**Supplementary material for:**

The ClpCP complex modulates respiratory metabolism in *Staphylococcus aureus* and is regulated in a SrrAB-dependent manner.

**Authors**

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**Figure S1**

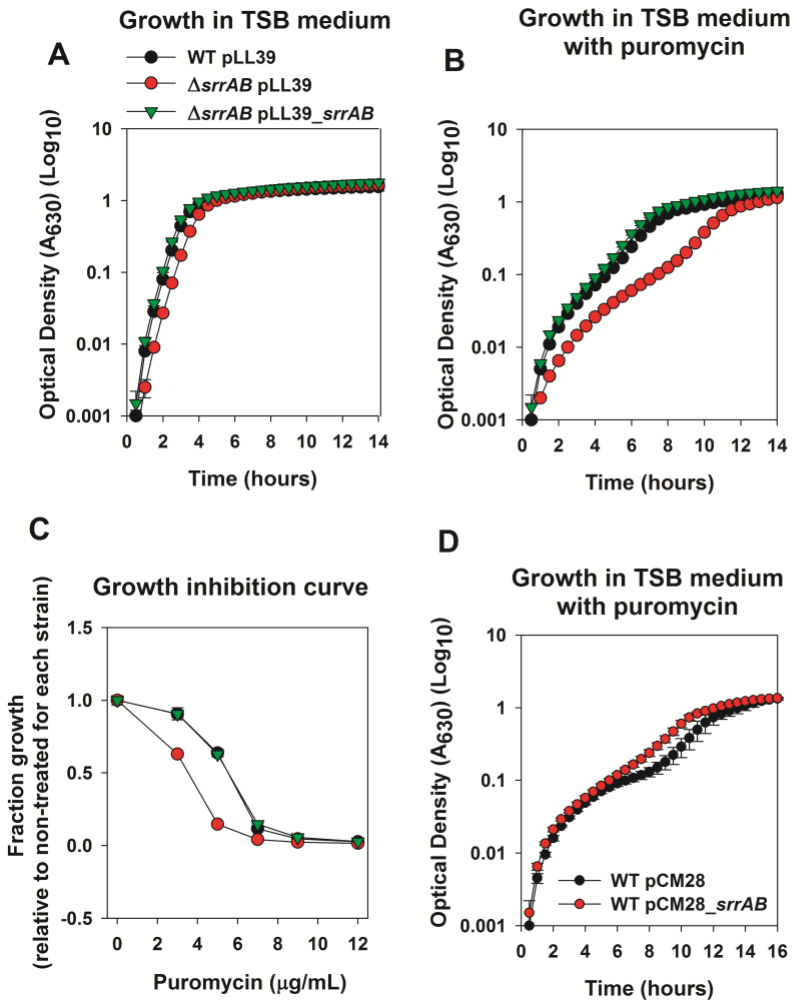


**Figure S1. A Δ*srrAB* strain has increased sensitivity to puromycin.** Panel A and B; A Δ*srrAB* strain is deficient in growth upon co-culture with puromycin. Growth profiles are presented for the WT (JMB1100) with the pLL39 episome (empty vector) and the Δ*srrAB* strain (JMB1467) with pLL39 or pLL39_*srrAB* in tryptic soy broth (TSB) medium in the absence (Panel A) or presence (Panel B) of puromycin. Panel C; The growth of a Δ*srrAB* strain is inhibited at lower concentrations of puromycin than the WT. The WT with pLL39 or the Δ*srrAB* strain with pLL39 or pLL39_*srrAB* was diluted into TSB containing varying amounts of puromycin. The strains were cultured for 5 hours and optical

densities were recorded. Data are presented as fraction growth achieved relative to the non-treated control for each strain. Panel D; Increased dosage of *srrAB* improves the puromycin resistance of the WT strain. Growth profiles are presented for the WT carrying the multicopy vector pCM28 or pCM28_*srrAB* and diluted into TSB in the presence of puromycin. Data in Panels A-D represent the average of duplicate cultures and error bars represent standard deviations. Error bars are shown in all figures but may not be visible where error is low. Data in panel E are representative of at least three independent experiments.

