**Supplemental information**

**Supplemental Figure 1. Growth of *S. aureus* ATCC29213 in supMH and MEM.** Growth of bacteria inoculated into supMH or MEM media, as measured by photometric absorbance at 600nm. Error bars indicate standard deviation of four separate measurements.

**Supplemental Figure 2. Colony morphologies of evolved isolates. A.** Growth phenotypes on blood agar. Isolate labels in blue indicate SCV morphology, and black labels are consistent with the parental strain. **B.** Pigmentation phenotypes on blood agar. Isolate labels in dull yellow indicate moderately increased pigmentation, bright yellow show markedly increased pigmentation, and black labels are consistent with the parental strain.

**Supplemental Table 1. Virulence activity of selected isolates relative to parental strain.**

**Supplemental Table 2. Growth rates of selected isolates relative to parental strain.**

**Supplemental Table 3. Real time PCR quantitation of *sigB* and *agrA* expression relative to parental strain.**
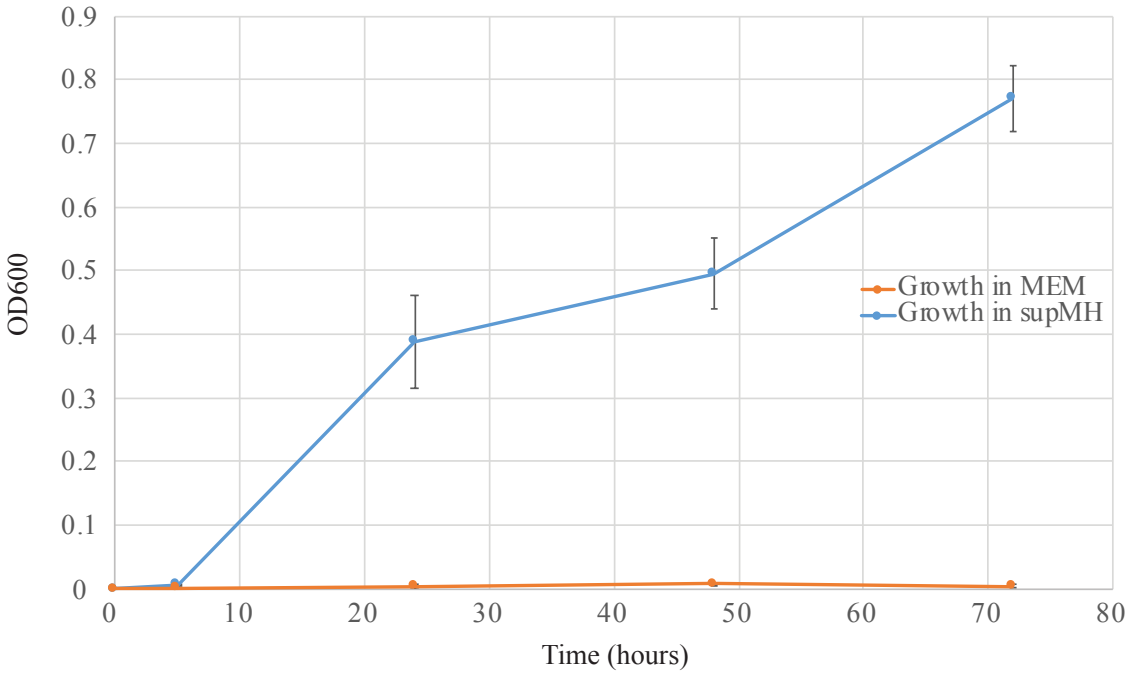
**Supplemental Table 4. Coding variants identified by whole genome sequencing.**
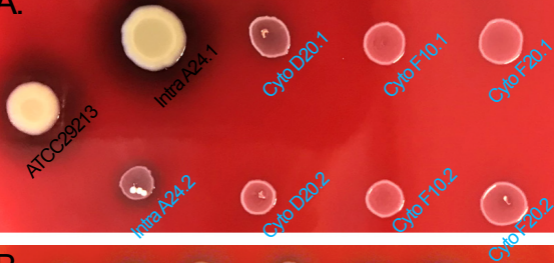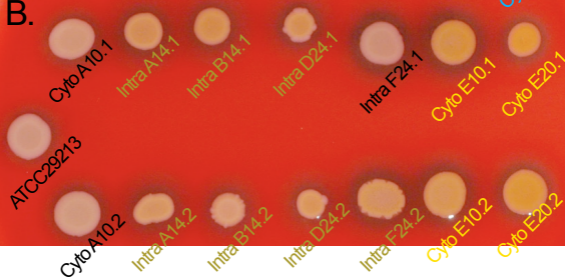
**Supplemental Table 5. Virulence of single gene mutant strains relative to parental strain.**

**Supplemental Table 6. Virulence activity of complemented gene deletion strains.**

**Supplemental Table 7. Virulence activity of epistatic mutations.**

**Supplemental Table 8. Oligonucleotide sequences**

A.

ATCC29213   Intra A24.1   Cyto D20.1   Cyto F10.1   Cyto F20.1

Intra A24.2   Cyto D20.2   Cyto F10.2   Cyto F20.2

B.

