CLAIMS

1. A fusion construct of formula (I):

X-A-linker-B-Z (I)

wherein **:**

(1) A and B are identical or different and are independently:

(a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO: 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO: 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), a SACOL1611 polypeptide (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), a SACOL1944 polypeptide (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193), based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

(b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a);

(c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b);

(d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or

(e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c);

(2) the linker is an amino acid sequence of at least one amino acid or is absent;

(3) X is absent or is an amino acid sequence of at least one amino acid; and

(4) Z is absent or is an amino acid sequence of at least one amino acid.

2. The construct of claim 1, wherein (1) (a) is a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0442 polypeptide as set

forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120), or a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164).

3. The construct of claim 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c); and the other one of A and B is (a') a polypeptide comprising a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (c').

4. The construct of claim 2, wherein at least one of A and B is (a) a polypeptide comprising a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d); and the other one of A and B is (a') a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b') a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a'); (c') a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a') or (b'); (d') a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a') to (c'); or (e') a polypeptide comprising an immunogenic variant comprising at least 12 consecutive amino acids of any one of (a') to (d').

5. The construct of claim 2, wherein A and B are identical or different and are (a) a polypeptide comprising a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-K (SEQ ID NOs: 11 and 109 to 120); (b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a); (c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b); (d) a

polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or (e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (d).

6. The construct of any one of claims 1-2 and 4, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: KDTINGKSNKSRNW (SEQ ID NO: 34); and KDGGKYTLESHKELQ (SEQ ID NO: 1).

7. The construct of claim 6, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences : STQNSSSVQDKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAG RVYTPKRNITLNKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKN VTFKLVKSVNDIEQV (SEQ ID NO: 30); DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITL NKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTK (SEQ ID NO :32); and DKQLQKVEEVPNNSEKALVKKLYDRYSKDTINGKSNKSRNWVYSERPLNENQVRIHLEGTYTVAGRVYTPKRNITL NKEVVTLKELDHIIRFAHISYGLYMGEHLPKGNIVINTKDGGKYTLESHKELQKDRENVKINTADIKNVTFKLVKSVN DIEQV (SEQ ID NO : 33).

8. The construct of any one of claims 1-2 and 4-5, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: QFGFDLKHKKDALA (SEQ ID NO: 21); TIKDQQKANQLAS (SEQ ID NO: 22); KDINKIYFMTDVDL (SEQ ID NO: 23); and DVDLGGPTFVLND (SEQ ID NO: 24).

9. The construct of claim 8, wherein said immunogenic fragment (d) comprises one or more of the following amino acid sequences: RASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTD LKRGQADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKH IVSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISSLTG (SEQ ID NO : 12); ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDL KRGQADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKHI VSQFGFDLKHKKDALALEKAKNKVDKSIETRSEAISSISSLTG (SEQ ID NO: 13); ASLSSEIKYTAPHDVTIKDQQKANQLASELNNQKIPHFYNYKEVIHTKLYKDNLFDVKAKEPYNVTITSDKYIPNTDL KRGQADLFVAEGSIKDLVKHKKHGKAIIGTKKHHVNIKLRKDINKIYFMTDVDLGGPTFVLNDKDYQE (SEQ ID NO: 14); KDINKIYFMTDVDLGGPTFVLNDKDYQEIRKYTKAKHIVSQFGFDLKHKKDALA (SEQ ID NO : 15); KDINKIYFMTDVDLGGPTFVLNDKDY (SEQ ID NO : 17); KDINKIYFMTDVDLGGPTFVLNDKD (SEQ ID NO : 16); KDINKIYFMTDVDLGGPTFVLND (SEQ ID NO : 19); KHIVSQFGFDLKHKKDALA (SEQ ID NO: 20) and SQFGFDLKHKKDALA (SEQ ID NO: 18).

10. The construct of any one of claims 1-3, wherein said immunogenic fragment (c') comprises one or more of the

following amino acid sequences: PYNGVVSFKDATGF (SEQ ID NO: 165); AHPNGDKGNGGIYK (SEQ ID NO: 167); SISDYPGDEDISVM (SEQ ID NO: 169); RGPKGFNFNENVQA (SEQ ID NO: 172); QFESTGTIKRIKDN (SEQ ID NO: 175); and GNSGSPVLNSNNEV (SEQ ID NO: 178).

11. The construct of claim 10, wherein said immunogenic fragment (d) comprises the following amino acid sequence: TQVKDTNIFPYNGVVSFKDATGFVIGKNTIITNKHVSKDYKVGDRITAHPNGDKGNGGIYKIKSISDYPGDEDISVMN IEEQAVERGPKGFNFNENVQAFNFAKDAKVDDKIKVIGYPLPAQNSFKQFESTGTIKRIKDNILNFDAYIEPGNSGSP VLNSNNEVIGVVYGGIGKIGSEYNGAVYFTPQIKDFIQKHIEQ (SEQ ID NO: 39).

12. The construct of any one of claims 1 to 11, wherein the linker comprises at least four identical or different amino acids selected from the group consisting of glycine, serine, alanine, aspartate, glutamate and lysine.

13. The construct of any one of claims 1 to 12, wherein the linker comprises (GGGGS)n (SEQ ID NO: 67), (ERKYK)n (SEQ ID NO: 61); or (EAAAK)n (SEQ ID NO: 63), wherein n=1 to 5.