**Figure S2**

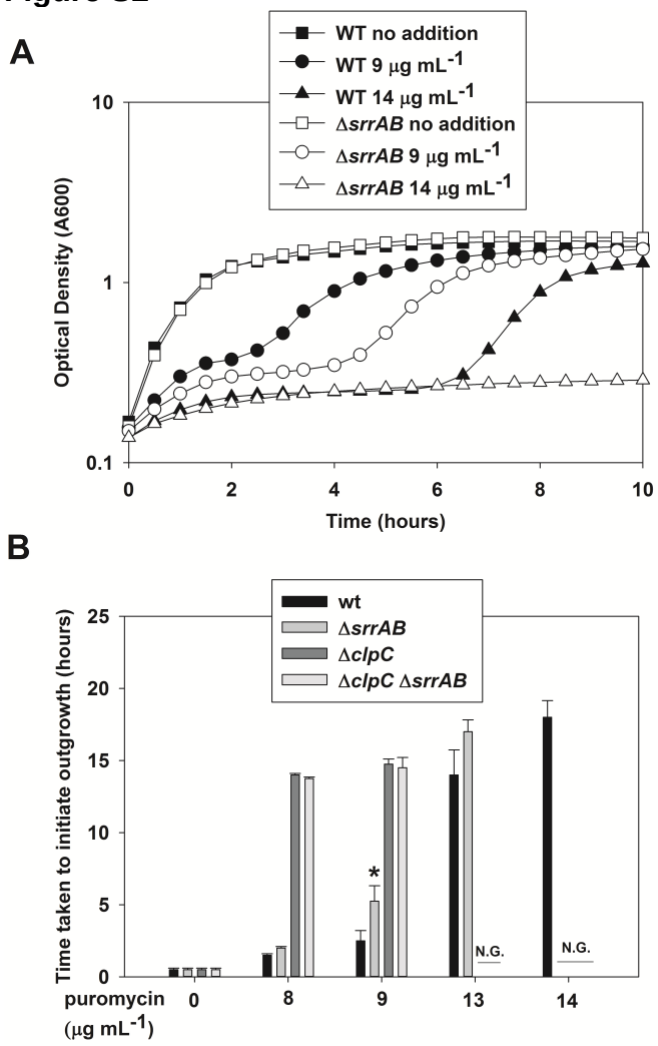


**Figure S2. A The puromycin sensitivity phenotypes associated with the Δ*srrAB* and Δ*clpC* mutation are not additive.** Panel A; Representative growth profiles are displayed for the WT (JMB 1100) and Δ*srrAB* (JMB 1467) strains in TSB medium in the presence or absence of various concentrations of puromycin. Panel B; The time taken for strains to initiate outgrowth is plotted vs. the concentration of puromycin present in the TSB medium. The data in Panel B represents the average of triplicate cultures and error bars represent standard deviations. Students t-tests were conducted against the WT and * represents $p < 0.05$.

