immune response in a subject including a subject infected or suspected to be infected with or immunised against *S. aureus*, are specifically contemplated.

In various examples, the conjugate is encoded by an expression construct comprising at least one nucleic acid sequence encoding a multivalent polynucleotide as herein described and at least one nucleic acid sequence encoding a particle-forming protein.

Pharmaceutical compositions

The present invention also relates to pharmaceutical compositions comprising an effective amount of a multivalent construct as contemplated herein, such as a multivalent polypeptide, polynucleotide, or conjugate thereof or a pharmaceutically acceptable salt or solvent thereof, and a pharmaceutically acceptable carrier.

In one example, the pharmaceutical composition comprises an effective amount of a polypeptide as disclosed herein or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition comprises an effective amount of a polynucleotide as disclosed herein or a pharmaceutically acceptable salt or solvate thereof, and a pharmaceutically acceptable carrier.

The pharmaceutical compositions may comprise an effective amount of two or more peptides of the invention, two or more peptide conjugates of the invention, or one more peptides of the invention and one or more peptide conjugates of the invention in combination.

The term "pharmaceutically acceptable carrier" refers to a carrier (including excipients, adjuvants, or vehicles) that may be administered to a subject together with the multivalent construct or conjugate as contemplated herein, or a pharmaceutically acceptable salt or solvent thereof, and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers that may be used in the compositions include, but are not limited to, ion exchangers, alumina, aluminium stearate, lecithin, self-emulsifying drug delivery systems (SEDDS) such as d- α -tocopherol polyethyleneglycol 1000 succinate, surfactants used in pharmaceutical dosage forms such as Tweens, polyoxyethylene alkyl ethers, polyethoxylated fatty acids or other similar polymeric delivery matrices, serum proteins, such as human serum albumin, buffer substances such as tris(hydroxymethyl)aminomethane (TRIS), ethylenediaminetetraacetic acid (EDTA), phosphates, glycine, sorbic acid, potassium sorbate, antioxidant chemicals such as ascorbic acid, sodium bisulfite, sodium metabisulfite, sodium sulfite, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene-polyoxypropylene-block polymers, polyethylene glycol and wool fat. Cyclodextrins such as α -, β -, and γ -cyclodextrin, or chemically modified derivatives such as hydroxyalkylcyclodextrins, including 2- and 3-hydroxypropyl-3-cyclodextrins, or other solubilized derivatives may also be advantageously used to enhance delivery. Oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents, which are commonly used in the formulation of pharmaceutically acceptable dosage forms such as emulsions and or suspensions.

Specifically contemplated adjuvants include potassium aluminium sulfate (Alum), and squalene, including oil-in-water emulsions of squalene such as MF59, and AddaVax™ as exemplified in the *in vivo* work presented herein in the Examples.

Further specifically contemplated adjuvants include receptor ligands or agonists, such as TLR agonists including TLR2 agonists and/or TLR7 agonists.

In one example, the adjuvant comprises one or more Pam (S-[2,3-bis(palmitoyloxy)propyl]) moieties. For example, the adjuvant comprises, consists essentially of, or consists of any one or more of the group consisting of: Pam1, Pam1Cys, Pam1CSK4, PEG-Pam1, PEG-Pam1Cys, PEG-Pam1CSK4, Pam2, Pam2Cys, Pam2CSK4, PEG-Pam2, PEG-Pam2Cys, PEG-Pam2CSK4, Pam3, Pam3Cys, Pam3CSK4, PEG-Pam3, PEG-Pam3Cys, and PEG-Pam3CSK4.

In particularly contemplated examples, the adjuvant comprises Pam2CSK4 and/or Pam3CSK4. For example, the adjuvant consists essentially of, or consists of Pam2CSK4. In another example, the adjuvant consists essentially of, or consists of Pam3CSK4. In another example, adjuvant consists essentially of, or consists of Pam2CSK4 and Pam3CSK4.

Other adjuvants contemplated for use in certain examples include saponin-based adjuvants such as the saponin QS21, oil-in-water emulsion containing saponins such as saponin QS21, and liposomes containing saponins such as saponin QS21; aluminium, such as amorphous aluminium hydroxyphosphate sulfate (AAHS), aluminium hydroxide, and aluminium phosphate; Monophosphoryl lipid (MPL), including aluminium salts thereof, and mixtures of MPLs and saponins such as QS21, for example AS01_B.

In the context of multivalent vaccine constructs comprising polynucleotides, such as an mRNA vaccine construct contemplated herein, pharmaceutically acceptable carriers will frequently be chosen to maintain mRNA stability prior to, during and after administration. Representative pharmaceutically acceptable carriers that are suitable for use in mRNA vaccine compositions include, but are not limited to, lipids and fats such as SM-102, DSPC (1,2-distearoyl-sn-glycero-3-phosphocholine), 4-

hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate, PEG2000-DMG (1,2-dimyristoyl-rac-glycero 3-methoxypolyethylene glycol-2000) and acetamides such as 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, cholesterol, buffers such as tromethamine, tromethamine hydrochloride, acetic acid, sodium salts such as sodium acetate, sodium chloride, potassium salts such as potassium chloride, monobasic potassium, dibasic sodium potassium phosphate dihydrate, and sugars such as sucrose.

The compositions are formulated to allow for administration to a subject by any chosen route, including but not limited to oral or parenteral (including topical, transdermal, intradermal, subcutaneous, intramuscular and intravenous) administration.

For example, the compositions may be formulated with an appropriate pharmaceutically acceptable carrier (including excipients, diluents, auxiliaries, and combinations thereof) selected with regard to the intended route of administration and standard pharmaceutical practice. For example, the compositions may be administered orally as a powder, liquid, tablet or capsule, or topically as an ointment, cream or lotion. Suitable formulations may contain additional agents as required, including emulsifying, antioxidant, flavouring or colouring agents, and may be adapted for immediate-release, delayed-release, modified-release, sustained-release, pulsed-release, or controlled-release.

The compositions may be formulated to optimize bioavailability, immunogenicity, or to maintain plasma, blood, or tissue concentrations within the immunogenic or therapeutic range, including for extended periods. Controlled delivery preparations may also be used to optimize antigen concentration at the site of action, for example.

The compositions may be formulated for periodic administration, for example to provide continued exposure. Strategies to elicit a beneficial immunological response, for example those that employ one or more "booster" vaccinations, are well known in the art, and such strategies may be adopted.

The compositions may be administered via the parenteral route. Examples of parenteral dosage forms include aqueous solutions, isotonic saline or 5% glucose of the active agent, or other well-known pharmaceutically acceptable excipients. Those familiar with the art will understand that a variety of excipients, solubilizing agents, buffers, and the like can be utilized as pharmaceutical carriers for delivery of the therapeutic agent.

Examples of dosage forms suitable for oral administration include, but are not limited to tablets, capsules, lozenges, or like forms, or any liquid forms such as syrups, aqueous solutions, emulsions and the like, capable of providing a therapeutically effective amount of the composition. Capsules can contain any standard pharmaceutically acceptable materials such as gelatin or cellulose. Tablets can be formulated in accordance with conventional procedures by compressing mixtures of the active ingredients with a solid carrier and a lubricant. Examples of solid carriers include starch and sugar bentonite. Active ingredients can also be administered in a form of a hard-shell tablet or a capsule containing a binder, e.g., lactose or mannitol, a conventional filler, and a tableting agent.

Examples of dosage forms suitable for transdermal administration include, but are not limited, to transdermal patches, transdermal bandages, and the like.

Examples of dosage forms suitable for topical administration of the compositions include any lotion, stick, spray, ointment, paste, cream, gel, etc., whether applied directly to the skin or via an intermediary such as a pad, patch or the like.

Examples of dosage forms suitable for suppository administration of the compositions include any solid dosage form inserted into a bodily orifice particularly those inserted rectally, vaginally and urethrally.

Examples of dosage of forms suitable for injection of the compositions include delivery via bolus such as single or multiple administrations by intravenous injection, subcutaneous, subdermal, and intramuscular administration or oral administration.

Examples of dosage forms suitable for depot administration of the compositions and include pellets of the multivalent constructs or conjugates thereof, or solid forms wherein the multivalent constructs or conjugates thereof are entrapped in a matrix of biodegradable polymers, microemulsions, liposomes or are microencapsulated.

Examples of infusion devices for the compositions include infusion pumps for providing a desired number of doses or steady state administration, and include implantable drug pumps.

Examples of implantable infusion devices for compositions include any solid form in which the multivalent constructs or conjugates thereof are encapsulated within or dispersed throughout a biodegradable polymer or synthetic, polymer such as silicone, silicone rubber, silastic or similar polymer.

Examples of dosage forms suitable for transmucosal delivery of the compositions include depositories solutions for enemas, pessaries, tampons, creams, gels, pastes, foams, nebulised solutions, powders and similar formulations containing in addition to the active ingredients such carriers as are known in the art to be appropriate. Such dosage forms include forms suitable for inhalation or insufflation of the compositions, including compositions comprising solutions and/or suspensions in pharmaceutically acceptable, aqueous, or organic solvents, or mixture thereof and/or powders. Transmucosal administration of the compositions may utilize any mucosal membrane but commonly utilizes the nasal, buccal, vaginal and rectal tissues. Formulations suitable for nasal administration of the compositions may be administered in a liquid form, for example, nasal spray, nasal drops, or by aerosol administration by nebulizer, including aqueous or oily solutions of the polymer particles. Formulations may be prepared as aqueous solutions for example in saline, solutions employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bio-availability, fluorocarbons, and/or other solubilising or dispersing agents known in the art.

Examples of dosage forms suitable for buccal or sublingual administration of the compositions include lozenges, tablets and the like. Examples of dosage forms suitable for ophthalmic administration of the compositions include inserts and/or compositions comprising solutions and/or suspensions in pharmaceutically acceptable, aqueous, or organic solvents.

Examples of formulations of compositions, including vaccines, may be found in, for example, Sweetman, S. C. (Ed.). Martindale. The Complete Drug Reference, 33rd Edition, Pharmaceutical Press, Chicago, 2002, 2483 pp.; Aulton, M. E. (Ed.) Pharmaceutics. The Science of Dosage Form Design. Churchill Livingstone, Edinburgh, 2000, 734 pp.; and, Ansel, H. C, Allen, L. V. and Popovich, N. G. Pharmaceutical Dosage Forms and Drug Delivery Systems, 7th Ed., Lippincott 1999, 676 pp..

Excipients employed in the manufacture of drug delivery systems are described in various publications known to those skilled in the art including, for example, Kibbe, E. H. Handbook of Pharmaceutical Excipients, 3rd Ed., American Pharmaceutical Association, Washington, 2000, 665 pp. The USP also provides examples of modified-release oral dosage forms, including those formulated as tablets or capsules. See, for example, The United States Pharmacopeia 23/National Formulary 18, The

United States Pharmacopeial Convention, Inc., Rockville MD, 1995 (hereinafter "the USP"), which also describes specific tests to determine the drug release capabilities of extended-release and delayed-release tablets and capsules. The USP test for drug release for extended-release and delayed-release articles is based on drug dissolution from the dosage unit against elapsed test time. Descriptions of various test apparatus and procedures may be found in the USP. Further guidance concerning the analysis of extended-release dosage forms has been provided by the F.D.A. (See Guidance for Industry. Extended-release oral dosage forms: development, evaluation, and application of in vitro/in vivo correlations. Rockville, MD: Center for Drug Evaluation and Research, Food and Drug Administration, 1997).

While the composition may comprise one or more extrinsic adjuvants, advantageously in some examples this is not necessary. In certain examples, the multivalent construct or conjugates thereof is self adjuvanting.

Therapeutic methods and uses

The constructs, compositions and methods described herein are in certain examples used in therapeutic methods, for example, in eliciting an immunological response in a subject in need thereof, such as the vaccination of a subject, or in the treatment or prevention of one or more diseases, disorders, pathologies, or conditions in a subject in need thereof.

Methods of vaccinating or eliciting an immune response in a subject comprising administering to the subject an effective amount of a multivalent construct or conjugate thereof as disclosed herein are specifically contemplated. Typically, such administration will be in the form of a pharmaceutical composition comprising one or more pharmaceutically acceptable carriers. Specifically contemplated compositions include those comprising an adjuvant, such as Alum, or squalene-based emulsions such as AddaVax™. The use of a multivalent construct or conjugate thereof as described herein for vaccinating or eliciting an immune response in a subject, and the use of such multivalent constructs or conjugates thereof in the manufacture of a medicament for vaccinating or eliciting an immune response in a subject are also specifically contemplated.

A "subject" as used herein is an animal, usually a mammal, including a mammalian companion animal, or a human. While the multivalent constructs specifically contemplated herein are optimised for administration to and efficacy in humans, certain examples will in certain circumstance find application in other species, including one or representative companion animals including feline, equine, and canine, or one or more representative agricultural animals, including bovine, ovine, caprine, cervine, and porcine.

In one specifically contemplated example, the constructs, compositions, and methods are directed to the treatment or prevention of mastitis, for example mastitis in bovine, and particularly in dairy bovine.

It will be appreciated that the various methods of therapy contemplated herein will typically embody the administration of an effective amount of the multivalent construct.

An "effective amount" is an amount sufficient to effect beneficial or desired results including clinical results. An effective amount can be administered in one or more administrations by various routes of administration.

The effective amount will vary depending on, among other factors, the disease or symptom(s) indicated, the severity of the disease or symptom(s), the age and relative health of the subject, the potency of the compound administered, the mode of administration and the treatment desired. A

person skilled in the art will be able to determine appropriate dosages having regard to these any other relevant factors.

The efficacy of a composition can be evaluated both in vitro and in vivo. For example, the composition can be tested in vitro or in vivo for its ability to induce a cell-mediated immune response.

