**Supplementary file 1**

***Supplementary file 1a.***

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| **Antibody** | **Antigen** | **Peptide sequence** | **Framework** |
| M-C1 | Cyclic peptide, loop TMS 1-2 | ELSGINFKDTLVEFSKINR | VH3-23 kappa 3 |
| M-C7.1 | Cyclic peptide, loop TMS 7-8 | LGFKTLGVPEEKV | VH1A kappa 1 |
| M-C7.2 | Cyclic peptide, loop TMS 7-8 | LGFKTLGVPEEKV | VH3-23 kappa 1 |
| M-C7.3 | Cyclic peptide, loop TMS 7-8 | LGFKTLGVPEEKV | VH1A kappa 1 |
| M-C9.1 | Cyclic peptide, loop TMS 9-10 | DALYDGNHLT | VH1A kappa 1 |
| M-C9.2 | Cyclic peptide, loop TMS 9-10 | DALYDGNHLT | VH3-23 kappa 1 |
| M-C13.1 | Cyclic peptide, loop TMS 13-14 | DIYTIEMHTSVLR | VH1A kappa 1 |
| M-C13.2 | Cyclic peptide, loop TMS 13-14 | DIYTIEMHTSVLR | VH1A kappa 1 |

***Supplementary file 1b.***

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| Strain name | Characteristics |
| *S. aureus* SA113 WT (ATCC 35556) | Restriction-deficient *S. aureus* strain derived from NCTC 8325 [1] |
| *S. aureus* SA113*∆mprF* | *mprF* deletion mutant of SA113, gene replaced by erythromycin resistance cassette [2]. |
| *S. aureus* SA113*∆spa* | *spa* deletion mutant of SA113. Constructed in this study. Markerless. |
| *S. aureus* SA113*∆spa∆mprF* | *spa* and *mprF* double deletion mutant of SA113. Constructed in this study. Ermr |
| *S. aureus* DAP-R MRSA 703 | Daptomycin resistant clinical CA-MRSA isolate possessing a single point mutation in *mprF* (S295L) [3] |
| *S. aureus* USA300 LAC | CA-MRSA WT strain [4] |
| *E. coli* TG1 | Strain for phage display usage [5] |

***Supplementary file 1c.***

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| **Plasmid** | **Characteristics** | **Short name in figures** |
| pKOR1 | *E. coli/S. aureus* shuttle vector to allow allelic replacement with inducible counter-selection in staphylococci [6] | - |
| pRB474 | *E. coli/S. aureus* shuttle vector [7] | pRB |
| pRB474mprF | *mprF* cloned in *E. coli/S. aureus* shuttle vector pRB474 [2] | pRB-MprF |
| pRB474mprF-GFP | N-terminally GFP-tagged *mprF* cloned in *E. coli/S. aureus* shuttle vector pRB474 [8] | pRB-MprF-GFP |
| pRB474mprFdelCys flag | C-terminally FLAG®-tagged, cysteine codon-depleted *mprF* cloned in *E. coli/S. aureus* shuttle vector pRB474; constructed in this study  | WT (-Cys) |
| pRB474mprFdelCys T263C flag | C-terminally FLAG®-tagged, cysteine codon-depleted *mprF* with artificial cysteine insertion cloned in *E. coli/S. aureus* shuttle vector pRB474; as indicated, each plasmid bears another amino acid substituted against cysteine (T263C, A99C, or T480C) | pRB-T263C |
| pRB474mprFdelCys A99C flag | pRB-A99C |
| pRB474mprFdelCys T480C flag | pRB-T480C |

***Supplementary file 1d.***

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| **Primer** | **5’ 🡪 3’ sequence** | **Usage** |
| A99C fw | GCATTGAATTGTATTGTAGGTTTCGGTGGCTTTATTGGTGCAGGCG | Forward primer for construction of pRB-A99C by site-directed mutagenesis |
| A99C rev | CCGAAACCTACAATACAATTCAATGCATTGATGATATAACTTACTC | Reverse primer for construction of pRB-A99C by site-directed mutagenesis |
| T263C fw | GTTGTATTACTAGGATTTAAATGTTTAGGTGTCCCTGAGGAAAAAG | Forward primer for construction of pRB-T263C by site-directed mutagenesis |
| T263C rev | CTTTTTCCTCAGGGACACCTAAACATTTAAATCCTAGTAATACAAC | Reverse primer for construction of pRB-T263C by site-directed mutagenesis |
| T480C fw | GGAACGTTATATGCATTAGATATTTATTGTATTGAAATGCATACATCTGTATTGCG | Forward primer for construction of pRB-T480C by site-directed mutagenesis |
| T480C rev | CGCAATACAGATGTATGCATTTCAATACAATAAATATCTAATGCATATAACGTTCC | Reverse primer for construction of pRB-T480C by site-directed mutagenesis |
| mprF C199+204S fw | TACTCTACTTTAGTGTCGTCTGTTGAATGGTTAGCAG | Primer for cysteine depletion of pRB474 encoded native *mprF* by site-directed mutagenesis |
| mprF C199+204S rev | AACAGACGACACTAAAGTAGAGTACAATCCTACAAAACG |
| mprF C217A fw | TTCGCTGGTGTAATTGTTGACGC |
| mprF C217A rev | ACCAGCGAAATATAATACAACTGC |
| mprF C380A fw | GCTGCTTTATTACTTTTACTGAATGTAGTTGG |
| mprF C380A rev | TAAAGCAGCACTAGTATGAATTGCC |
| mprF C526S fw | GATAGCGAGGAGATTATTAATCAG |
| mprF C526S rev | CTCGCTATCTTCAATTTTAGAAG |
| mprF C717S fw | GTAATTGCATTTAGTAGTTTAATGCCAACATACTTTAATGATG |
| mprF C717S rev | CTACTAAATGCAATTACTTCATTTTCTTCATTTCGCATTACACC |
| Spa-del\_attB1 | ggggacaagtttgtacaaaaaagcaggccaatattccatggtccagaact | Construction of a markerless *spa* knockout mutant using the pKOR1 vector system [6] |
| Spa-del | gtcgagatctataaaaacaaacaatacacaacg |
| Spa-del\_attB2 | ggggaccactttgtacaagaaagctgggatcagcaagaaaacacacttcc |
| Spa-del rev | aaaagatctaacgaattatgtattgcaata |
| MprF\_USA300\_fw | cagatatcaatatgacaaaag | Amplification of *mprF* in USA300 |
| MprF\_USA300\_rev | cttaaatattcttatctgtacc | Amplification of *mprF* in USA300 |
| MprF\_USA300\_600 | gtcatttttcttaccattattc | Sequencing of *mprF* in USA300 |
| MprF\_USA300\_1200 | gtgcttgtttattacttttac | Sequencing of *mprF* in USA300 |
| MprF\_USA300\_1800 | gttaggtgatgaaaatgcc | Sequencing of *mprF* in USA300 |
| MprF\_USA300\_2200 | gatggttgccagagttag | Sequencing of *mprF* in USA300 |
| MprF\_USA300\_800rev | cttcttagctgatgtacc | Sequencing of *mprF* in USA300 |

**Supplemental references**

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