Cyto A10.1   Intra A14.1   Intra B14.1   Intra D24.1   Intra F24.1   Cyto E10.1   Cyto E20.1

ATCC29213

Cyto A10.2   Intra A14.2   Intra B14.2   Intra D24.2   Intra F24.2   Cyto E10.2   Cyto E20.2

| | Cytotoxicity | | | Invasiveness | | |
|---|---|---|---|---|---|---|
| | **Supplemental Table 1: Virulence activity of selected isolates relative to parental strain** | | | | | |
| Strain | Fold difference | SEM | *p** | Fold difference | SEM | *p** |
| Cyto A10.1 | 1.11 | 0.02 | 4.78E-04 | 1.96 | 0.94 | 2.57E-01 |
| Cyto A10.2 | 1.16 | 0.03 | 8.48E-04 | 0.49 | 0.14 | 1.78E-03 |
| Cyto B10.1 | 1.10 | 0.03 | 6.83E-03 | 1.73 | 0.52 | 6.70E-02 |
| Cyto B10.2 | 1.18 | 0.04 | 4.04E-03 | 0.91 | 0.25 | 6.31E-01 |
| Cyto C10.1 | 1.22 | 0.04 | 7.74E-04 | 0.87 | 0.05 | 1.58E-01 |
| Cyto C10.2 | 1.08 | 0.01 | 3.98E-04 | 1.46 | 0.05 | 2.62E-04 |
| Cyto D10.1 | 1.09 | 0.02 | 2.38E-04 | 0.52 | 0 | 8.39E-05 |
| Cyto D10.2 | 1.12 | 0.02 | 5.19E-06 | 2.89 | 0.59 | 8.50E-04 |
| Cyto E10.1 | 1.11 | 0.02 | 1.35E-03 | 1.09 | 0.61 | 8.81E-01 |
| Cyto E10.2 | 1.09 | 0.02 | 3.34E-03 | 1.74 | 0.08 | 9.87E-06 |
| Cyto F10.1 | 1.07 | 0.03 | 3.72E-02 | 19.48 | 1.98 | 7.24E-08 |
| Cyto F10.2 | 1.06 | 0.02 | 6.63E-03 | 4.75 | 0.34 | 2.32E-08 |
| Cyto A20.1 | 1.14 | 0.04 | 1.06E-03 | 10.93 | 1.75 | 7.50E-06 |
| Cyto A20.2 | 1.08 | 0.03 | 2.35E-02 | 6.14 | 0.53 | 6.08E-08 |
| Cyto B20.1 | 1.07 | 0.03 | 1.37E-02 | 5.66 | 0.7 | 2.00E-06 |
| Cyto B20.2 | 1.03 | 0.03 | 3.93E-01 | 18.48 | 0.61 | 1.32E-12 |
| Cyto C20.1 | 1.06 | 0.04 | 1.37E-01 | 10.75 | 1.59 | 3.77E-06 |
| Cyto C20.2 | 0.97 | 0.03 | 4.23E-01 | 91.58 | 6.94 | 2.83E-09 |
| Cyto D20.1 | 1.31 | 0.11 | 8.68E-03 | 11.58 | 0.62 | 2.32E-10 |
| Cyto D20.2 | 1.28 | 0.08 | 1.66E-03 | 47.7 | 11.4 | 1.15E-04 |
| Cyto E20.1 | 1.09 | 0.04 | 1.75E-02 | 152 | 11.98 | 3.95E-09 |
| Cyto E20.2 | 1.03 | 0.04 | 4.92E-01 | 58.68 | 7.99 | 8.05E-07 |
| Cyto F20.1 | 1.30 | 0.10 | 9.02E-03 | 30.2 | 2.9 | 3.51E-08 |
| Cyto F20.2 | 1.45 | 0.13 | 2.37E-03 | 33.33 | 1.81 | 1.29E-10 |
| Intra A14.1 | 1.12 | 0.13 | 1.67E-05 | 6.2 | 1.3 | 1.33E-03 |
| Intra A14.2 | 1.54 | 0.11 | 1.30E-14 | 151.25 | 11.91 | 3.92E-09 |
| Intra B14.1 | 1.10 | 0.50 | 2.90E-01 | 1.73 | 0.33 | 1.17E-02 |
| Intra B14.2 | 1.18 | 0.26 | 2.73E-03 | 1.75 | 0.51 | 1.42E-01 |
| Intra C14.1 | 1.16 | 0.43 | 7.50E-02 | 15.65 | 1.21 | 5.90E-09 |
| Intra C14.2 | 1.20 | 0.22 | 1.22E-03 | 13.33 | 1.22 | 3.44E-08 |
| Intra D14.1 | 1.16 | 0.27 | 7.89E-05 | 6.41 | 2.11 | 3.37E-03 |
| Intra D14.2 | 1.04 | 0.23 | 1.96E-01 | 10.23 | 0.91 | 3.32E-08 |
| Intra E14.1 | 1.14 | 0.37 | 1.52E-02 | 8.74 | 1.01 | 5.04E-07 |
| Intra E14.2 | 1.13 | 0.31 | 3.80E-02 | 5.87 | 0.7 | 7.10E-06 |
| Intra F14.1 | 1.14 | 0.12 | 1.76E-04 | 20.68 | 3.59 | 9.96E-06 |
| Intra F14.2 | 1.19 | 0.16 | 1.54E-04 | 18.03 | 1.6 | 2.07E-08 |
| Intra A24.1 | 1.75 | 0.24 | 4.08E-11 | 109.03 | 9.09 | 7.00E-09 |
| Intra A24.2 | 1.62 | 0.71 | 1.34E-06 | 66.38 | 2.58 | 4.13E-12 |
| Intra B24.1 | 1.79 | 0.32 | 1.88E-11 | 205.25 | 5.76 | 1.44E-13 |
| Intra B24.2 | 1.91 | 0.43 | 3.38E-07 | 78.73 | 16.23 | 3.14E-05 |
| Intra C24.1 | 1.26 | 0.25 | 2.73E-07 | 9.93 | 0.94 | 6.66E-08 |
| Intra C24.2 | 1.27 | 0.45 | 7.15E-04 | 25.5 | 1.65 | 8.19E-10 |
| Intra D24.1 | 1.78 | 0.27 | 1.48E-07 | 109 | 1.91 | 1.41E-15 |
| Intra D24.2 | 1.83 | 0.48 | 1.28E-05 | 717.75 | 80.16 | 3.67E-07 |
| Intra E24.1 | 1.27 | 0.61 | 1.57E-02 | 80.88 | 7.5 | 2.04E-08 |
| Intra E24.2 | 1.25 | 0.38 | 6.39E-03 | 47.7 | 4.03 | 8.99E-09 |
| Intra F24.1 | 1.20 | 0.21 | 1.03E-03 | 36.98 | 1.43 | 4.31E-12 |
| Intra F24.2 | 1.07 | 0.43 | 3.85E-01 | 5.83 | 1.2 | 1.23E-03 |