5 For in vivo studies, the composition can be fed to or injected into an animal (e.g., a mouse) and its effects on eliciting an immune response are then assessed. Based on the results, an appropriate dosage range and administration route can be determined.

The composition may be administered as a single dose or a multiple dose schedule. Multiple doses may be used in a primary immunisation schedule and/or in a booster immunisation schedule.

10 The present invention also relates to a method of vaccinating or eliciting an immune response in a subject comprising administering to the subject an effective amount of a pharmaceutical composition as herein described. The present invention further relates to use of a pharmaceutical composition as herein described for vaccinating or eliciting an immune response in a subject, and to the use of one or more multivalent constructs or conjugates thereof in the manufacture of a
15 medicament for vaccinating or eliciting an immune response in a subject.

In one specific example, the method of eliciting an immune response in a subject comprises administering to the subject an effective amount of a multivalent polypeptide construct as herein described. The present invention also relates to use of a conjugate of the invention for eliciting an immune response, and to use of a peptide conjugate of the invention in the manufacture of a
20 medicament for eliciting an immune response in a subject.

In certain examples, eliciting an immune response comprises raising or enhancing an immune response. In exemplary examples, eliciting an immune response comprises eliciting a humoral and a cell mediated response.

In certain examples, eliciting an immune response provides immunity.

25 The immune response is elicited for treating a disease or condition, or ameliorating one or more symptoms associated with a disease or condition. A person skilled in the art will appreciate that the multivalent constructs and conjugates described herein are useful for treating or preventing *S. aureus* infection and/or a disease or condition associated with *S. aureus* infection.

The term "treatment", and related terms such as "treating" and "treat", as used herein relates
30 generally to treatment, of a human or a non-human subject, in which some desired therapeutic effect is achieved. The therapeutic effect may, for example, be inhibition, reduction, amelioration, halt, or prevention of a disease or condition.

In certain examples, the compositions are used to elicit systemic and/or mucosal immunity. Enhanced systemic and/or mucosal immunity may be reflected in an enhanced TFH, TH1, and/or TH17
35 immune response. The enhanced immune response may include an increase in the production of immunoglobulins (Ig), such as IgG1, IgG2a, and/or IgG3, IgM, and/or IgA.

The present invention further relates to a method of vaccinating a subject comprising administering to the subject an effective amount of a polypeptide as herein described. The present invention also relates to use of a conjugate of the invention for eliciting an immune response, and to
40 use of a peptide conjugate of the invention in the manufacture of a medicament for eliciting an immune response in a subject.

The invention is further described with reference to the following examples. It will be appreciated that the invention as claimed is not intended to be limited in any way by these examples.

EXAMPLES

Example 1: Construction and characterisation of multivalent SSL polypeptides

This example describes the construction of various multivalent polypeptide constructs comprising multiple *S. aureus* SSL polypeptides, and their characterisation including for use as vaccines as contemplated herein.

Two fusion proteins comprising wildtype or functionally inactive forms of SSLs 3, 7 and 11 were produced, characterised and tested for their capacity to elicit a specific antibody response and reduce the *S. aureus* burden in a model of peritoneal infection. The utility of two different adjuvant formulations was also compared in these studies, an Alum-based formulation was used as the benchmark and compared to AddaVax™, an analogue of MF59, an adjuvant that elicits a broader range of humoral and cellular immune responses.

Results

SSLs are important for bacterial survival.

All *ssl* genes, including the *ssl3*, *ssl7*, and *ssl11* genes, were knocked out of the *S. aureus* JSNZ strain to create a Δ *ssl* strain. Recombplemented Δ *ssl* strains were then prepared in which one or more selected *ssl* genes were introduced. Survival of the Δ *ssl* strain was reduced by approximately 1-log when compared to wildtype bacteria ($p < 0.01$, Figure 2a) in a human whole blood killing (WBK) assay. Recombplemented Δ *ssl* strains confirmed to express SSLs 3, 7 or polySSL 7311 proteins had significantly enhanced survival compared to *S. aureus* Δ *ssl* bacteria ($p < 0.05$), whereas restoration of SSL11 did not increase bacterial CFU in this assay (Figure 2a). *In vivo* survival of wildtype and *S. aureus* Δ *ssl* strains was compared in mice infected intra-peritoneally. *S. aureus* Δ *ssl* developed attenuated disease relative to mice given wildtype bacteria. Mice infected with the *S. aureus* Δ *ssl* strain had significantly reduced weight loss on days 3 and 4 of infection (~5%, $p < 0.05$, Figure 2b), accompanied by a two-log reduction in median *S. aureus* CFU in the spleen ($p < 0.01$) and liver ($p < 0.0001$) on day 5 of infection (Figure 2c).

Design, construction and characterisation of a polySSL 7311 fusion protein

Mutant or wildtype *ssl7*, *ssl11* and *ssl3* genes from *S. aureus* JSNZ were sequentially cloned into the pET32a.3C expression vector, which expresses proteins with a thioredoxin tag to facilitate protein production. Successful incorporation of all genes into this vector was confirmed by sequencing and the plasmid transformed into the *E. coli* Rosetta-gami 2(DE3)pLysS strain for protein production. The final gene constructs produced a 70kD polySSL 7311 recombinant fusion protein containing the wild-type proteins and polySSL 7311m composed of the functional mutants. These were purified by nickel-affinity chromatography, followed by cleavage of the thioredoxin tag with 3C protease and further purification by ion-exchange and size-exclusion chromatography.

To verify normal protein conformation, the wildtype polySSL 7311 protein was tested for individual functional activity. The polySSL 7311m protein was tested in parallel to confirm loss of SSL3 function.

SSL3 inhibits TLR-2 function and human myelomonocytic THP-1 cells were stimulated with the TLR-2 ligand lipoteichoic acid (LTA), and the production of Tumour Necrosis Factor (TNF) measured.

This activity was ablated in the presence of SSL3 or polySSL 7311, but production was unaffected by the presence of a similar concentration of polySSL 7311m (Figure 3a).

SSL7 inhibits end-stage complement. polySSL 7311 and SSL7 completely inhibited the deposition of C5b-9 onto the surface of *S. aureus*, while poly SSL7311m showed no inhibition (Figure 3b). Wildtype SSL7 and SSL7311 proteins bound to human IgA but SSL7311M displayed minimal IgA binding. This confirmed that SSL7311M was devoid of both SSL7 functions (Figure 3c).

5 Similarly, wildtype SSL11 and polySSL 7311 displayed glycan mediated binding to PSGL-1, whereas mutant versions of these proteins lacked this capability (Figure 3d).

Collectively, these data show that a multivalent fusion polypeptide comprising the SSLs 3, 7 and 11 retains the immune evading activities of each individual SSL, supporting the use of a multivalent SSL polypeptide construct for stimulating anti-SSL immune responses as contemplated herein. All further testing used the mutant form of the poly SSL7311 vaccine which is referred to as the PolySSL7311 vaccine.

The PolySSL7311 vaccine elicits specific IgG and proliferation responses to all components

The PolySSL7311 vaccine was tested for its ability to stimulate specific humoral and cellular responses to SSLs 3, 7 and 11 in mice after delivery in one of two analogues of adjuvants that are licenced for human use, AdjuPhos® which is an Alum-based formulation and AddaVax™, an oil-in-water based formulation similar to MF59 (Figure 4a). Robust anti-SSL7 IgG responses were detected on day 25, after two immunisations (mean endpoint titre = 10^5 for AdjuPhos® or AddaVax™, $p < 0.001$ or $p < 0.0001$ Vs control). Seroconversion of all mice and production of anti-SSL3 IgG antibodies did not occur until day 39, after three immunisations (mean endpoint titres = $\sim 10^4$ for AdjuPhos® or AddaVax™ treated groups, $p < 0.0001$ or $p < 0.001$ Vs control). Development of anti-SSL11 IgG responses required three immunisations before significantly increased titres were detectable relative to the control group (mean endpoint titres = $\sim 10^4$ for AdjuPhos® or AddaVax™ treated groups, $p < 0.0001$ or $p < 0.01$ Vs control). Therefore, the PolySSL7311 vaccine stimulates antibody responses to all three components when delivered in either adjuvant, with the highest responses to SSL7, then SSL3 and weaker responses to SSL11. Splenocytes were harvested from fully immunised mice on day 39, in conjunction with a PBS treated control group, and tested for their capacity to proliferate in response to exposure to SSLs 3, 7 and 11 (Figure 4b). Both the PolySSL7311 vaccinated groups had a higher median stimulation index compared to the PBS control group, but only the group vaccinated with AddaVax™ had a significantly enhanced proliferation response compared to the PBS control group ($p < 0.01$ for SSLs 3, 7 and 11).

The SSL7311 vaccine attenuates S. aureus infection

Vaccinated mice were challenged with *S. aureus* via the intra-peritoneal route to determine whether the specific immune responses raised to each vaccine component were sufficient to reduce signs of disease and the *S. aureus* tissue burden. As delivery in either AdjuPhos® or AddaVax™ was able to elicit specific immunity in vaccinated mice, the utility of each adjuvant was tested in the challenge model. A decline in body weight in response to *S. aureus* challenge is an important objective measure of disease severity. Vaccinated mice lost a comparable amount of body weight compared to the control mice in the first 24h after challenge but showed a more rapid recovery at subsequent time points, with significant differences between these groups at day 4 for AdjuPhos® ($p < 0.05$) and day 3 for AddaVax™ ($p < 0.05$) (Figure 5). The most significant reduction in *S. aureus* burden was evident in mice vaccinated with the PolySSL7311 vaccine emulsified in AddaVax™, the median *S. aureus* CFU was reduced by approximately 2-log in vaccinated mice relative to adjuvant treated controls: liver = 3.5×10^3 Vs 1.6×10^5 CFU, $p < 0.0001$; kidney = 3.5×10^3 Vs 1.6×10^5 CFU, $p < 0.05$) (Figure 5).

The *S. aureus* burden was reduced to a lesser extent in mice vaccinated with vaccine adsorbed to AdjuPhos®: median *S. aureus* CFU was reduced by 0.5 – 1 log in vaccinated mice relative to adjuvant treated controls: liver = 5.2×10^4 Vs 3.7×10^5 CFU, $p < 0.05$); kidney = 1.9×10^4 Vs 7.9×10^4 CFU, ns) (Figure 5).

5 *Protection from S. aureus challenge requires delivery of SSLs 3, 7 and 11 in the PolySSL7311 vaccine*

Fusing all three vaccine candidate antigens into the PolySSL7311 protein offers benefits in terms of simplifying vaccine formulation and also promotes a superior protective immune response compared to a mixture of the individual SSLs. Mice were vaccinated with PolySSL7311 (Vaccine) or
10 equivalent quantities of individual SSLs 3, 7 and 11 (SSLs), delivered in AdjuPhos® or AddaVax™, followed by an intra-peritoneal challenge with *S. aureus*. All mice lost approximately 10% of baseline body weight 24h after challenge, but mice given the vaccine in either AdjuPhos® or AddaVax™ tended to recover more rapidly (Figure 6). Mice vaccinated with PolySSL7311 in AddaVax™ had a ~3 log reduction in *S. aureus* in the kidneys (0.9×10^2 Vs 2.7×10^5 CFU, $p < 0.01$) and a ~2.5 log drop in *S.*
15 *aureus* in the liver (3.5×10^3 Vs 2.8×10^6 , $p < 0.001$). In contrast, the *S. aureus* burden was comparable in the control and SSLs treatment groups (Figure 6). There was no significant difference in kidney or liver CFU between mice vaccinated with polySSL7311, SSLs or controls administered in AdjuPhos®, suggesting delivery of materials in this adjuvant is of marginal efficacy (Figure 6).

20 *Immunisation with PolySSL7311 leads to enhanced antibody titres to each of the components*

Development of antibody titres to each of the vaccine components was compared in fully vaccinated control, SSLs, or PolySSL7311 treated mice. Similar trends were observed in groups of mice immunised with either adjuvant. Comparable anti-SSL3 IgG responses developed irrespective of whether mice were given SSLs or the SSL7311 vaccine. In contrast IgG responses to both SSL7
(mean endpoint titre = $\sim 10^6$ Vs $\sim 10^5$ for AdjuPhos® ($p < 0.01$) and AddaVax™ ($p < 0.05$)) and
25 SSL11 (mean endpoint titre = $\sim 10^5$ Vs $\sim 10^2$ for AdjuPhos® ($p < 0.0001$) and 10^4 Vs $\sim 10^2$ AddaVax™ ($p < 0.05$)) were significantly enhanced in the PolySSL7311 vaccinated mice, when compared to the SSLs group (Figure 7a).

30 *The PolySSL7311 vaccine elicits antibodies that neutralise SSL7*

Immune sera were tested for the capacity to neutralise the activity of each component in the SSL7311 vaccine. The quantitative assays used to characterise and confirm the functional integrity of the wildtype version of the SSL7311 vaccine (Figure 3) were modified to incorporate an incubation step with murine sera to determine whether this prevented the interaction between individual SSLs and their respective ligands. Diluted sera from vaccinated or control mice were incubated with SSL3 protein and tested for the capacity to restore TLR2 signalling by THP-1 cells. Only sera from mice
35 immunised with individual SSL proteins administered in AdjuPhos® showed any capacity (median = $\sim 10\%$, $p < 0.001$) to attenuate the function of SSL3 (Figure 7b). In contrast, incubation of immune serum from PolySSL7311 vaccinated mice was able to partially prevent binding of SSL7 to human IgA and enabled cleavage of complement C5 in the presence of SSL7. Sera from mice immunised with either SSLs (median of $\sim 20\%$ in AdjuPhos®, $p < 0.05$; $\sim 30\%$ in AddaVax™, $p < 0.001$) or
40 PolySSL7311 (median of $\sim 50\%$ in AdjuPhos®; $\sim 40\%$ in AddaVax™, $p < 0.0001$) partially neutralised binding of SSL7 to IgA relative to controls ($\sim 10\%$ IgA binding) (Figure 7b). Only incubation of SSL7 with immune sera from PolySSL7311 vaccinated mice was able to largely restore complement C5 cleavage and deposition of C5b-9 onto the surface of heat-killed *S. aureus* (median of $>90\%$ C5b-9

deposition for mice given the PolySSL7311 vaccine in either adjuvant Vs negligible detection of C5b-9 in the control group ($p < 0.001$ for AdjuPhos® and $p < 0.01$ for AddaVax™) (Figure 7b). Assays designed to demonstrate the possible production of neutralising antibodies to SSL11 were confounded by non-specific binding of wildtype SSL11 to murine serum at dilutions expected to show an antibody-mediated effect, which prevented quantification of carbohydrate-specific binding by SSL11.