**Figure S3.**

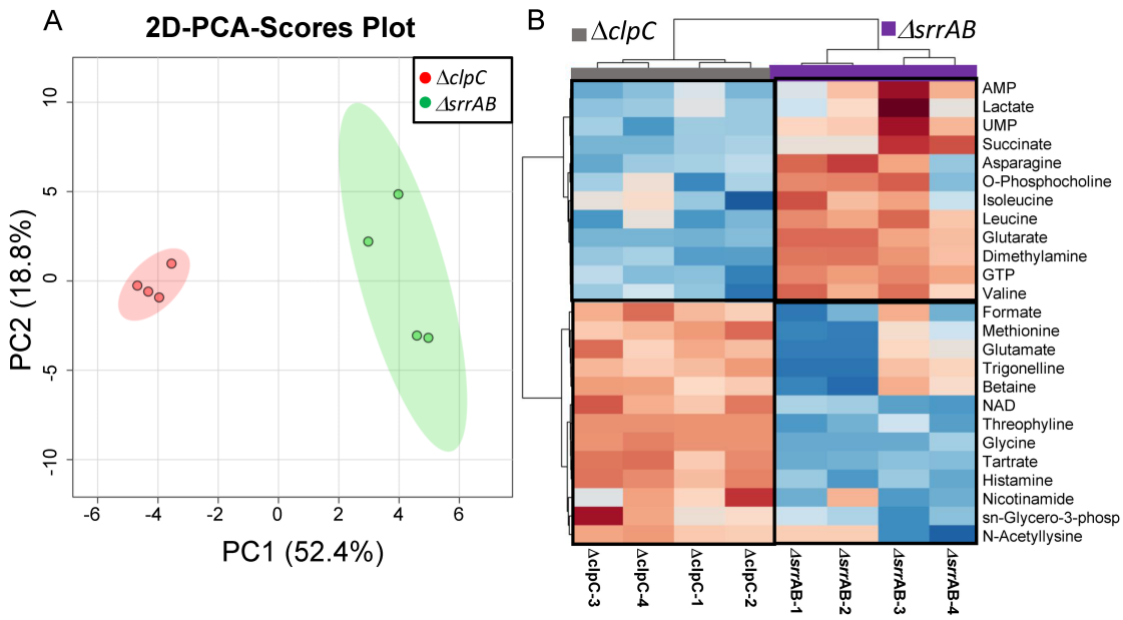


**Figure S3. 2D PCA scores plot and corresponding hierarchical clustering analysis (HCA) of the metabolic profiles of Δ*clpC* and Δ*srrAB*, following aerobic growth for 48 hours.** Panel A: 2D-PCA scores plot separating the metabolic profiles of Δ*clpC* (JMB 8025) and Δ*srrAB* (JMB 1467), with PC1 and PC 2 accounting for 52.4% and 18.8% of the variance, respectively. Panel B: Heatmap visualization of the top 25 metabolites contributing the most to the separation of the Δ*clpC* and Δ*srrAB* strains based of their distinct metabolite profiles, and indicating elevated levels of GTP, AMP, and lactate in Δ*srrAB* compared to Δ*clpC*. Levels of AMP, UMP, succinate, and citrulline are also increased in the Δ*srrAB* strain, indicating that the Δ*clpC* and Δ*srrAB* mutations are not affecting central metabolism in exactly the same way. Boxed regions indicate regions of higher (red) or lower (blue) metabolite levels between groups, with relative scale ranging from +2 (red) to -2 (blue); The heatmap also reveals

that, within these top 25 metabolites whose levels are significantly altered between the two strains, a comparable distribution of increased or decreased metabolite levels is observed between Δ*clpC* and Δ*srrAB.*

**Figure S4.**

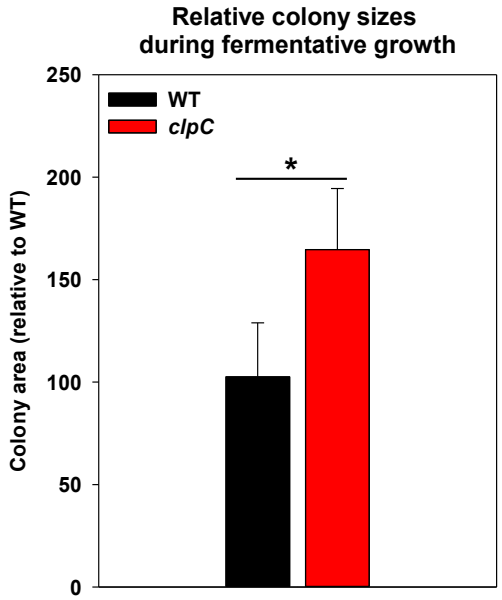


**Figure S4. Quantification of colony size of the WT and Δ*clpC* strains after fermentative growth.** Relative colony sizes of the WT (JMB1100) and Δ*clpC* (JMB 8025) strains were determined using the particle size tool in the ImageJ software. Sizes for at least ten colonies for each strain were determined.