*2-tailed T-test

**Supplemental Table 2: Growth rate of selected isolates relative to parental strain**

| Strain | Fold Difference | SEM | p* |
|---|---|---|---|
| Cyto A10.1 | 0.88 | 0.01 | 4.32E-01 |
| Cyto A10.2 | 0.82 | 0.02 | 2.63E-01 |
| Cyto B10.1 | 0.89 | 0.00 | 4.86E-01 |
| Cyto B10.2 | 0.93 | 0.09 | 6.65E-01 |
| Cyto C10.1 | 0.92 | 0.05 | 6.20E-01 |
| Cyto C10.2 | 1.09 | 0.06 | 5.74E-01 |
| Cyto D10.1 | 1.02 | 0.04 | 9.15E-01 |
| Cyto D10.2 | 1.07 | 0.04 | 6.38E-01 |
| Cyto E10.1 | 1.09 | 0.03 | 5.82E-01 |
| Cyto E10.2 | 1.03 | 0.04 | 8.47E-01 |
| Cyto F10.1 | 1.21 | 0.05 | 2.42E-01 |
| Cyto F10.2 | 1.24 | 0.06 | 4.09E-01 |
| Cyto A20.1 | 0.87 | 0.07 | 4.21E-01 |
| Cyto A20.2 | 0.95 | 0.07 | 7.73E-01 |
| Cyto B20.1 | 1.00 | 0.03 | 9.95E-01 |
| Cyto B20.2 | 1.27 | 0.12 | 1.30E-01 |
| Cyto C20.1 | 1.09 | 0.03 | 5.71E-01 |
| Cyto C20.2 | 1.01 | 0.07 | 9.54E-01 |
| Cyto D20.1 | 1.35 | 0.01 | 3.83E-02 |
| Cyto D20.2 | 1.32 | 0.01 | 5.45E-02 |
| Cyto E20.1 | 1.37 | 0.03 | 3.18E-02 |
| Cyto E20.2 | 1.16 | 0.03 | 3.29E-01 |
| Cyto F20.1 | 1.20 | 0.04 | 2.13E-01 |
| Cyto F20.2 | 1.13 | 0.02 | 3.37E-01 |
| Intra A14.1 | 1.73 | 0.10 | 6.30E-04 |
| Intra A14.2 | 1.67 | 0.05 | 8.96E-04 |
| Intra B14.1 | 1.44 | 0.04 | 1.35E-02 |
| Intra B14.2 | 1.49 | 0.07 | 8.25E-03 |
| Intra C14.1 | 1.82 | 0.02 | 1.32E-04 |
| Intra C14.2 | 1.67 | 0.04 | 8.46E-04 |
| Intra D14.1 | 1.29 | 0.11 | 1.05E-01 |
| Intra D14.2 | 1.54 | 0.10 | 5.27E-03 |
| Intra E14.1 | 1.57 | 0.07 | 3.32E-03 |
| Intra E14.2 | 1.74 | 0.04 | 3.43E-04 |
| Intra F14.1 | 1.94 | 0.07 | 4.67E-05 |
| Intra F14.2 | 1.65 | 0.01 | 1.03E-03 |
| Intra A24.1 | 1.85 | 0.09 | 2.06E-05 |
| Intra A24.2 | 1.78 | 0.11 | 1.54E-05 |
| Intra B24.1 | 1.49 | 0.08 | 8.42E-03 |
| Intra B24.2 | 1.69 | 0.05 | 6.83E-04 |
| Intra C24.1 | 1.66 | 0.04 | 8.99E-04 |
| Intra C24.2 | 1.56 | 0.13 | 5.31E-03 |
| Intra D24.1 | 1.88 | 0.10 | 1.30E-04 |
| Intra D24.2 | 1.78 | 0.05 | 2.50E-04 |
| Intra E24.1 | 1.55 | 0.04 | 3.41E-03 |
| Intra E24.2 | 1.23 | 0.07 | 1.67E-01 |
| Intra F24.1 | 1.90 | 0.02 | 5.52E-05 |
| Intra F24.2 | 1.69 | 0.16 | 1.84E-03 |