Discussion

There is an urgent need to develop alternative strategies to treat or prevent infection with *S. aureus*. The data presented herein have demonstrated for the first time that a vaccine combining three functionally inactive secreted *S. aureus* immune evasion factors attenuates development of *S. aureus* peritonitis. The reduction in bacterial load required delivery of these antigens as a fusion protein, supporting the inventors' view that this format improves presentation of these antigens to the immune system. These results confirm that the important contribution of the SSLs to *S. aureus* survival *in vivo*.

One design consideration for the polySSL7311 protein was ease of production. Delivery of antigens in this format unexpectedly enhanced development of IgG to SSLs 7 and 11 and elicited antibodies that neutralise the activities of SSL7. Healthy individuals developed an IgG response to SSL7 but lacked the capacity to neutralise either IgA binding or inhibition of complement C5 by SSL7, suggesting functional antibodies are not typically produced after exposure to *S. aureus*. SSL7 is reported to be a potent inhibitor of neutrophil responses to whole *S. aureus*, dampening immune-complex mediated peritonitis and purportedly shows potential for inhibiting complement-mediated disease in murine models.

Generation of functional antibodies against SSL7 through delivery of PolySSL7311 in AddaVax™ was herein shown to be associated with attenuation of bacterial burden, suggesting to the inventors that SSL7 contributes significantly to *S. aureus* virulence. The impact of SSL7 on infection may be under-estimated in murine model given the interaction between complement C5 and SSL7 is enhanced in the presence of human IgA. In contrast, location of SSL3 within the polySSL7311 protein elicited a strong cellular proliferation response and a robust IgG response, but an impaired capacity to elicit a neutralising antibody response, potentially due to masking of key epitopes.

The comparison of AdjuPhos® and AddaVax™ presented herein produced comparable antibody responses, whereas significant attenuation of the bacterial burden required vaccination with AddaVax™.

Key issues for validating *S. aureus* vaccine candidates have been proposed, including use of appropriate challenge model(s), determining a meaningful threshold for what constitutes protection from challenge and confirming cross-strain protection. The PolySSL7311 vaccine was tested in a systemic (peritoneal) model of infection because survival of an *S. aureus*Δ*ssl* strain was significantly reduced in this model. The potential for this vaccine to confer protection in other settings is addressed below. A recent report described a multi-component vaccine comprised of several conserved *Staphylococcal* transporter proteins and α-haemolysin which had a significant impact on dermonecrosis in a skin infection model, but only conferred partial protection against bacteraemia, highlighting the risk of over-dependence on a single model. The definition of what constitutes a significant reduction in *S. aureus* burden is also subject to debate.

The PolySSL7311 vaccine significantly reduced *S. aureus* tissue burden (>2 -log) when administered in AddaVax™, and was tested using a mouse-adapted *S. aureus* strain (JSNZ) that is

both transmissible and virulent in mice. Although the *ssIs* are retained in both veterinary and murine strains, suggesting they provide a significant survival advantage across a broad range of species, testing the protective efficacy of the polySSL7311 vaccine in clinically relevant strains is an important step towards demonstrating the broader applicability of this approach.

The data presented herein in a mouse model of peritoneal inflammation strongly supports the inventors' view that the multivalent polySSL constructs contemplated herein will have broad applicability in tackling *Staphylococcal* disease.

Methods

Ethics statement

Written informed consent was obtained from all donors and blood samples were handled as per the Declaration of Helsinki and University of Auckland Human Participants Ethics Committee guidelines. Animal experiments approved by the University of Auckland Animal Ethics Committee and were conducted in accordance with the University of Auckland's Code of Ethical Conduct and the Animal Welfare Act 1999.

Bacteria

A streptomycin resistant mouse-adapted strain of *S. aureus* (JSNZ) was used for all gene knockout and *in vivo* studies. *S. aureus* gene knockout strains were produced by allelic exchange with the pIMAY plasmid, and selected genes reintroduced by complementation as described elsewhere^{47, 48}.

S. aureus was cultured at 37° C in either tryptic soy broth (TSB, Difco) with vigorous shaking, or plated onto tryptic soy agar (TSA) for enumeration of bacteria. Mice were inoculated with *S. aureus* grown to mid log-phase and administered in PBS. The challenge dose was confirmed by serial 10-fold dilution of the inoculum, plating of triplicate spots, and enumeration of colony forming units (CFU) after overnight incubation at 37° C. Heat-killed *S. aureus* JSNZΔ*spa* was prepared by incubation of an overnight culture of bacteria at 56° C for 1 h, washed in PBS and pelleted bacteria stored at -20° C. *E. coli* was grown at 37° C unless specified otherwise in Luria-Bertani (LB) broth (1% w/v Bacto-tryptone (Oxoid), 0.5 % w/v yeast extract (Oxoid), 1% NaCl) with vigorous shaking, or on LB agar (LB supplemented with 15 g/L agar). Where antibiotic selection was required, *S. aureus* was cultured with 0.5 g/mL Streptomycin or *Escherichia coli* with 0.5 mg/mL Ampicillin, 0.03 mg/mL Chloramphenicol, 0.015 mg/mL Kanamycin or 0.0125 mg/mL Tetracycline (Sigma-Aldrich, Australia).

Cloning, expression and purification of recombinant proteins

E. coli strains DH5α, AD494(DE3)pLysS and Rosetta gammi 2(DE3)pLysS were used for cloning and production of recombinant proteins from *S. aureus* JSNZ. Briefly, mutant SSL11j was created *via* the *ss/11*.R179A mutation to attenuate binding to glycoproteins; wildtype SSL7j sequence was mutated in several locations to eliminate interactions with IgA (*ss/7*.L79A.P82A) and C5 (*ss/7*.H117A.S119A). The N-terminus of SSL3 is prone to degradation, therefore a truncated but functional form of wildtype SSL3,²² was amplified from *S. aureus* JSNZ genomic DNA by PCR and sub-cloned into the expression vector pET32a-3C. Targeted mutations (see Figure 15) were introduced into SSL3j to prevent interactions with TLR2 (*ss/3*.F186A.F188A) and attenuate carbohydrate binding (*ss/3*.T327A). Wildtype and mutant *ss/* genes were cloned into pET32a-3C and transformed into *E. coli* DH5α. PCR positive plasmids were sequence confirmed, and then individually transformed into *E. coli* AD494(DE3)pLysS to be expressed as N-terminal thioredoxin fusion proteins. Fusion proteins were purified by Ni₂₊ affinity chromatography followed by cleavage with 3C protease as detailed

elsewhere^{28, 50}, then further purified by ion exchange chromatography (MonoQ or MonoS, GE Healthcare).

Wildtype and mutant *ssl7*, *ssl3* and *ssl11* genes were successively cloned in tandem (Figure 1) and the plasmid transformed into AD494(DE3)pLysS. Expression of soluble fusion protein required separation of each gene with a linker, use of *E. coli* Rosetta gammi 2(DE3)pLysS and induction of protein production at 28° C. The polySSL protein was purified as described above, then further purified by size exclusion chromatography (Superdex S75, GE Healthcare). All recombinant proteins were confirmed to be ≥95% pure by SDS-PAGE and coomassie blue staining. For functional assays, wildtype SSL3j protein was treated with Endotoxin Removal Beads (Milttenyi Biotech) and confirmed to contain <1 Endotoxin Units/0.01 mg with an Endosafe-PTS™ reader (Charles River).

Preparation of human whole blood and serum

Peripheral blood from healthy volunteers was used to examine the activity of the *S. aureus*Δ*ssl* strains and purified recombinant proteins. Blood was collected into heparinised tubes and either directly combined with live *S. aureus* or further processed to obtain peripheral blood leukocyte (PBL) lysates.²⁴ Human plasma for use in pull-down assays was collected from heparin treated blood by centrifugation. Blood collected in untreated tubes was left to clot for 30 min at room temperature prior to centrifugation at 1,250 *g* for 20 min at 4°C as a source of normal human serum (NHS) and stored at -80°C.

Whole blood killing (WBK) assay

A WBK assay was used to compare survival of *S. aureus* knockout strains.⁴⁸ Briefly, mid log phase cultures of *S. aureus* were washed, re-suspended in Hanks balanced salt solution and ~1 x 10⁴ CFU bacteria added to 70% v/v whole blood for 20h at 37° C with gentle shaking. At 0 and 20h bacteria were serially diluted in PBS and plated in triplicate onto TSA for enumeration of CFU.

Characterisation of SSL7311 proteins by pull down assay

Binding profiles of individual and fusion proteins to target ligands was confirmed using pull down assays. Recombinant proteins were coupled to Cyanogen bromide-activated sepharose and combined with either serum or PBL lysate at a ratio of 1:10 as previously described.²⁴ Eluted proteins were separated by 12.5% SDS-PAGE and transferred onto a nitrocellulose membrane for detection of bound proteins by Western blotting. Membranes were blocked overnight at 4°C in Tris Buffered Saline-0.1% Tween (TBS-T) supplemented with 5% w/v skim milk powder. Membranes were probed with primary or secondary antibody in TBS-T- 2.5% w/v skim milk powder and washed three times with TBS-T for 5 min between each antibody incubation. Immobilised protein-antibody complexes were detected with ECL Western Blotting Substrate and visualized with a GelDoc2000 (BioRad). The identity of bound proteins was confirmed using anti-human antibodies to C5, CD162 or human IgA and bound antibodies were detected with horse radish peroxidase (HRP) conjugated goat anti-mouse or anti-rabbit IgG.

Confirmation of functional activity of the SSL7311 proteins

Retention or loss of functions associated with SSL7 – binding to human IgA and inhibition of complement C5 cleavage - were determined by ELISA.²⁷ Reagents were added at 50 µL/well to Maxisorb (Nunc) plates for all ELISAs unless specified otherwise, samples were assayed in duplicate, and plates were washed in PBS-0.05% v/v Tween-20 (Sigma) ≥ three times between 1h RT incubation steps. Binding of HRP conjugated secondary antibodies was detected with TMB (Sigma)

substrate and the reaction stopped using 10% v/v HCl. Plates were read on an Ensign plate reader (Perkin Elmer) and final absorbance calculated by subtracting the 570 nm from 450 nm values.

Human IgA was purified from pooled healthy donor plasma by SSL7 C5- mutant sepharose affinity chromatography and anti-SSL7 IgG removed by passing the preparation through a Protein G column (GE Healthcare).²⁷ Microtitre plates were coated overnight at 4° C with SSL7 protein diluted to 10 µg/mL in PBS, washed and blocked with 200 µL/well Assay Buffer (PBS-1% bovine serum albumin (BSA; Gibco)), incubated with purified human IgA at 6.25×10^{-2} mg/mL and IgA binding detected with anti-IgA:HRP. Inhibition of SSL7-IgA binding was tested by incubation of serum diluted 1:200 from immunised or control (baseline) mice on SSL7 coated microtitre plates for 1h at RT before the addition of IgA. Percent neutralising activity was calculated as follows: (control – test) / control x 100.

For detection of MAC complex (C5b-9) formation, microtitre plates were coated overnight with $\sim 5 \times 10^6$ CFU/well heat-killed *S. aureus*Δspa then washed and blocked with 200 µL/well 1% w/v human serum albumin (Calbiochem) in PBS. NHS (2.5% v/v) and SSL7 were incubated in GHB (150 mM NaCl, 60 mM HEPES, 0.1% w/v bovine skin type B gelatin (Sigma Aldrich) pH 7.35 supplemented with 0.06 mM CaCl₂ and 0.4 mM MgCl₂) for 1h at 37° C. Deposition of C5b-9 onto the *S. aureus* was detected with mouse anti-human C5b-9 and goat anti-mouse IgG:HRP. Prevention of SSL7-C5 binding was tested by incubating serum (diluted 1:200) from immunised or control mice with 0.5 µM SSL7 for 1h at RT prior to the addition of 2.5% NHS. Percent neutralising activity was calculated as follows: (test – control serum) / 1% inhibition (NHS alone (maximum readout) – control serum) / 100).

SSL3-mediated inhibition of signalling through TLR2 was determined using a cytokine stimulation assay. THP-1 myelomonocytic cells (ATCC®™ TIB-202, 10^5 cells/well) were combined with SSL3 and 2 µg/mL lipoteichoic acid from *S. aureus* (LTA; InvivoGen) in a round-bottomed microtitre plate for ~20h. Supernatants were harvested and TNF production quantified by ELISA. Sera from immunised or control mice was incubated with 0.48nM SSL3 for 1h at a dilution of 1:25 prior to the addition of cells and LTA, to assess its capacity to interfere with the interaction between SSL3 and TLR2. Percent neutralising activity was calculated as described for the MAC complex assay.

Infection and vaccination of mice

Female Crl:CD1(ICR)-Elite certified as *S. aureus*-free, were supplied by the Vernon Jansen Unit (University of Auckland, Auckland, New Zealand), housed in individually ventilated cages with water and chow given *ad libitum*, and monitored daily by qualified staff.