14. The construct of any one of claims 1 to 13, wherein said X comprises a polyhistidine of 6 to 10 amino acids.

15. The construct of any one of claims 1 to 13, wherein said X is absent.

16. The construct of any one of claims 1 to 15, wherein said Z is absent.

17. An isolated nucleic acid molecule encoding the construct defined in any one of claims 1 to 16.

18. A vector comprising the isolated nucleic acid defined in claim 17.

19. A host cell comprising the vector defined in claim 18.

20. The cell of claim 19, which is a live attenuated form of *Staphylococcus aureus*.

21. The cell of claim 20, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.

22. The cell of claim 21, wherein the live attenuated form of *Staphylococcus aureus* having a stabilized SCV phenotype is a Δ*hemB*Δ720 *S. aureus*.

23. A composition comprising:

(A) at least one of the constructs defined in any one of claims 1 to 16; at least one of the nucleic acid molecules defined in claim 17; at least one of the vectors defined in claims 18; or at least one of the cells defined in any one of claims 19 to 22; and

(B) (i) a polypeptide defined in any one of claims 1 to 11;

(ii) a live attenuated *Staphylococcus aureus*;

(iii) a pharmaceutically acceptable excipient;

(iv) an adjuvant; or

(v) a combination of at least two of (i) to (iv).

24. The composition of claim 23, wherein the live attenuated form of *Staphylococcus aureus* expresses:

(a) a polypeptide comprising a SACOL0029 polypeptide as set forth in any one of the sequences depicted in FIG. 24 (SEQ ID NOs: 5 and 121 to 131), a SACOL0264 polypeptide (SEQ ID NO: 185), a SACOL0442 polypeptide as set forth in any one of the sequences depicted in FIG. 22D (SEQ ID NOs: 29 and 82 to 92), a SACOL0718 polypeptide (SEQ ID NO: 186), a SACOL0720 polypeptide as set forth in any one of the sequences depicted in FIGs. 23I-J (SEQ ID NOs: 11 and 109 to 120), a SACOL1353 polypeptide (SEQ ID NO : 187), a SACOL1416 polypeptide (SEQ ID NO : 188), SACOL1611 (SEQ ID NO : 189), a SACOL1867 polypeptide as set forth in any one of the sequences depicted in FIG. 25D (SEQ ID NOs: 152 to 164), a SACOL1912 polypeptide (SEQ ID NO : 43), SACOL1944 (SEQ ID NO : 190), a SACOL2144 polypeptide (SEQ ID NO : 191), a SACOL2365 polypeptide (SEQ ID NO : 192), a SACOL2385 polypeptide (SEQ ID NO : 50) or a SACOL2599 polypeptide (SEQ ID NO : 193) based on the gene nomenclature from the *Staphylococcus aureus* COL (SACOL) genome set forth in NCBI Reference Sequence NC_002951.2;

(b) a polypeptide encoded by a gene from a same operon as a gene encoding the polypeptide of (a);

(c) a polypeptide comprising an immunogenic fragment of at least 13 consecutive amino acids of (a) or (b);

(d) a polypeptide comprising an amino acid sequence at least 60% identical overall to the sequence of the polypeptide of any one of (a) to (c); or

(e) a polypeptide comprising an immunogenic variant comprising at least 13 consecutive amino acids of any one of (a) to (c).

25. The composition of claim 23 or 24, wherein the live attenuated form of *Staphylococcus aureus* has a stabilized small colony variant (SCV) phenotype.

26. The composition of any one of claims 23 to 25, wherein the adjuvant comprises alum, an oil, saponin, cyclic-diguanosine-5'-monophosphate (c-di-GMP), polyphosphasine, indolicidin, pathogen-associated molecular patterns (PAMPS), liposome or a combination of at least two thereof.

27. Use of an effective amount of the construct defined in any one of claims 1 to 16; of the nucleic acid molecule defined in claim 17; of the vector defined in claim 18; of the cell defined in any one of claims 19 to 22; or of the composition defined in any one of claims 23 to 26 in the manufacture of a medicament for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal.

28. The use of claim 27, wherein said Staphylococcal IMI is caused by one or more *Staphylococcus aureus* strains.

29. The use of claim 27 or 28, wherein said mammal is a cow.

30. The construct defined in any one of claims 1 to 16; the nucleic acid molecule defined in claim 17; the vector defined in claim 18; the cell defined in any one of claims 19 to 22; the composition defined in any one of claims 23 to 26 or a combination of at least two thereof, for use in the prevention and/or treatment of a Staphylococcal intramammary infection (IMI) in a mammal.

31. A kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal comprising:

(A) (i) at least one of the constructs defined in any one of claims 1 to 16; (ii) at least one of the nucleic acid molecules defined in claim 17; (iii) at least one of the vectors defined in claim 18; (iv) at least one of the cells defined in any one of claims 19 to 22; or (v) a combination of at least two of (i) to (iv), and

(B) (i) the polypeptide defined in any one of claims 1 to 11;

(ii) a live attenuated *Staphylococcus aureus*;

(iii) a pharmaceutically acceptable excipient;

(iv) an adjuvant;

(v) instructions for using the kit for preventing and/or treating a Staphylococcal intramammary infection (IMI) in a mammal; or

(vi) a combination of at least two of (i) to (v).