*2-tailed T-test

| | agrA | | | sigB | | |
|---|---|---|---|---|---|---|
| Sample Name | Average RQ | SEM | p* | Average RQ | SEM | p* |
| Cyto A10.1 | 0.85 | 0.01 | 2.02E-02 | 1.20 | 0.02 | 1.24E-03 |
| Cyto A10.2 | 1.02 | 0.02 | 7.28E-01 | 1.39 | 0.03 | 4.27E-04 |
| Cyto B10.1 | 51.22 | 1.03 | 2.34E-06 | 0.73 | 0.01 | 1.71E-05 |
| Cyto B10.2 | 8.37 | 0.31 | 4.39E-05 | 1.32 | 0.02 | 1.27E-04 |
| Cyto C10.1 | 3.59 | 0.07 | 8.97E-06 | 2.26 | 0.03 | 6.82E-06 |
| Cyto C10.2 | 1.54 | 0.02 | 3.94E-04 | 2.01 | 0.02 | 1.58E-06 |
| Cyto D10.1 | 2.38 | 0.03 | 5.57E-03 | 1.28 | 0.01 | 5.67E-05 |
| Cyto D10.2 | 1.44 | 0.04 | 1.92E-01 | 1.78 | 0.05 | 2.13E-04 |
| Cyto E10.1 | 3.24 | 0.16 | 2.09E-03 | 1.80 | 0.06 | 5.70E-04 |
| Cyto E10.2 | 4.14 | 0.10 | 3.43E-04 | 2.24 | 0.14 | 2.15E-03 |
| Cyto F10.1 | 1.41 | 0.04 | 2.17E-01 | 1.82 | 0.03 | 2.83E-05 |
| Cyto F10.2 | 1.36 | 0.04 | 2.80E-01 | 2.09 | 0.03 | 7.35E-06 |
| Cyto A20.1 | 1.19 | 0.01 | 1.18E-03 | 2.09 | 0.08 | 6.06E-04 |
| Cyto A20.2 | 1.27 | 0.01 | 2.35E-04 | 2.10 | 0.02 | 7.67E-05 |
| Cyto B20.1 | 1.37 | 0.00 | 4.95E-05 | 1.66 | 0.02 | 6.01E-04 |
| Cyto B20.2 | 1.45 | 0.07 | 6.91E-03 | 1.39 | 0.16 | 1.32E-01 |
| Cyto C20.1 | 4.87 | 0.06 | 7.93E-07 | 1.13 | 0.01 | 9.52E-02 |
| Cyto C20.2 | 2.91 | 0.18 | 9.01E-04 | 2.47 | 0.03 | 3.46E-05 |
| Cyto D20.1 | 1.29 | 0.02 | 8.65E-04 | 2.17 | 0.12 | 1.45E-03 |
| Cyto D20.2 | 1.18 | 0.03 | 8.81E-03 | 1.85 | 0.03 | 3.52E-05 |
| Cyto E20.1 | 1.56 | 0.13 | 2.33E-02 | 0.87 | 0.01 | 8.86E-03 |
| Cyto E20.2 | 1.04 | 0.06 | 6.15E-01 | 1.45 | 0.02 | 2.16E-04 |
| Cyto F20.1 | 1.22 | 0.08 | 8.06E-02 | 1.37 | 0.02 | 4.31E-04 |
| Cyto F20.2 | 1.17 | 0.03 | 1.74E-02 | 1.28 | 0.02 | 1.03E-03 |
| Intra A14.1 | 0.60 | 0.01 | 1.08E-04 | 0.90 | 0.04 | 8.14E-02 |
| Intra A14.2 | 0.53 | 0.03 | 4.22E-04 | 0.96 | 0.03 | 3.19E-01 |
| Intra B14.1 | 0.98 | 0.06 | 7.93E-01 | 1.05 | 0.06 | 5.27E-01 |
| Intra B14.2 | 9.38 | 0.56 | 2.49E-04 | 0.79 | 0.03 | 3.00E-03 |
| Intra C14.1 | 0.47 | 0.03 | 2.14E-04 | 1.28 | 0.02 | 5.78E-04 |
| Intra C14.2 | 1.02 | 0.04 | 7.88E-01 | 0.87 | 0.01 | 7.27E-04 |
| Intra D14.1 | 0.76 | 0.06 | 6.26E-02 | 1.07 | 0.05 | 4.73E-01 |
| Intra D14.2 | 215.42 | 8.79 | 3.75E-05 | 1.74 | 0.04 | 5.52E-04 |
| Intra E14.1 | 1.39 | 0.07 | 1.97E-02 | 0.96 | 0.01 | 4.66E-01 |
| Intra E14.2 | 0.84 | 0.07 | 1.96E-01 | 1.23 | 0.13 | 2.61E-01 |
| Intra F14.1 | 0.60 | 0.01 | 3.17E-03 | 1.00 | 0.03 | 9.41E-01 |
| Intra F14.2 | 9.65 | 0.36 | 4.07E-05 | 0.51 | 0.01 | 1.17E-03 |
| Intra A24.1 | 1.27 | 0.02 | 4.03E-04 | 1.09 | 0.01 | 1.70E-01 |
| Intra A24.2 | 4.39 | 0.15 | 5.34E-05 | 1.10 | 0.02 | 1.72E-01 |
| Intra B24.1 | 4.16 | 0.02 | 1.05E-08 | 1.22 | 0.05 | 4.60E-02 |
| Intra B24.2 | 8.26 | 0.26 | 2.34E-05 | 1.09 | 0.02 | 1.74E-01 |
| Intra C24.1 | 1.49 | 0.07 | 3.76E-03 | 1.15 | 0.02 | 6.43E-02 |
| Intra C24.2 | 7.31 | 0.22 | 1.93E-05 | 1.01 | 0.02 | 9.52E-01 |
| Intra D24.1 | 4.35 | 0.14 | 5.06E-05 | 1.48 | 0.03 | 3.33E-04 |
| Intra D24.2 | 2.80 | 0.04 | 1.28E-05 | 1.17 | 0.04 | 2.76E-02 |
| Intra E24.1 | 110.13 | 7.95 | 3.61E-04 | 2.43 | 0.06 | 3.37E-05 |
| Intra E24.2 | 3.16 | 0.35 | 7.62E-03 | 1.22 | 0.03 | 5.74E-03 |
| Intra F24.1 | 13.23 | 0.16 | 3.73E-07 | 1.12 | 0.02 | 5.70E-03 |
| Intra F24.2 | 0.63 | 0.06 | 1.61E-02 | 0.86 | 0.03 | 1.33E-02 |