Mice aged 7-8 weeks were weighed prior to infection by intra-peritoneal injection with $\sim 10^8$ CFU *S. aureus* and monitored twice daily. Systemically infected mice were monitored twice daily for clinical signs of infection such as ruffled fur, hunching and reduced activity. Mice that met one or more of the following pre-determined endpoints: loss of $\geq 15\%$ of baseline body weight in 24h; $\geq 20\%$ weight loss over the course of the study; more than two clinical signs of infection; or were found moribund; were euthanised immediately by CO₂ inhalation. Selected tissues were excised, homogenised and *S. aureus* enumerated as described elsewhere.¹⁶

Mice aged 5-6 weeks were vaccinated subcutaneously three times, two weeks apart, with 10 µg of 7311M protein emulsified 1:1 in AddaVax™ or adsorbed to Adju-Phos (InvivoGen) and challenged by intra-peritoneal injection with $\sim 10^8$ CFU *S. aureus* JSNZ two weeks after the final vaccination. Mice were monitored, weighed, euthanised and tissue processed as described above.

Quantification of anti-SSL3, 7 and 11 IgG

Endpoint titres to SSLs 3, 7 and 11 were measured by ELISA, using the conditions described above for SSL7-IgA binding. Sera were serial 10-fold diluted in Assay Buffer starting at a 1:100 for SSL7 or SSL11, or 1:200 for SSL3 coated wells. Baseline serum samples were included on every assay plate and endpoint titres were calculated as the reciprocal of those values that were $\geq 2 \times$ average baseline values + 2 x standard deviation.

Detection of cellular and cytokine responses

Spleens were collected from individual mice and processed to obtain splenocyte suspensions for quantification of cell proliferation. Briefly, 2×10^6 splenocytes/well were transferred to round-bottomed microtitre plates and stimulated with 50 $\mu\text{g/mL}$ of purified recombinant SSL3, 7 or 11 mutant proteins or medium alone for 72h. Cells were pulsed with 0.25 $\mu\text{Ci/well}$ tritiated thymidine (Perkin-Elmer) for the final 6h of the culture period prior to quantification of thymidine uptake. Results are expressed as a stimulation index (SI): stimulated cells/medium alone control from triplicate wells per condition.

Statistics

Data were collated and analysed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla California USA). Specifics of the statistical analyses performed are described in the Brief description of the Figures.

Example 2: Characterisation of different multivalent constructs

This example describes the characterisation of a number of different multivalent SSL polypeptide constructs, including those having a different order of each SSL within the polySSL protein. As can be seen from Figure 8, the order of the SSL3, the SSL7, and the SSL11 had an effect on different aspects of SSL functionality. The polySSL 7311 described in Example 1 did not induce the same high levels of anti-SSL3 or anti-SSL11 antibody titres as were elicited by other polySSL polypeptides (Figure 8A). Of the various polySSL polypeptides tested in this example, polySSL 7311 polypeptide provided less effective protection after *S. aureus* challenge (Figure 8B).

Mice were vaccinated subcutaneously on days 0, 14 and 28 with PBS or 10 μg of protein administered in AddaVax. Blood samples were collected on day 39, prior to challenge, for determination of endpoint titres (Figure 8a). Mice were then challenged systemically with $\sim 5 \times 10^7$ CFU *S. aureus* JSNZ on day 42 and tissues harvested on day 46 (Figure 8b). Data are combined from three independent studies, each containing $n=6$ mice per treatment group.

As can be seen, each of the polySSL polypeptide constructs exemplified in this Example provided effective immunological responses and protection from *S. aureus* infection.

Example 3: Vaccine composition

This example describes the development and characterisation of vaccine compositions comprising a representative multivalent SSL polypeptide construct together with different adjuvants and other carriers.

Protective immunity to *S. aureus* requires a combination of specific antibody recognition and the stimulation of phagocyte function. Enhanced phagocyte function through the induction of Th1 and Th17 cellular immunity has been proposed to be important for combatting *S. aureus* infection. Induction of these responses is orchestrated by the host's innate immune response. Without wishing to be bound by any theory, the inventors consider that the selection of a suitable adjuvant in an anti-

virulence vaccine is important to the induction of an appropriate protective immune response to staphylococcal infection.

Mice were vaccinated subcutaneously on days 0, 14 and 28 with Alum alone (A1); 10 µg of PolySSL (vaccine) delivered in Alum; or 10 µg of PolySSL delivered in one of 4 different combination adjuvants: Combination 1, alum + Pam3CSK4 (TLR2 ligand); Combination 2, alum + CL429 (TLR2 and NOD2 ligands); Combination 3, alum + CL413 (TLR2 and TLR7 ligands); and Combination 4, alum + MPLA (TLR4 ligand). Mice were then challenged systemically with $\sim 5 \times 10^7$ CFU *S. aureus* JSNZ on day 42 and tissues harvested on day 46.

As can be seen in Figure 9, the use of various combination adjuvants enabled protection against infection that was significantly improved over that conferred through the use of alum alone.

Example 4: Vaccine compositions comprising multiple multivalent polypeptide constructs

This example describes the preparation of compositions (such as vaccine compositions) comprising multiple different multivalent SSL polypeptide constructs.

It would be advantageous for certain examples of anti-virulence vaccines to confer protection against the virulence factor variants from all the major disease-causing strains of *S. aureus*.

To account for SSL allelic variation across staphylococcal strains prevalent around the globe, six polySSLs were constructed to cover the globally prevalent disease-causing clonal complexes (CC1, CC5, CC8, CC22, CC30 and CC93) – see Figure 10 (Figure adapted from Monarco M. et al. (2017) Curr Top Microbiol Immunol 409:21-29), which shows the most common clones in bold.

The gene of each SSL was mutated at specific amino acids to eliminate their *in vivo* function whilst maintaining immune recognition (see Figure 15, Figure 16, and Figure 17).

All the recombinant polySSLs variants were soluble and stable and were produced to high purity in bacteria (see Figure 11).

Challenge studies presented herein show that combinations of recombinant polySSLs in a vaccine can protect against a wide variety of strains, including strains that are not explicitly covered by that vaccine. That is, vaccine compositions exemplified herein provide cross reactivity including for strains from which an SSL polypeptide is not present in the vaccine.

Vaccination with either polySSL JSNZ, or combinations of polySSL from CC5 and CC22 (PolySSL CC5/CC22), or CC8 and CC30 (PolySSL CC8/CC30) can induce cross-reactive SSL-specific antibodies in mice infected with *S. aureus* strain JSNZ, CC5, CC8, CC22, or CC30 (Figure 12). Female CD1 mice were immunised with one of three different vaccine mixtures on Day 0, 14 and 28 of the study. Sera was collected on Day 11, 25 and 39, and examined via enzyme-linked immunosorbent assay (ELISA) for specific IgG antibody production against PolySSL components SSL3, SSL7 and SSL11. IgG Titres for components generated from a range of the *S. aureus* strains of interest were measured to determine if cross-reactive antibodies were produced. SSL7 from the polySSL is highly immunogenic and resulted in median titres of 10^5 log across each strain tested and each vaccine used. SSL3 had median titres of 10^4 - 10^5 log across strains and showed higher variability. SSL11 was the least immunogenic with much lower median titres, especially at days 11 and 25. By day 39 median titres were generally 10^4 log but some variability between strains was observed.

Combination vaccines as assessed in this Example generated neutralizing antibodies against SSL variants that were not included in the vaccine, as shown in the heatmaps of percentage inhibition of TLR2 activation by SSL3 (Figure 13 A) and inhibition of SSL7 binding to IgA (Figure 13 B).

Functional assays described in Example 1 were used to measure the level of functional antibodies following immunization. Sera from Day 39 were used to test the ability to neutralize the ability of SSL3 variants from the indicated strains to inhibit TLR2 signalling or the ability of SSL7 to bind IgA. Data has been summarized as a heat map where darker colouring is higher neutralization.

Antibodies neutralised the function of SSL3 from *S. aureus* JSNZ, CC5, CC8 and ST93 with variable success. PolySSL CC5/CC22 showed the highest cross-neutralization (Figure 13A). Antibodies neutralised the function of SSL7 from *S. aureus* JSNZ, CC5, CC8, CC22, CC30 and ST93 with variable success. PolySSL CC5/CC22 and PolySSL CC8/CC30 showed the highest cross-neutralization, but not necessarily towards the same strains (Figure 13B).

Immunisation with only one polySSL or with a combination of two polySSLs provided cross-protection to a range of *S. aureus* strains (Figure 14). Following immunization, mice were challenged with $\sim 7 \times 10^7$ colony forming units (CFU) of *S. aureus* on Day 42.

Bacterial burden in the kidney and liver was measured on Day 46. $n=6$ mice were immunised with each vaccine and data is combined from one-three independent studies. CFU log change of the vaccinated groups have been normalized to the unvaccinated control group. As can be seen in Figure 14, statistically significant reductions in bacterial load in both liver and kidney were observed in vaccinated mice compared to control.

The data presented in this Example strongly supports the prophylactic and therapeutic efficacy of the polySSL constructs and vaccine compositions comprising same. Individual polySSL polypeptides and combinations of two or more such polySSL polypeptides were effective against a wide range of *S. aureus*, eliciting strong reactivity and cross-reactivity to various *S. aureus* strains including strains from which an SSL was not present in the polySSL or vaccine.

Publications

Monarco M. et al. (2017) Curr Top Microbiol Immunol 409:21-29.

The entire disclosures of all applications, patents and publications cited above and below, if any, are herein incorporated by reference.

Where in the foregoing description reference has been made to integers or components having known equivalents thereof, those integers are herein incorporated as if individually set forth.

It should be noted that various changes and modifications to the presently preferred examples described herein will be apparent to those skilled in the art. Such changes and modifications may be made without departing from the spirit and scope of the invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications be included within the present invention.

The invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features.

Aspects of the invention have been described by way of example only, and it should be appreciated that variations, modifications and additions may be made without departing from the scope of the invention, for example when present the invention as defined in the indicative claims.

Furthermore, where known equivalents exist to specific features, such equivalents are incorporated as if specifically referred in this specification.

CLAIMS

1. A multivalent polypeptide comprising two or more Staphylococcal Superantigen-Like (SSL) virulence factors, wherein at least two of the Staphylococcal Superantigen-Like (SSL) virulence factors are independently selected from the group consisting of:
 - a. an SSL virulence factor capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity;
 - b. a mutated SSL virulence factor that in the absence of the mutation is capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity;
 - c. an antigenic fragment of (a) or (b);
 - d. an SSL virulence factor capable of binding to a complement molecule and/or of inhibiting complement activation;
 - e. a mutated SSL virulence factor that in the absence of the mutation is capable of binding to a complement molecule and/or of inhibiting complement activation;
 - f. an antigenic fragment of (d) or (e);
 - g. an SSL virulence factor capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis;
 - h. a mutated SSL virulence factor that in the absence of the mutation is capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis;
 - i. an antigenic fragment of (g) or (h).
2. The multivalent polypeptide according to claim 1, wherein when present in the multivalent polypeptide the or an activity of one or more of the SSL virulence factors is inhibited or ablated.
3. The multivalent polypeptide according to claim 1 or claim 2 wherein the antigenic fragment does not exhibit the or an activity of the SSL virulence factor of which it is a fragment.
4. The multivalent polypeptide according to any one of claims 1 to 3, wherein:
 - a. the SSL virulence factor capable of binding to a Toll-like receptor and/or of inhibiting Toll-like receptor activity is a Staphylococcal Superantigen-Like virulence factor 3 (SSL3); and/or
 - b. the SSL virulence factor capable of binding to a complement molecule and/or of inhibiting complement activation is a Staphylococcal Superantigen-Like virulence factor 7 (SSL7); and/or
 - c. the SSL virulence factor capable of binding to sialic acid and/or of inhibiting phagocyte chemotaxis is a Staphylococcal Superantigen-Like virulence factor 11 (SSL11); and/or
 - d. any combination of two or more of (a) to (c) above;
 - e. each of (a) to (c) above.
5. A multivalent polypeptide, wherein the multivalent polypeptide comprises at least one Staphylococcal Superantigen-Like virulence factor 3 (SSL3) polypeptide, at least one

Staphylococcal Superantigen-Like virulence factor 7 (SSL7) polypeptide, and at least one Staphylococcal Superantigen-Like virulence factor 11 (SSL11) polypeptide.

6. The multivalent polypeptide according to claim 4 or 5, wherein one or more of the at least one SSL3 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:
- a. an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 3;
 - b. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3;
 - c. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3, wherein said at least 50 consecutive amino acids comprise a Toll-like receptor binding region;
 - d. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 3, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
 - e. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 3;
 - f. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24;
 - g. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24, wherein said at least 50 consecutive amino acids comprise a Toll-like receptor binding region;
 - h. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 1 to 24, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
 - i. an amino acid sequence set forth in any one of SEQ ID NO: 1 to 24;
 - j. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 13 to 24;
 - k. an amino acid sequence set forth in any one of SEQ ID NO: 13 to 24;
 - l. an amino acid sequence according to any one of (a) to (k) above wherein when present the Toll-like receptor binding region is inactive;
 - m. an amino acid sequence according to any one of (a) to (l) above wherein when present the sialic acid binding region is inactive;
 - n. an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (m) above; or
 - o. any combination of any two or more of (a) to (n) above.