**Supplemental Table 1. Metabolite levels for the WT, Δ*srrAB*, Δ*clpC*, and Δ*srrAB* Δ*clpC* strains***

| | WT | Δ*clpC* | | Δ*srrAB* | | Δ*srrAB* Δ*clpC* | |
|---|---|---|---|---|---|---|---|
| Metabolite | fc | fc | p.value | fc | p.value | fc | p.value |
| Acetate | 1 | 1.6 ± 0.29 | 1.7E-03 | 1.4 ± 0.30 | 3.4E-02 | 1.7 ± 0.40 | 1.33E-03 |
| ADP | 1 | 1.9 ± 0.22 | 8.7E-05 | 1.8 ± 0.26 | 4.8E-04 | 1.6 ± 0.38 | 2.09E-03 |
| AMP | 1 | 1.0 ± 0.05 | 4.6E-01 | 1.2 ± 0.18 | 1.7E-02 | 0.8 ± 0.20 | 6.16E-02 |
| Asparagine | 1 | 1.2 ± 0.05 | 2.1E-04 | 1.5 ± 0.25 | 2.5E-03 | 1.3 ± 0.21 | 9.96E-03 |
| Aspartate | 1 | 0.9 ± 0.12 | 6.0E-01 | 1.1 ± 0.65 | 5.6E-01 | 1.3 ± 0.76 | 2.66E-01 |
| Betaine | 1 | 1.4 ± 0.04 | 1.4E-03 | 1.0 ± 0.30 | 7.8E-01 | 1.1 ± 0.14 | 5.57E-01 |
| Citrulline | 1 | 1.3 ± 0.33 | 1.4E-01 | 1.4 ± 0.30 | 1.4E-02 | 0.7 ± 0.14 | 3.00E-03 |
| Dimethylamine | 1 | 0.7 ± 0.14 | 8.9E-02 | 1.6 ± 0.81 | 3.4E-02 | 0.6 ± 0.40 | 8.57E-02 |
| Formate | 1 | 1.4 ± 0.10 | 4.6E-05 | 1.2 ± 0.19 | 3.5E-02 | 1.2 ± 0.09 | 4.44E-03 |
| Glutamate | 1 | 1.1 ± 0.13 | 2.2E-01 | 0.7 ± 0.23 | 6.2E-02 | 0.5 ± 0.05 | 9.55E-06 |
| Glutarate | 1 | 0.5 ± 0.25 | 4.8E-02 | 2.4 ± 1.99 | 1.5E-02 | 0.9 ± 0.59 | 7.39E-01 |
| Glycine | 1 | 2.5 ± 0.58 | 4.9E-04 | 1.0 ± 0.40 | 9.9E-01 | 2.0 ± 0.85 | 3.98E-03 |
| GTP | 1 | 0.8 ± 0.14 | 3.1E-02 | 1.4 ± 0.12 | 2.8E-05 | 0.5 ± 0.03 | 3.62E-06 |
| Histamine | 1 | 1.8 ± 0.17 | 1.0E-04 | 0.8 ± 0.10 | 5.6E-03 | 1.6 ± 0.23 | 2.03E-03 |
| Histidine | 1 | 1.0 ± 0.25 | 9.6E-01 | 1.2 ± 0.07 | 1.3E-01 | 1.4 ± 0.14 | 3.53E-03 |
| Isoleucine | 1 | 1.1 ± 0.14 | 2.9E-01 | 1.3 ± 0.10 | 8.8E-03 | 1.1 ± 0.12 | 6.39E-01 |
| Lactate | 1 | 1.3 ± 0.06 | 7.3E-04 | 1.5 ± 0.27 | 1.6E-03 | 1.2 ± 0.29 | 2.05E-01 |
| Leucine | 1 | 1.0 ± 0.14 | 7.3E-01 | 1.4 ± 0.18 | 2.1E-03 | 1.1 ± 0.25 | 5.20E-01 |
| Lysine | 1 | 1.2 ± 0.08 | 1.5E-02 | 1.3 ± 0.12 | 3.9E-03 | 1.2 ± 0.14 | 5.25E-03 |
| Methionine | 1 | 1.9 ± 0.18 | 6.5E-06 | 1.4 ± 0.21 | 1.1E-02 | 2.0 ± 0.43 | 2.69E-03 |
| NAD | 1 | 1.9 ± 0.07 | 2.1E-05 | 1.3 ± 0.17 | 1.4E-03 | 1.9 ± 0.20 | 4.22E-04 |
| NADP | 1 | 1.2 ± 0.26 | 3.4E-01 | 1.1 ± 0.27 | 4.9E-01 | 1.0 ± 0.22 | 8.50E-01 |
| N-alpha-Acetyllysine | 1 | 1.7 ± 0.62 | 3.6E-02 | 1.2 ± 1.04 | 5.8E-01 | 1.4 ± 1.11 | 1.91E-01 |
| Niacinamide | 1 | 1.7 ± 0.30 | 1.5E-03 | 1.2 ± 0.28 | 1.2E-01 | 1.6 ± 0.47 | 2.00E-02 |
| Nicotinamide N-oxide | 1 | 1.2 ± 0.09 | 1.7E-01 | 1.6 ± 0.7 | 2.9E-02 | 1.2 ± 0.33 | 1.96E-01 |
| O-Phosphocholine | 1 | 1.2 ± 0.28 | 1.7E-01 | 1.5 ± 0.49 | 3.0E-02 | 1.6 ± 0.49 | 1.11E-02 |
| Phenylalanine | 1 | 1.3 ± 0.06 | 6.4E-03 | 1.3 ± 0.19 | 6.3E-03 | 1.3 ± 0.23 | 5.21E-03 |
| sn-Glycero-3-phosphocholine | 1 | 1.6 ± 0.13 | 1.1E-03 | 1.3 ± 0.19 | 7.2E-03 | 1.1 ± 0.17 | 9.34E-02 |
| Succinate | 1 | 0.9 ± 0.03 | 2.6E-01 | 2.1 ± 0.95 | 1.7E-02 | 1.2 ± 0.45 | 3.32E-01 |
| Tartrate | 1 | 1.1 ± 0.01 | 4.8E-11 | 1.1 ± 0.01 | 6.8E-13 | 1.0 ± 0.01 | 7.74E-08 |
| Theophylline | 1 | 7.5 ± 0.72 | 2.5E-06 | 1.4 ± 0.62 | 3.5E-01 | 7.0 ± 1.85 | 3.16E-06 |
| Threonine | 1 | 1.3 ± 0.07 | 3.9E-03 | 1.5 ± 0.29 | 2.7E-03 | 1.2 ± 0.29 | 1.74E-01 |
| Trigonelline | 1 | 1.9 ± 0.69 | 1.5E-02 | 1.1 ± 1.26 | 8.9E-01 | 1.5 ± 1.07 | 1.09E-01 |
| Trimethylamine N-oxide | 1 | 1.2 ± 0.08 | 5.6E-03 | 0.9 ± 0.43 | 4.6E-01 | 0.9 ± 0.19 | 3.24E-01 |
| Tryptophan | 1 | 1.3 ± 0.11 | 9.1E-03 | 1.2 ± 0.15 | 4.5E-02 | 1.3 ± 0.16 | 2.32E-03 |
| Tyrosine | 1 | 1.4 ± 0.06 | 5.9E-06 | 1.2 ± 0.18 | 3.6E-02 | 1.3 ± 0.19 | 2.27E-02 |
| UMP | 1 | 1.1 ± 0.05 | 1.1E-01 | 1.5 ± 0.29 | 2.7E-03 | 0.9 ± 0.22 | 1.54E-01 |
| Valine | 1 | 0.9 ± 0.05 | 2.0E-01 | 1.2 ± 0.12 | 4.3E-03 | 1.0 ± 0.16 | 5.02E-01 |