*2-tailed T-test

Supplemental Table 4: Coding variants identified by whole genome sequencing

*0 indicates strain matches the reference genome, 1 indicates the presence of the alternate allele

| Supplementary Table 5: Virulence of single gene mutant strains relative to parental strain. | | | | | | |
|---|---|---|---|---|---|---|
| | Cytotoxicity | | | Invasivness | | |
| Strain | Fold Difference | SEM | p* | Fold Difference | SEM | p |
| ATCC29213 ΔaroE | 1.01 | 0.01 | 4.38E-01 | 0.28 | 0.07 | 5.47E-07 |
| ATCC29213 ΔbrnQ2 | 1.04 | 0.01 | 1.13E-03 | 0.17 | 0.05 | 1.65E-08 |
| ATCC29213 ΔctaB | 1.03 | 0.02 | 1.11E-01 | 11.44 | 0.95 | 2.82E-13 |
| ATCC29213 ΔmenF | 0.99 | 0.02 | 8.36E-01 | 1.00 | 0.14 | 9.56E-01 |
| ATCC29213 mnhA Ala722Thr | 1.01 | 0.02 | 4.98E-01 | 0.37 | 0.10 | 1.38E-05 |
| ATCC29213 ΔmurA | 1.07 | 0.02 | 1.00E-03 | 0.03 | 0.01 | 1.89E-10 |
| ATCC29213 ΔnupC | 1.02 | 0.01 | 9.94E-02 | 0.12 | 0.04 | 2.48E-09 |
| ATCC29213 ΔpurR | 1.23 | 0.05 | 8.75E-05 | 8.81 | 0.36 | 1.46E-18 |
| ATCC29213 ΔqoxD | 1.11 | 0.02 | 1.89E-06 | 24.23 | 4.69 | 8.76E-06 |
| ATCC29213 ΔsaeS | 1.00 | 0.01 | 9.94E-01 | 0.04 | 0.00 | 2.28E-10 |
| ATCC29213 Δ srrA | 1.00 | 0.01 | 7.33E-01 | 0.07 | 0.01 | 4.24E-10 |
| ATCC29213 tufA Ala271Val | 1.07 | 0.03 | 9.90E-02 | 0.05 | 0.01 | 3.07E-10 |
| ATCC29213 ΔubiE | 0.96 | 0.02 | 6.15E-02 | 2.59 | 0.42 | 1.46E-04 |
| ATCC29213 ΔunnamedA | 1.04 | 0.02 | 2.38E-02 | 0.09 | 0.01 | 6.25E-10 |
| ATCC29213 ΔunnamedB | 1.07 | 0.02 | 2.46E-04 | 5.24 | 0.76 | 3.60E-06 |
| ATCC29213 ΔyhcF | 1.00 | 0.01 | 8.27E-01 | 0.43 | 0.03 | 2.24E-06 |
| ATCC29213 ΔypfH | 1.02 | 0.02 | 2.83E-01 | 0.18 | 0.04 | 1.02E-08 |
| JE2 ebH ::bursa aurealis | 1.07 | 0.01 | 3.93E-04 | 2.02 | 0.39 | 3.53E-02 |
| JE2 ndhC ::bursa aurealis | 1.06 | 0.02 | 8.29E-03 | 16.60 | 4.21 | 3.78E-03 |
| JE2 menD ::bursa aurealis | 1.05 | 0.01 | 7.90E-03 | 1.83 | 0.53 | 1.39E-01 |
| JE2 sdrC ::bursa aurealis | 1.08 | 0.01 | 2.30E-05 | 7.94 | 0.47 | 1.09E-08 |