7. The multivalent polypeptide according to claim 4 or 5, wherein one or more of the at least one SSL7 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:
- a. an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 7;
 - b. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7;
 - c. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7, wherein said at least 50 consecutive amino acids comprise an immunoglobulin binding region;
 - d. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 7, wherein said at least 50 consecutive amino acids comprise a complement C5 binding region;
 - e. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7;
 - f. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48;
 - g. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48, wherein said at least 50 consecutive amino acids comprise an immunoglobulin binding region;
 - h. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 25 to 48, wherein said at least 50 consecutive amino acids comprise a complement C5 binding region;
 - i. an amino acid sequence set forth in any one of SEQ ID NO: 25 to 48;
 - j. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 37 to 48;
 - k. an amino acid sequence set forth in any one of SEQ ID NO: 37 to 48;
 - l. an amino acid sequence according to any one of (a) to (k) above wherein when present the immunoglobulin binding region is inactive;
 - m. an amino acid sequence according to any one of (a) to (l) above wherein when present the complement binding region is inactive;
 - n. an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (m) above; or
 - o. any combination of any two or more of (a) to (n) above.
8. The multivalent polypeptide according to claim 4 or 5, wherein one or more of the at least one SSL11 polypeptide comprises, consists essentially of, or consists of an amino acid sequence selected from the group consisting of:

- a. an amino acid sequence comprising an antigenic fragment from Staphylococcal Superantigen-Like virulence factor 11;
 - b. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 11;
 - c. at least 50 consecutive amino acids from Staphylococcal Superantigen-Like virulence factor 11, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
 - d. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11;
 - e. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 49 to 72;
 - f. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 49 to 72, wherein said at least 50 consecutive amino acids comprise a sialic acid binding region;
 - g. an amino acid sequence set forth in any one of SEQ ID NO: 49 to 72;
 - h. at least 50 consecutive amino acids from any one of the amino acid sequences set forth in any one of SEQ ID NO: 61 to 72;
 - i. an amino acid sequence set forth in any one of SEQ ID NO: 61 to 72;
 - j. an amino acid sequence according to any one of (a) to (i) above wherein when present the sialic acid binding region is inactive;
 - k. an amino acid sequence according to any one of (a) to (j) above wherein the SSL11 polypeptide does not exhibit chemotactic activity;
 - l. an amino acid sequence having at least about 90% amino acid sequence identity to any one of (a) to (k) above; or
 - m. any combination of any two or more of (a) to (l) above.
9. The multivalent polypeptide according to any one of claims 1 to 8, wherein the multivalent polypeptide comprises:
- a. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 3; and/or
 - b. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7; and/or
 - c. an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11.
10. The multivalent polypeptide according to any one of claims 1 to 9, wherein the multivalent polypeptide comprises:

- a. a Staphylococcal Superantigen-Like virulence factor 3 polypeptide comprising an amino acid sequence having at least 90% amino acid sequence identity to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 3 and in which one or more of the Toll-like receptor binding activity, the Toll-like receptor inhibitory activity, and/or the sialic acid binding activity is inactive; and/or
 - b. a Staphylococcal Superantigen-Like virulence factor 7 polypeptide comprising an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 7 and in which one or more of the immunoglobulin binding activity, the complement binding activity, and/or the complement inhibiting activity is inactive; and/or
 - c. a Staphylococcal Superantigen-Like virulence factor 11 polypeptide comprising an amino acid sequence corresponding to the amino acid sequence of Staphylococcal Superantigen-Like virulence factor 11 and in which the sialic acid binding activity and/or the phagocyte chemotactic activity is inactive.
11. The multivalent polypeptide according to any one of claims 1 to 10, wherein the multivalent polypeptide comprises an amino acid sequence encoding a Staphylococcal Superantigen-Like virulence factor 3 polypeptide, a Staphylococcal Superantigen-Like virulence factor 7 polypeptide, and a Staphylococcal Superantigen-Like virulence factor 11 polypeptide, wherein:
- a. either the amino acid sequence encoding the Staphylococcal Superantigen-Like virulence factor 3 polypeptide or the amino acid sequence encoding the Staphylococcal Superantigen-Like virulence factor 11 polypeptide is most proximal to the N-terminus of the multivalent polypeptide; or
 - b. the amino acid sequence encoding the Staphylococcal Superantigen-Like virulence factor 3 polypeptide is most proximal to the N-terminus of the multivalent polypeptide; or
 - c. the amino acid sequence encoding the Staphylococcal Superantigen-Like virulence factor 11 polypeptide is most proximal to the N-terminus of the multivalent polypeptide; or
 - d. the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus a Staphylococcal Superantigen-Like virulence factor 3 amino acid sequence, a Staphylococcal Superantigen-Like virulence factor 7 amino acid sequence, and a Staphylococcal Superantigen-Like virulence factor 11 amino acid sequence; or
 - e. the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus a Staphylococcal Superantigen-Like virulence factor 3 amino acid sequence, a Staphylococcal Superantigen-Like virulence factor 11 amino acid sequence, and a Staphylococcal Superantigen-Like virulence factor 7 amino acid sequence; or
 - f. the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus a Staphylococcal Superantigen-Like virulence factor 11 amino acid sequence, a

- Staphylococcal Superantigen-Like virulence factor 3 amino acid sequence, and a Staphylococcal Superantigen-Like virulence factor 7 amino acid sequence; or
- g. the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence comprising, consisting essentially of, or consisting of from N-terminus to C-terminus a Staphylococcal Superantigen-Like virulence factor 11 amino acid sequence, a Staphylococcal Superantigen-Like virulence factor 7 amino acid sequence, and a Staphylococcal Superantigen-Like virulence factor 3 amino acid sequence.
12. The multivalent polypeptide according to any one of claims 1 to 11, wherein the multivalent polypeptide does not exhibit one or more of the biological activities selected from the group consisting of: Toll-like receptor binding activity, Toll-like receptor inhibitory activity, sialic acid binding activity, phagocyte chemotactic activity, immunoglobulin binding activity, complement binding activity, complement inhibiting activity, and any combination of two or more thereof.
13. The multivalent polypeptide according to any one of claims 1 to 12, wherein:
- a. each Staphylococcal Superantigen-Like virulence factor is from the same clonal complex; or
 - b. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC1 clonal complex; or
 - c. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC5 clonal complex; or
 - d. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC8 clonal complex; or
 - e. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC15 clonal complex; or
 - f. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC22 clonal complex; or
 - g. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC30 clonal complex; or
 - h. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC45 clonal complex; or
 - i. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC93 clonal complex; or
 - j. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC97 clonal complex; or
 - k. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC121 clonal complex; or
 - l. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC398 clonal complex; or

- m. each Staphylococcal Superantigen-Like virulence factor is from any Sequence Type (ST) in the CC88 clonal complex.
14. The multivalent polypeptide according to any one of claims 1 to 13, wherein at least two of the Staphylococcal Superantigen-Like virulence factors are separated from one another by an intervening amino acid sequence.
15. The multivalent polypeptide according to claim 14, wherein the intervening amino acid sequence comprises a linker sequence or a proteolytic cleavage site.
16. The multivalent polypeptide of any one of the preceding claims, wherein the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence corresponding to at least about 50 contiguous amino acids from a sequence set forth in any one of SEQ ID NO.s: 73 to 144.
17. The multivalent polypeptide of any one of the preceding claims, wherein the multivalent polypeptide comprises, consists essentially of, or consists of an amino acid sequence corresponding to the sequence set forth in any one of SEQ ID NO.s: 73 to 144.
18. The multivalent polypeptide of any preceding claim, additionally comprising one or more amino acid sequences selected from the group consisting of:
- a. an amino acid sequence comprising an intracellular translocation domain;
 - b. an amino acid sequence comprising a domain associated with improved proteolytic processing;
 - c. an amino acid sequence comprising an adjuvant or a domain associated with enhanced immunological response or immunogenicity;
 - d. an amino acid sequence comprising a domain associated with enhanced epitope presentation or processing;
 - e. an amino acid sequence capable of inducing or increasing the activity of one or more of a B-cell, a CD4⁺ cell, a CD8⁺ cell, or an antigen-presenting cell; and
 - f. any combination of two or more of any of (a) to (e) above.
19. A polynucleotide construct encoding a multivalent polypeptide or polypeptide conjugate as claimed in any preceding claim.
20. The polynucleotide construct of claim 19, wherein said construct is a vaccine construct, such as an mRNA vaccine construct.
21. An isolated, purified, recombinant, or synthesised polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid sequence having at least about 90% or at least about 95% nucleic acid sequence identity to a nucleic acid sequence set forth in any one of SEQ ID NO.s: 145 to 156.
22. A polynucleotide, construct, vector, cell-free system, or cell capable of expressing a multivalent polypeptide or polypeptide conjugate as claimed in any preceding claim, or comprising a polynucleotide as claimed in any one of claims 30 to 32, or a polynucleotide encoding a multivalent polypeptide as claimed in any preceding claim.

23. The vector as claimed in claim 22, wherein the vector comprises, consists essentially of, or consists of a nucleotide sequence corresponding to the sequence of any one of SEQ ID NO.s: 145 to 156.
24. A pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of one or more multivalent polypeptides according to any preceding claim or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers.
25. A pharmaceutical composition comprising, consisting essentially of, or consisting of an effective amount of a polynucleotide, construct or vector according to any preceding or subsequent claim or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers.
26. The pharmaceutical composition of claim 24 or claim 25, wherein the composition comprises two or more multivalent polypeptides according to any one of the preceding claims.
27. The pharmaceutical composition of claim 26, wherein the composition comprises two or more multivalent polypeptides as defined in any one of claims 1 to 18.
28. The pharmaceutical composition of claim 27, wherein the composition comprises three or more, four or more, five or more, or six or more of the multivalent polypeptides as defined in any one of claims 1 to 18.
29. The pharmaceutical composition of any one of claims 24 to 28 comprising one or more adjuvants selected from the group consisting of: potassium aluminium sulfate (Alum); squalene; AddaVaxTM; Pam2CSK4; and Pam3CSK4.
30. The pharmaceutical composition of any one of claims 24 to 29, wherein the composition comprises two or more polynucleotides, constructs, or vectors according to any preceding or subsequent claim or any combination thereof, or a pharmaceutically acceptable salt or solvate thereof, together with one or more pharmaceutically acceptable carriers.
31. The pharmaceutical composition of any one of claims 24 to 30, wherein the pharmaceutical composition is an immunogenic composition.
32. The pharmaceutical composition of any one of claims 24 to 30, wherein the composition is a vaccine.
33. A method of treating or preventing a *Staphylococcus* infection, the method comprising administering to a subject in need thereof an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or a pharmaceutical composition according to any preceding claim.
34. A method of vaccinating a subject in need thereof, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or a pharmaceutical composition according to any preceding claim.
35. The method of claim 34, wherein the method is a method of vaccinating a subject against *Staphylococcus aureus*.

36. Use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as claimed in any preceding claim for treating or preventing a *Staphylococcus* infection in a subject, for vaccinating a subject, and/or for eliciting an immune response in a subject.
37. Use of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition thereof as claimed in any preceding claim in the manufacture of a medicament for treating or preventing a *Staphylococcus* infection in a subject, for vaccinating a subject, and/or for eliciting an immune response in a subject.
38. A method of eliciting in a subject in need thereof an immune response or conferring on a subject in need thereof a health benefit, the method comprising administering to the subject an effective amount of a multivalent polypeptide, a polynucleotide, construct, vector, or pharmaceutical composition as claimed in any preceding claim.
39. The method of claim 38, wherein the health benefit is decreased susceptibility to infection with *Staphylococcus aureus*, and/or reduced risk of symptoms associated with or sequelae of such infection.
40. The use according to claim 36 or 37 or the method according to claim 38 or 39, wherein the immune response is or is characterised by an increase in the number or activity of one or more immunoglobulins capable of specifically binding to a *Staphylococcus* antigen.
41. The use according to claim 36 or 37 or the method according to claim 38 or 39, wherein the immune response is or is characterised by an increase in the number or activity of one or more immune cells.