## Supplementary Table 2: Oligonucleotides used in this study.

| **RT- PCR primers** | |
|---|---|
| *clpC* For | CGTCGTTTCCAACCTGTACAAG |
| *clpC*Rev | TGGTGTGCTTCGTAACGATCTC |
| *spa*For | GATGGTAACGGAGTACATGTCGTT |
| *spa* Rev | GTGCCGTTTGCTTTTGCAAT |
| *cydB* For | GCCTTGGATTGTTCGTGGTT |
| *cydB*Rev | CCGCCTGCTTGTGTTGCT |

| **Cloning primers** | |
|---|---|
| pCM28_IsceI_yeast For | AAAAACCTACAGAAGCTTGCATGCCTGCAAGTTACGCTAGGGATAACAGGGTAATATAG |
| srrB_pCM28 reverse | TGATTACGAATTCATGATCGAATGCTAGCGGATCTTTTATTCTGGTTTTGGTAGTTTA |
| spa (0113) Pro For HindIII | CCCAAGCTTTGTAGAATTCACAATTCTAGCTATTATCACTTCTCAAAAT |
| spa(0113) Pro Rev Kpn1 | CCCGGTACCAATAAATGTTTT TCT TTT TCA AAT TAA TACCCCCTGTATG |
| clpC hindIII | CCCAAGCTTATTATATTATTGATGGGCTTTTAGATAAAATG |
| clpC kpnI | CCCGGTACCGTGGTGTCTTTCCAACGTGCTC |