*2-tailed T-test

| Supplemental Table 6: Virulence of complemented gene deletion strains | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cytotoxicity | | | Invasiveness | | |
| Strain Background | Complemented Mutation | Fold Difference | SEM | p* | Fold Difference | SEM | p* |
| ATCC29213 ΔaroE | *aroE* Thr182Ile | 0.99 | 0.01 | 5.08E-01 | 4.20 | 0.74 | 3.59E-02 |
| ATCC29213 ΔsaeS | *saeS* Ala106Thr | 0.99 | 0.03 | 7.57E-01 | 2.33 | 0.09 | 5.85E-04 |
| ATCC29213 ΔbrnQ2 | *brnQ2* Asp198Tyr | 1.02 | 0.02 | 3.93E-01 | 1.55 | 0.47 | 9.14E-02 |
| ATCC29213 ΔnupC | *nupC* Pro189Leu | 0.98 | 0.04 | 5.91E-01 | 1.32 | 0.24 | 2.73E-01 |
| ATCC29213 ΔunnamedA | *unnamedA* Asp486Glu | 1.05 | 0.05 | 8.20E-01 | 2.87 | 0.49 | 3.70E-02 |
| ATCC29213 ΔyhcF | *yhcF* Thr74Ile | 0.96 | 0.02 | 1.16E-01 | 8.81 | 1.57 | 7.45E-04 |
| ATCC29213 ΔctaB | *ctaB* Thr222Ile | 1.05 | 0.04 | 6.36E-01 | 6.15 | 1.16 | 1.00E-03 |

*2-tailed paired t-test comparing mutant complementation vector to wild type complementation vector

| | Cytotoxicity | | | | Invasiveness | | | |
|---|---|---|---|---|---|---|---|---|
| **Supplementary Table 7: Virulence activity of epistatic mutations** | | | | | | | | |
| Strain | Fold difference relative to ATCC29213 | SEM | Signfiicance relative to ATCC29213 | Significance relative to double mutant* | Fold difference relative to ATCC29213 | SEM | Signfiicance relative to ATCC29213 | Significance relative to double mutant* |
| ATCC29213 *tufA* Ala271Val | 1.23 | 0.05 | 8.67E-02 | 5.83E-02 | 0.07 | 0.01 | 7.50E-07 | 6.62E-06 |
| ATCC29213 Δ*purR* | 1.07 | 0.03 | 2.19E-01 | 1.15E-01 | 8.24 | 1.87 | 3.70E-02 | 6.73E-03 |
| ATCC29213 *tufA* Ala271Val Δ*purR* | 0.97 | 0.03 | 4.23E-01 | N/A | 18.32 | 2.63 | 5.85E-04 | N/A |