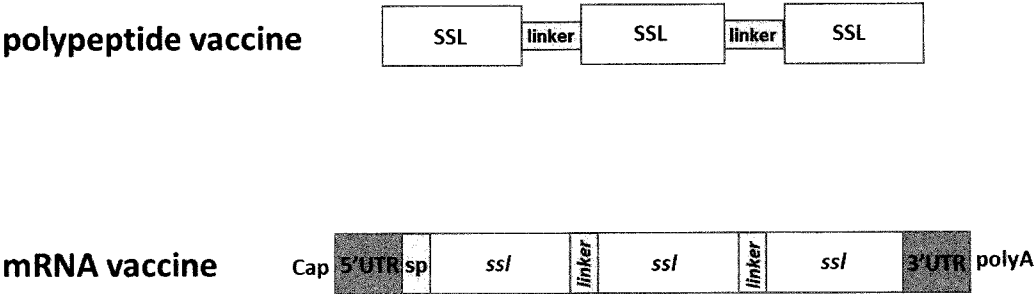


Figure 1

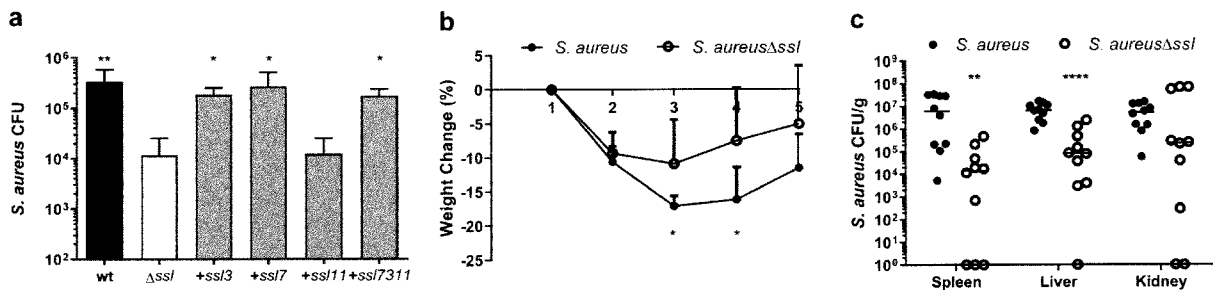


Figure 2

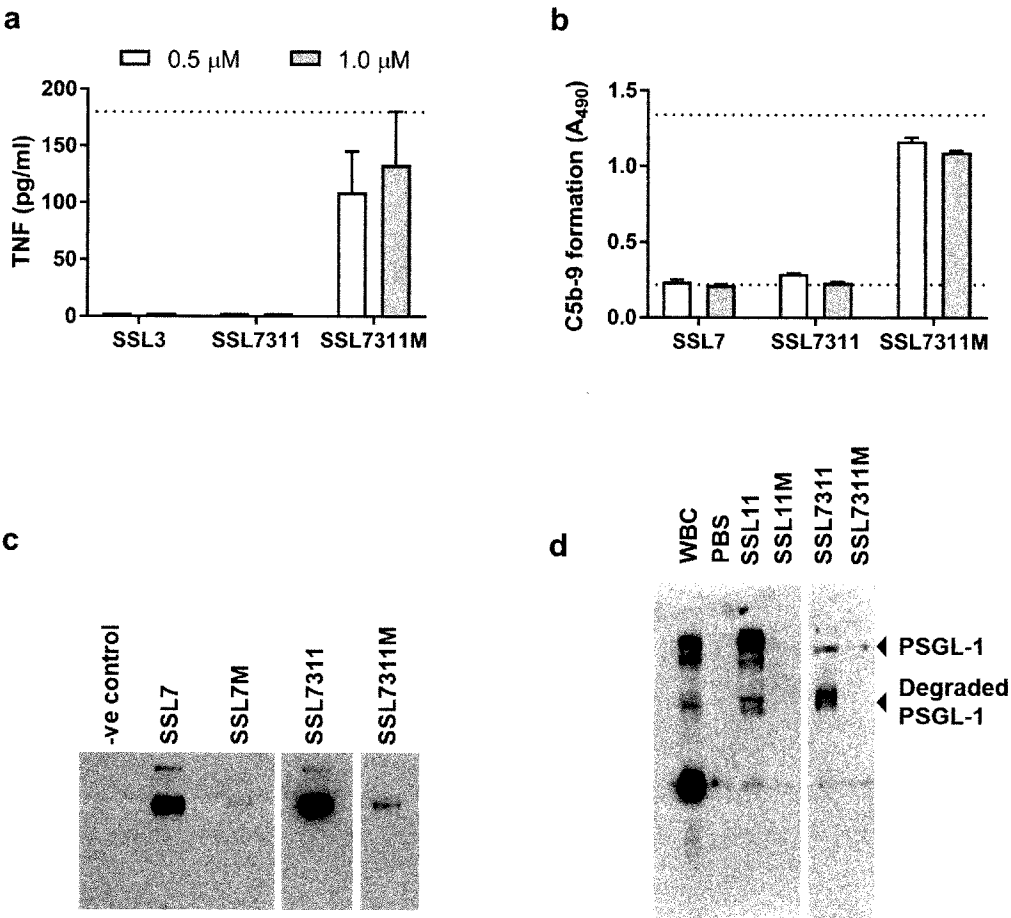


Figure 3

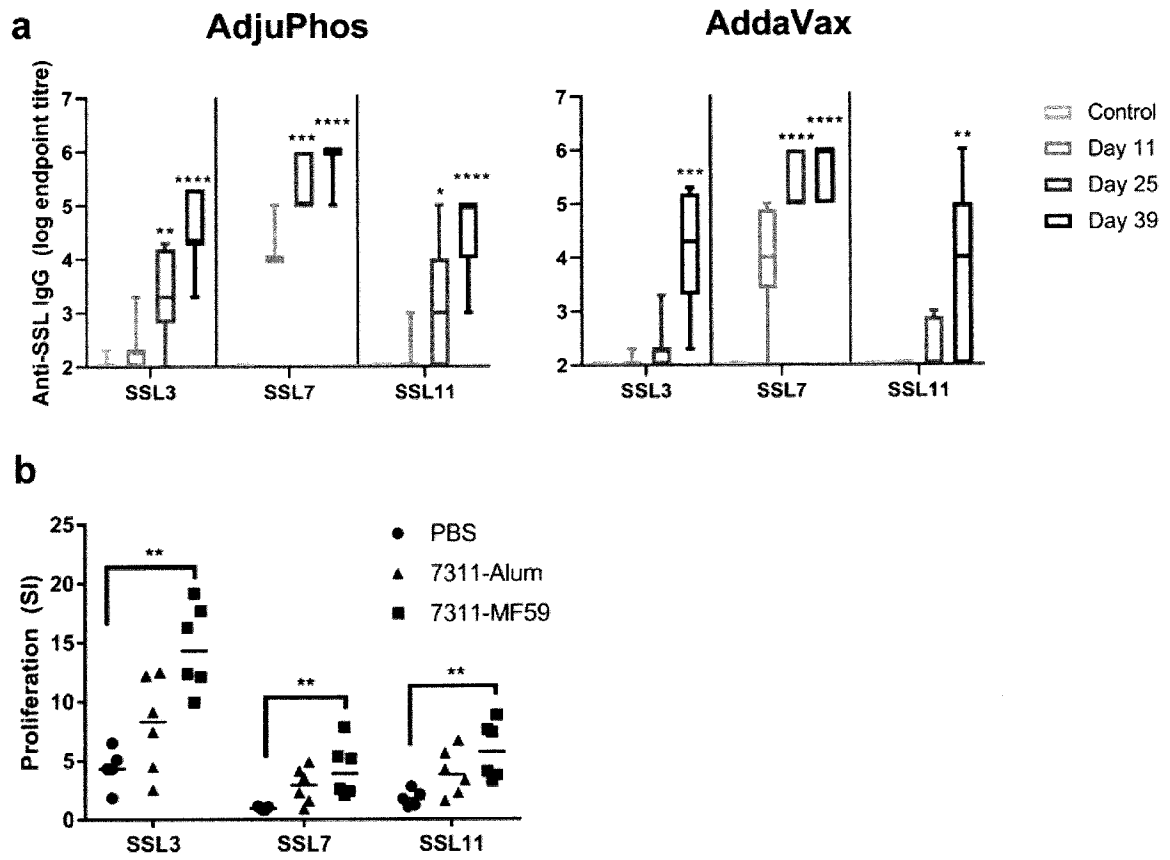


Figure 4

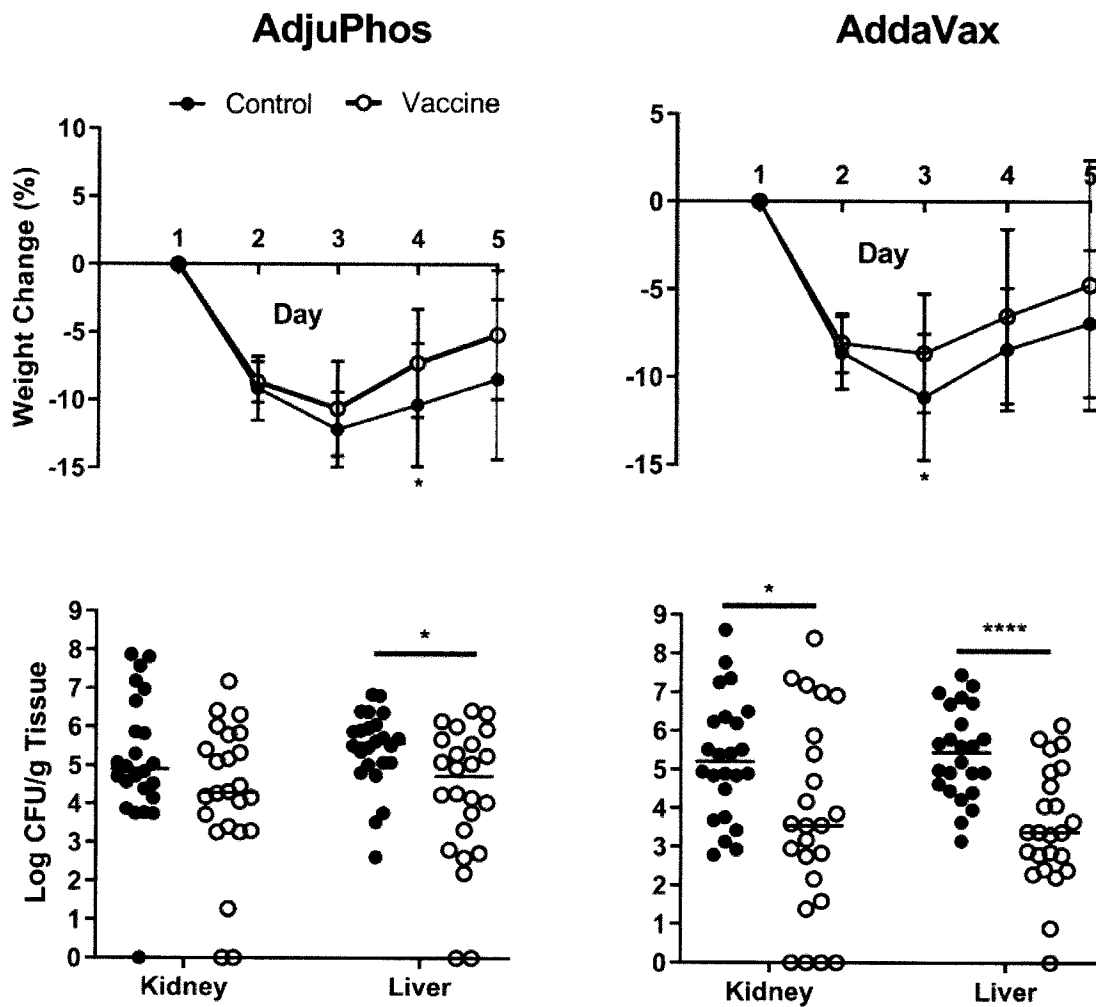


Figure 5

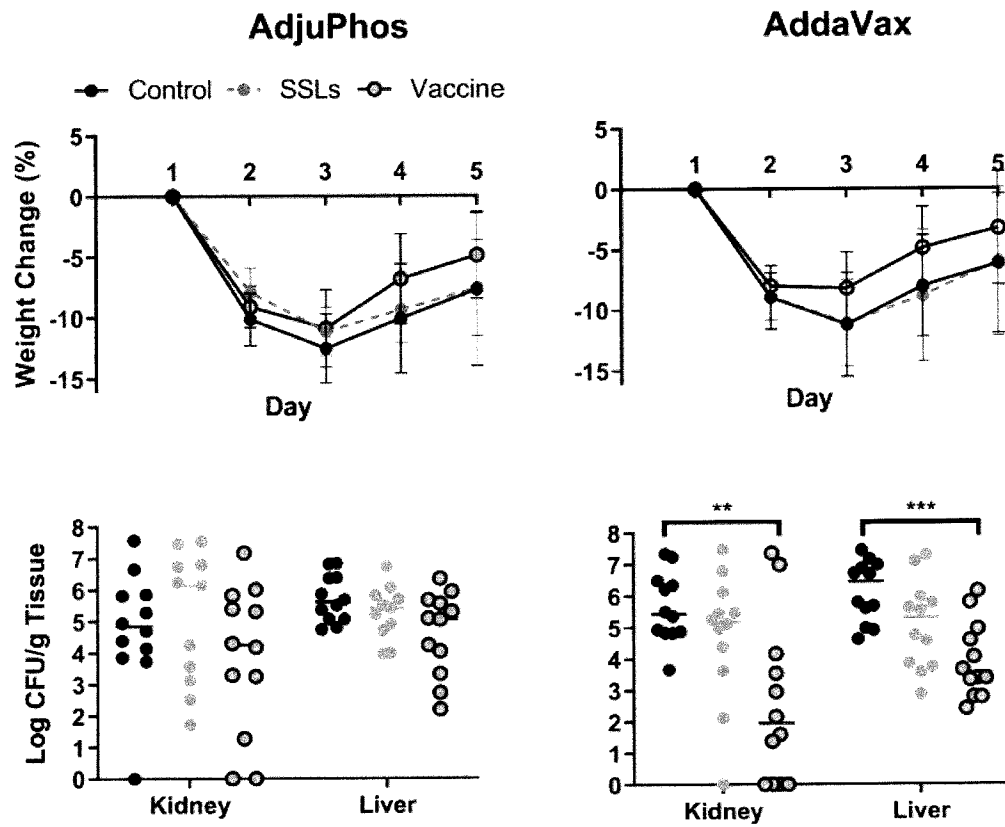


Figure 6

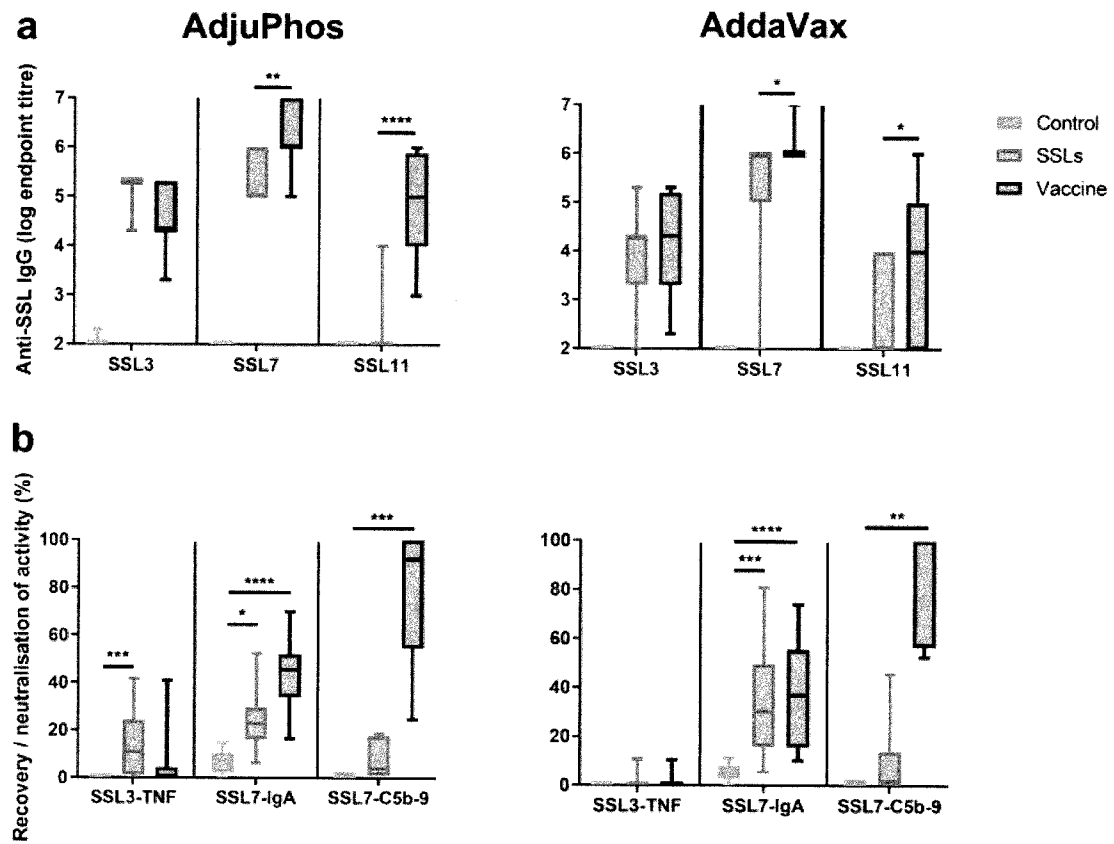


Figure 7

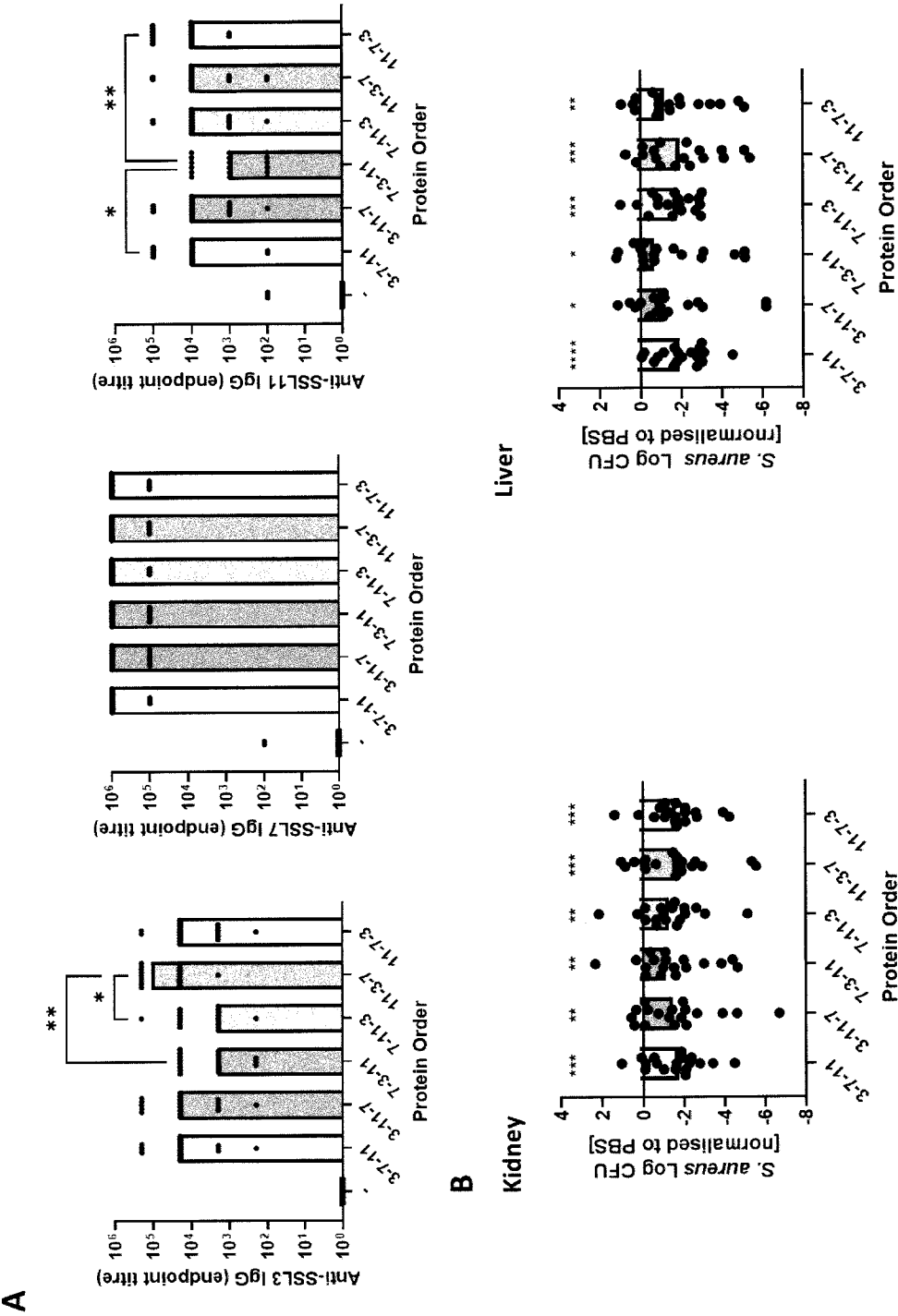


Figure 8

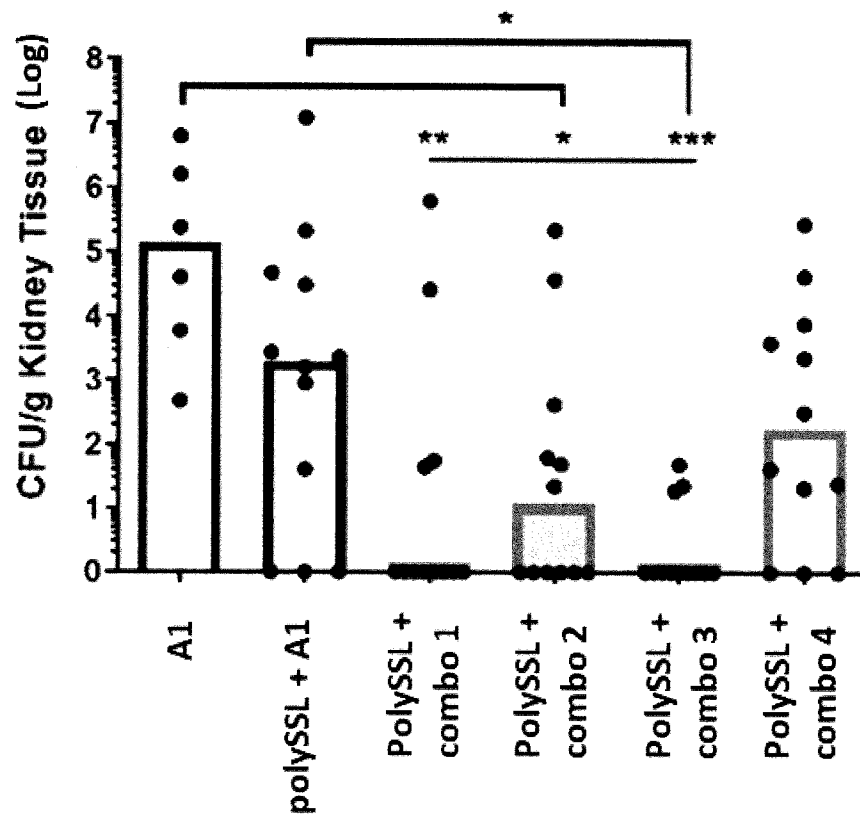


Figure 9

Clonal Complex (CC)	Sequence Types (ST) within Clonal Cluster
CC1	ST1, ST872, ST1005, ST1115, ST1336, ST573/772
	ST5, ST73, ST111, ST125, ST149, ST221, ST225, ST228, ST496, ST526, ST575, ST835, ST930, ST998
CC5	ST8, ST94, ST247, ST250, ST254, ST576, ST609, ST612, ST985, ST995, ST1337, ST72, ST239-241
	ST15, ST582
CC15	ST22, ST1117
CC22	ST30, ST36, ST39, ST982
CC30	ST93