* 2-tailed T-test

**Supplementary Table 8. Summary of oligonucleotides used**

| Oligonucleotide Name | Oligonucleotide Sequence | Purpose / Notes |
|---|---|---|
| | **PCR and Cloning Oligonucleotides** | |
| TT_F | AACAGTGGCGCCTGTCACTTTGCTTGAT | Use to amplify TT element from pCN50. |
| TT_R | TTATCTGAATTCAGGCGCGCCTATTCTAAATG | Use to amplify TT element from pCN50. |
| aroE_expression_F_sbfI | GTTTTT CCTGCAGG CCGCTTGAAAAATTATAACGAAGTT | Use to amplify aroE for cloning into complementation vector |
| aroE_expression_R_xmaI | GTTTTT CCCGGG ATCGGATCAATATTGTGTGCTAAAC | Use to amplify aroE for cloning into complementation vector |
| Brnq2_expression_F_sbfI | GTTTTT CCTGCAGG TGAAATTGTGAATAAAAGTCATCAGT | Use to ampify BrnQ2 for cloning into complementation vector |
| Brnq2_expression_R_xmaI | GTTTTT CCCGGG TCAAAGGACCGAATAAACAATCATA | Use to ampify BrnQ2 for cloning into complementation vector |
| nupC_expression_F_sbfI | GTTTTT CCTGCAGG ATATGGCCTTGAAAGTGTCTATGAT | Use to ampify nupC for cloning into complementation vector |
| nupC_expression_R_xmaI | GTTTTT CCCGGG GTCCAAGCATCTCACCTATATCAAT | Use to ampify nupC for cloning into complementation vector |
| unnamedA_expression_F_sbfI | GTTTTT CCTGCAGG ATAGCATGCAAATCAACACAAAGTA | Use to ampify unnamedA for cloning into complementation vector |
| unnamedA_expression_R_xmaI | GTTTTT CCCGGG TTCCTCCTTTTCTATCCGTAAAACT | Use to ampify unnamedA for cloning into complementation vector |
| YhcF_expression_F_sbfI | GTTTTT CCTGCAGG TACCAAAGATAAAGTGTTGTTGGTG | Use to ampify yhcF for cloning into complementation vector |
| YhcF_expression_R_xmaI | GTTTTT CCCGGG ATCCTTGAGGAACTTTGAAAGAGAT | Use to ampify yhcF for cloning into complementation vector |
| ctaB_expression_f_sbfI | GTTTTTCCTGCAGGTTTGTTTTTTGGATAATTTTTAACAGCGTGA | Use to ampify ctaB for cloning into complementation vector |
| ctaB_expression_r_xmaI | GTTTTTCCCGGGATTGCACTAATGACAATACATGTCG | Use to ampify ctaB for cloning into complementation vector |
| sae_operon_F_sbfI | GTTTTT CCTGCAGG GTTGTTTTTATTGTGGCAAAAGGTTTAT | Use to amplify sae operon for cloning into complementation vector |
| sae_operon_R_xmaI | GTTTTT CCCGGG GCTATTTTATTATTAGGCGGCATACAG | Use to amplify sae operon for cloning into complementation vector |
| BrnQ2_amp_FV2 | TGTAGTGTTATTCCACGAGACGTTA | Use to amplify brnQ2 lcous for confirmation of successful recombineering |
| BrnQ2_amp_RV2 | CTTGATACATTGAAACGTACACAGC | Use to amplify brnQ2 lcous for confirmation of successful recombineering |
| srrA_amp_FV2 | TGATAGAGGACGTACAGTGGTTACA | Use to amplify srrA lcous for confirmation of successful recombineering |
| srrA_amp_RV2 | CCAGGATTTTCAATTAATGTTTGAC | Use to amplify srrA lcous for confirmation of successful recombineering |
| ctaB_amp_F | TGTGTTTATTTATTTCCCTTTTCCA | Use to amplify ctaB lcous for confirmation of successful recombineering |
| ctaB_amp_R | CTTTTTGCCAAACAATTTATACACC | Use to amplify ctaB lcous for confirmation of successful recombineering |
| MenF_amp_F | GTAAAAACTTGCTTCGTTAAAGCTG | Use to amplify menF lcous for confirmation of successful recombineering |
| MenF_amp_R | GACGTGAAAAATTGCAAGATAAGTT | Use to amplify menF lcous for confirmation of successful recombineering |
| MnhA_amp_F | AATTTGCCCTAAAATTGGAAGTATC | Use to amplify mnhA lcous for confirmation of successful recombineering |
| MnhA_amp_R | AATGAAGACGTTCTTTTTCAATCAC | Use to amplify mnhA lcous for confirmation of successful recombineering |
| MurA1_amp_F | TTACAATACTCGACGCAGGTAAAAT | Use to amplify murA lcous for confirmation of successful recombineering |
| MurA1_amp_R | AGCTTTAGCATTACCTTTACCGATT | Use to amplify murA lcous for confirmation of successful recombineering |
| TufA_amp_F | TTTTACAATGTCCAAGACTCAACAA | Use to amplify tufA lcous for confirmation of successful recombineering |
| TufA_amp_R | ATCACTATGCTGAAGTTCCAAAATC | Use to amplify tufA lcous for confirmation of successful recombineering |
| UbiE_amp_F | ACATTCCATTTTGATTGGAGATTTA | Use to amplify ubiE lcous for confirmation of successful recombineering |
| UbiE_amp_R | TGTTGACTGTTAAATTGGTCTTGAA | Use to amplify ubiE lcous for confirmation of successful recombineering |
| unnamedA_amp_F | ATATGGCGATAAATCTGTGATGAAT | Use to amplify unnamedA lcous for confirmation of successful recombineering |
| unnamedA_amp_R | TGGAACAAGCCTAGAGTTAGAAGAA | Use to amplify unnamedA lcous for confirmation of successful recombineering |
| unnamedB_amp_F | TTATTTGGTCAAGATAGCTCGATTC | Use to amplify unnamedB lcous for confirmation of successful recombineering |
| unnamedB_amp_R | AGAATCACAAGATGTTGTCGTGTTA | Use to amplify unnamedB lcous for confirmation of successful recombineering |
| agrA-F | TGA TAA TCC TTA TGA GGT GCT T | Real time PCR primer for agrA |
| agrA-R | CAC TGT GAC TCG TAA CGA AAA | Real time PCR primer for agrA |
| sigB-F | TGT CGT GAG ATG TTG GG | Real time PCR primer for sigB |
| sigB-R | CGA TTC CAG CTT CAT GT | Real time PCR primer for sigB |
| 16SrRNA-F | CTGGAACTGAGACACGGTCC | Real time PCR primer for 16sRRNA |
| 16SrRNA-R | GACCTTCATCACTCACGCGG | Real time PCR primer for 16sRRNA |
| | **Recombineering Oligos*** | |
| BrnQ2_deletion_oligo | T*T*A*T*TCCTGTTGATATTTAATTGGATCTTGTTTTACAAATATGCCCATA GCGAAGAGCGTAAACCCAATGACCCATGTATTTTTATTCAT | Use to engineer brnQ2 deletion in S. aureus |
| srrA_deletion_oligo | C*T*A*T*TTAGCCGGCTCATCATTAGATTTAACCTCAAATTTATACCCTCTG ATTCTATCCTCATCATCTACGATAAGTATTTCGTTCGACAT | Use to engineer srrA deletion in S. aureus |
| ctaB_deletion_oligo | C*T*A*G*ATCAAAGTAAGTAATGAAACAATTACAGCTAACACGAAAAAGTTA ACTCTGCTAATATTTTGTGACAAAGTATGCTCTTTGCTCAT | Use to engineer ctaB deletion in S. aureus |
| MenF_deletion_oligo | T*T*A*T*GATTTCCCATTCATATCGACTCCTAATGCATTCATCATAGGATAT ATCTCTTTGACAATATCGTCCTCTAATACGCCCGTAGCCAT | Use to engineer menF deletion in S. aureus |
| unnamedA_deletion_oligo | C*T*A*T*CCGTAAAACTGGTAATAATTTTTAATAAGTTCGTACATAATTACAG GTGCGCCATTACCTTTAGGTGGTTTAATAGTTATTGTCAT | Use to engineer unnamedA deletion in S. aureus |
| mnhA_Ala722Thr_repair_up | C*A*A*A*AAAAGAGAAATAATTAAAATTTCTGTATCACTCTTGATGaCtCTaAT gGTTGTATCATTAATTTTTATTACACAACAAACAGATGG | Use to engineer mnhA Ala722Thr mutation in S. aureus. Lower case indicates mismatches. |
| tufa_Ala271Val_repair_top | G*T*T*G*AAATGTTCCGTAAATTATTAGACTACGCTGAAGtaGGaGAtAAtATa GcaGCATTATTACGTGGTGTTGCTCGTGAAGACGTACAA | Use to engineer tufA Ala271Val mutation in S. aureus. Lower case indicates mismatches. |
| unnamedB_deletion_oligo | T*T*A*G*AATAATACGATGGTTAAGATGAAATATATGACAAAGATAAAACAA TTAGATAAGATATTGCTAGTAAAATCAGATAAAATTGCAT | Use to engineer unnamedB deletion in S. aureus |
| MurA1_deletion_oligo | T*T*A*A*TCGTTAATACGTTCAATGTCTGCACCTAATTGCTTCAATTTTTCA CCCGTTAATTTATTTCCACCTTTGATTACTATTTTATCCAT | Use to engineer murA deletion in S. aureus |
| UbiE_deletion_oligo | T*T*A*A*TCACCTTTGGTATTATCTTTTTCTTTATAGCCAAGGTGCATAAAA ACGCGATGTACTTGCTCTTTATTTGCTTTATTGTCAGCCAT | Use to engineer ubiE deletion in S. aureus |
| | **Counterselection Oligonucleotides** | |
| ctaB_v2_sgRNA_down | AAAAACACTAACATCATACCAAAACCG | Targets to PAM internal to ctaB deletion |
| ctaB_v2_sgRNA_up | AGCTCGGTTTTGGTATGATGTTAGTG | Targets to PAM internal to ctaB deletion |
| MenF_sgRNA_down | AAAAACCAATCTGAGAAACATCATCCG | Targets to PAM internal to menF deletion |
| MenF_sgRNA_up | AGCTCGGATGATGTTTCTCAGATTGG | Targets to PAM internal to menF deletion |
| unnamedA_v2_sgRNA_down | AAAACCTCTCGTTCGGATGCAATCCG | Targets to PAM internal to unnamedA deletion |
| unnamedA_v2_sgRNA_up | AGCTCGGATTGCATCCGAACGAGAGG | Targets to PAM internal to unnamedA deletion |
| MnhA_sgRNA_down | AAAAACTTGGCACCACATGTTTCGCCG | Targets to PAM adjacent to engineered missense mutations in mnhA deletion |
| MnhA_sgRNA_up | AGCTCGGCGAAACATGTGGTGCCAAG | Targets to PAM adjacent to engineered missense mutations in mnhA deletion |
| TufA_sgRNA_down | AAAAACATGATTTCAACTTCTTCACCG | Targets to PAM adjacent to engineered missense mutations in tufA deletion |
| TufA_sgRNA_up | AGCTCGGTGAAGAAGTTGAAATCATG | Targets to PAM adjacent to engineered missense mutations in tufA deletion |
| unnamedB_sgRNA_down | AAAACGTCACTTCGACATTGCCGCCG | Targets to PAM internal to unnamedB deletion |
| unnamedB_sgRNA_up | AGCTCGGCGGCAATGTCGAAGTGACG | Targets to PAM internal to unnamedB deletion |
| MurA1_sgRNA_down | AAAAACCCAGGCAATGCAACAATACCG | Targets to PAM internal to murA deletion |
| MurA1_sgRNA_up | AGCTCGGTATTGTTGCATTGCCTGGG | Targets to PAM internal to murA deletion |
| srrA_sgRNA_down | AAAAACCCTGGATACCATCCATTTCCG | Targets to PAM internal to srrA deletion |
| srrA_sgRNA_up | AGCTCGGAAATGGATGGTATCCAGGG | Targets to PAM internal to srrA deletion |
| UbiE_sgRNA_down | AAAACCCTGTAAAACTACGTACTCCG | Targets to PAM internal to ubiE deletion |
| UbiE_sgRNA_up | AGCTCGGAGTACGTAGTTTTACAGGG | Targets to PAM internal to ubiE deletion |
| BrnQ2_sgRNA_down | AAAAACAATAATGGTAAACCAATCCCG | Targets to PAM internal to brnQ2 deletion |
| BrnQ2_sgRNA_up | AGCTCGGGATTGGTTTACCATTATTG | Targets to PAM internal to brnQ2 deletion |

*Asterisk (*) indicates phosphothioate bond