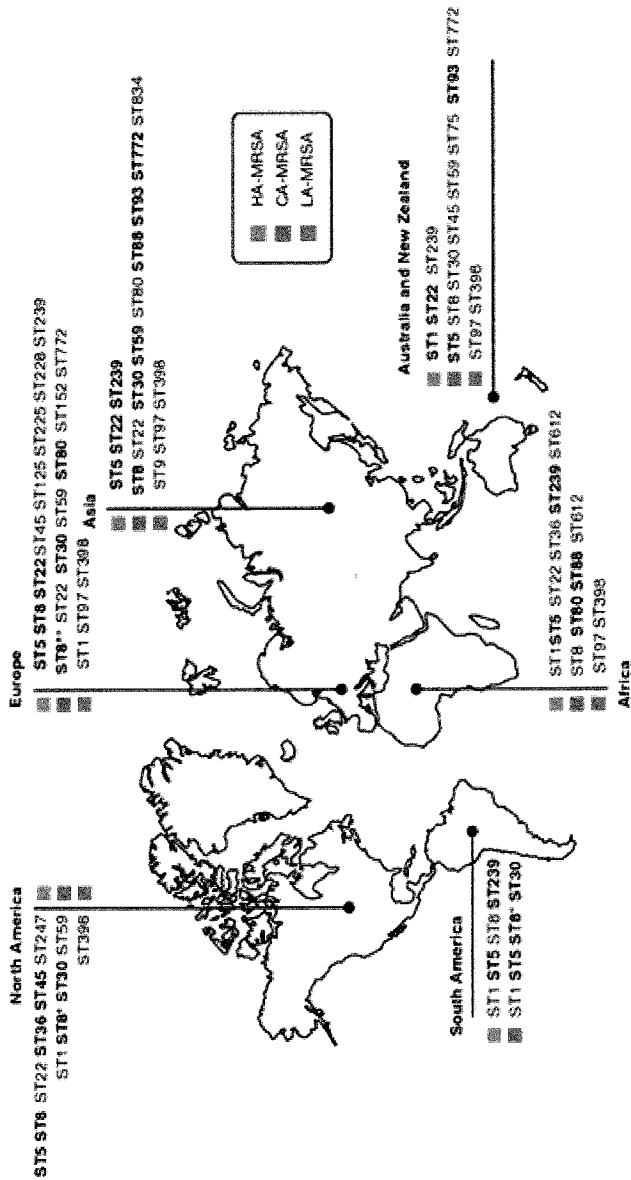


Figure 10

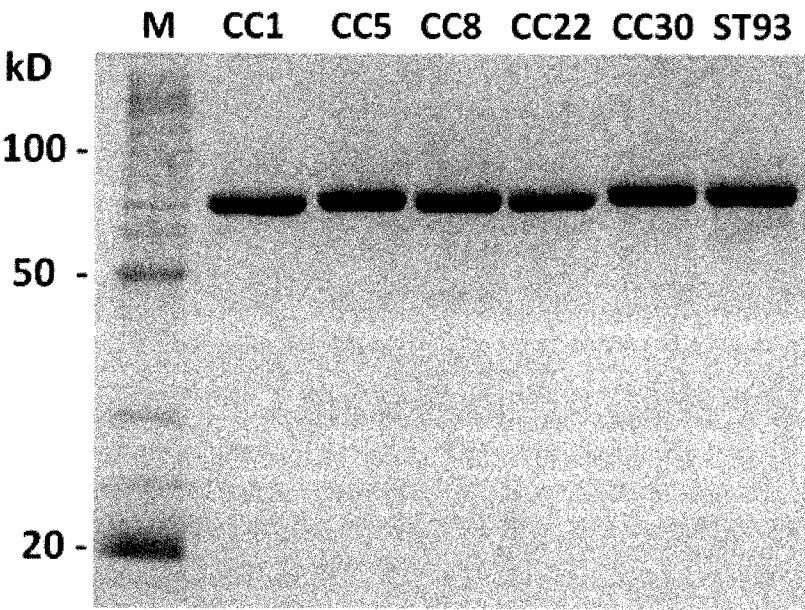


Figure 11

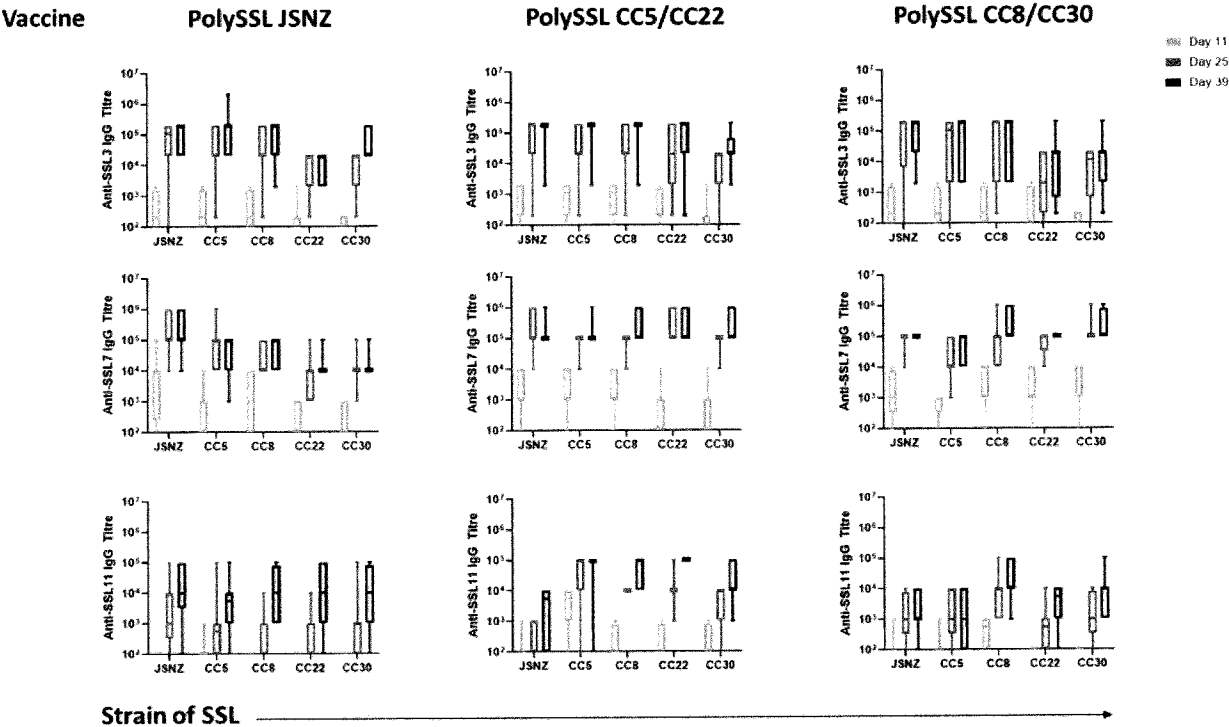


Figure 12

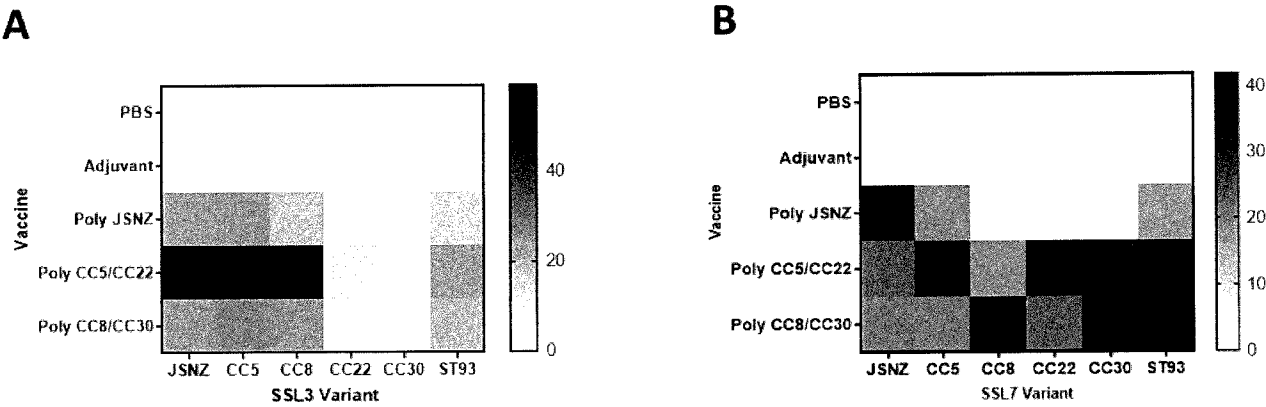
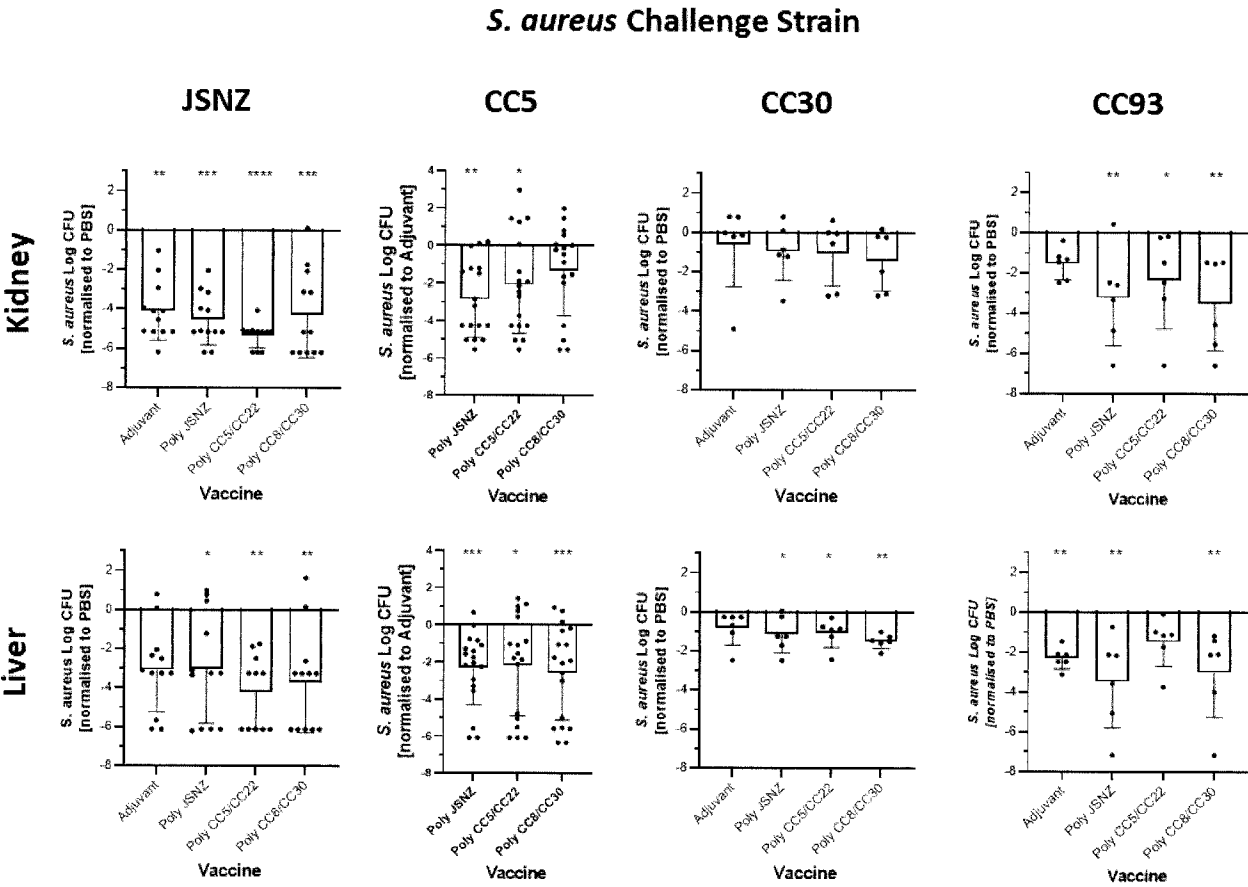


Figure 13



		+ + + +	+ + + + +	
CC5	QAQTDMPKYEDLRAYYTKPSFEFEKQ	FGMLKPWTTVRFMNVIPNRFTYKIALVGKDEK		60
JSNZ	QAQTDITPKYEDLRAYYTKPSFEFEKQ	FGMLKPWTTVRFMNVIPNRFTYKIALVGKDEK		60
CC8	QAQTDMPKYEDLRAYYTKPSFEFEKQ	FGMLKPWTTVRFMNVIPNRFTYKIALVGKDEK		60
CC1	QAQTDMPKYEDLRAYYTKPSFEFEKQ	FGMLKPWTTVRFMNVIPNRFTYKIALVGKDEK		60
ST93	TPPIEINPKYKDLRAYYTKPSFEFEKQ	FGMLKPWTTVRFMNIIPNRFTYKIALVGKDDK		60
CC22	QAQKEINPKYKDLRTYYTKPSLEFEKQ	FGMLKPWTTVRFMNVIPDWFYKIALVGKDDK		60
CC30	QAQKEINPKYKDLRTYYTKPSLEFEKQ	FGMLKPWTTVRFMNVIPDWFYKIALVGKDDK		60
	::,***;***;*****;*****;*****;***;*****;*			
		+ +		
CC5	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVDHKAELSVTKKDNQGMISRDV			120
JSNZ	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVNHKAELSITKKDNQGMISRDV			120
CC8	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVNHKVELSITKKDNQGMISRDV			120
CC1	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVNHKVELSITKKDNQGMISRDV			120
ST93	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVDHKAELNITKEDNKKGKISSDV			120
CC22	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVDHKARISITKEDEKKGKISRDD			120
CC30	KYKDGEYDNIDVFIVLEDNKYQLKKYSVGGITKTNSKKVDHKAELSITKKDEKKGKISHDD			120
	*****;*.:.:.*;*.:.:.* *			
		% % % % % %		
CC5	SEYMITKEEISLKELDFKLKQLIEKHNLYGNMGSGTIVIKMKNGGKYTFELHKKLQEHR			180
JSNZ	SEYMITKEEISLKELDFKLKQLIEKHNLYGNMGSGTIVIKMKNGGKYTFELHKKLQEHR			180
CC8	SEYMITKEEISLKELDFKLKQLIEKHNLYGNMGSGTIVIKMKNGGKYTFELHKKLQEHR			180
CC1	SEYMITKEEISLKELDFKLKQLIEKHNLYGNMGSGTIVIKMKNGGKYTFELHKKLQEHR			180
ST93	SEYKITKEEISLKELDFKLKQLIEQHNLYGNIGSGTIVIKMKNGGKYTFELHKKLQEHR			180
CC22	SEYKITKEEISLKELDFKLKQLIEQHNLYGNIGSGTIVIKTKNGGKYTFELHKKLQEHR			180
CC30	SEYKITKEEISLKELDFKLKQLIEQHNLYGNIGSGTIVIKTKNGGKYTFELHKKLQEHR			180
	*** *****;*****;***** *****			
		%		
CC5	MADVIEGTNIDKIEVNIK--	198		
JSNZ	MADVIEGTNIDKIEVNIK--	198		
CC8	MADVIDGTNIDNIEVNIK--	194		
CC1	MADVIDGTNIDNIEVNIK--	198		
ST93	MADVIDGTNIDSIEVNIK--	198		
CC22	MADVIDGTSIDRIEVNLKSS	200		
CC30	MADVIDGTSIERIEVNLKSS	200		
	***** **.*; *****;			

+ TLR2 binding region ¹% Sialic acid binding site ²⁻⁶

Figure 15

% C5 binding region 8

Figure 16

Figure 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2024/050129

A. CLASSIFICATION OF SUBJECT MATTER

C07K 14/31 (2006.01) A61K 39/00 (2006.01) A61K 39/085 (2006.01) A61K 39/116 (2006.01) A61P 31/04 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN Caplus, Biosis, Medline, Wpids, Biotechabs: Keyword search surrounding Staphylococcal superantigens and SSLs and their use in multivalent vaccines. Keyword search of specifically SSLs 3/7/11.

GenomeQuest: Sequence search of SEQ DID NOs: 73-156, Sequence search of SSL3/7/11 at 90% variability

Applicant/Inventors name searched in internal databases provided by IP Australia and external database (DOCDB and DWPI): asn=((AUCKLAND NEAR UNISERVICES NEAR LIMITED) OR (AUCKLAND NEAR UNISERVICES)) AND inn=((CLOW NEAR Fiona) OR (CLOW NEAR F)) OR

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"D" document cited by the applicant in the international application	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
4 March 2025

Date of mailing of the international search report
07 February 2025

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INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/NZ2024/050129
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/034682 A1 (UMC UTRECHT HOLDING B.V.) 14 March 2013 Examples 3-5, pages 44-55, claims 1-3, 7-11, pages 60-61	1-4, 6-10, 12-16, 18-20, 22, and 24-41
A	VENKATASUBRAMANIAM, A., <i>et al.</i> , 2019, "TBA ₂₂₅ , a Fusion Toxoid Vaccine for Protection and Broad Neutralization of Staphylococcal Superantigens", Scientific Reports, 9(3279) , pages 1-13 Results, pages 4-8, Figures 1-7, Discussion, pages 8-10, Figures 8-9	1-41
A	YOKOYAMA, R., <i>et al.</i> , 2012, "Staphylococcal Superantigen-like Protein 3 Binds to the Toll-like Receptor 2 Extracellular Domain and Inhibits Cytokine Production Induced by Staphylococcus aureus, Cell Wall Component, or Lipopeptide in Murine Macrophages", Infection and Immunity, 80(8) , pages 2816-2825 Abstract, Results, pages 2819-2820, final paragraph page 2824	1-41
A	LANGLEY, R., <i>et al.</i> , 2005, "The Staphylococcal Superantigen-Like Protein 7 Binds IgA and Complement C5 and Inhibits IgA-FcαRI Binding and Serum Killing of Bacteria", The Journal of Immunology, 174(5) , pages 2926-2933 Abstract, Results, pages 2928-2932, Figures 3, 5-10	1-41
A	CHEN, C., <i>et al.</i> , 2019, "Staphylococcal Superantigen-like Protein 11 Mediates Neutrophil Adhesion and Motility Arrest, a Unique Bacterial Toxin Action", Scientific Reports, 9(4211) , pages 1-11 Abstract, Results and Discussion, pages 1-8, Figures 1-3	1-41
A	LANGLEY, R. J., <i>et al.</i> , 2015, "Chapter 32 - Bacterial Superantigens and Superantigen-like Toxins", The Comprehensive Sourcebook of Bacterial Protein Toxins (Fourth Edition), Academic Press, pages 911-974 pages 911, 926, and 949-957	1-41
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INTERNATIONAL SEARCH REPORT

International application No.

PCT/NZ2024/050129

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/NZ2024/050129	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2013/034682 A1	14 March 2013	WO 2013034682 A1	14 Mar 2013
		AR 087811 A1	16 Apr 2014
		AU 2012306282 A1	20 Mar 2014
		US 2014248273 A1	04 Sep 2014
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2019)			