#### **Supplementary Information**

## Bayesian Modeling and Intrabacterial Drug Metabolism Applied to Drug-resistant Staphylococcus

#### aureus

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**Figure S1. Comparative model external ROC curves.** Each of the three models (MRSA\_1a, MRSA\_1b, and Broad\_MSSA) were assessed with an external test set composed of 374 in-house compounds. Both MRSA\_1a and MRSA\_1b models, having similar true-positive rates for the first (i.e., highest scoring) 15% of compounds, perform significantly better than the Broad\_MSSA model.



Figure S2. Demonstration of the bactericidal activity of JSF-3151. A) determination of its minimum bactericidal concentration versus MRSA and MSSA strains and B) time-kill kinetics versus MRSA ATCC 43300. In each plot, the error bars were set as  $\pm$  the standard error based on three independent experiments. The dashed line in B) corresponds to the lower level limit of detection.



**Figure S3.** *In vivo* profiling of JSF-3151 in mice. A) Pharmacokinetic profile of JSF-3151 whereas two female CD-1 mice received a single dose of compound administered orally at 25 mg/kg in 20% DMA/80% PEG300. B) Groups of 12 female outbred Swiss Webster mice were infected with 1.0 x 10<sup>8</sup> CFU of a MRSA COL strain and then administered single doses of vehicle (10% DMSO in sterile saline), JSF-3151 (24 mg/kg) or vancomycin (100 mg/kg) 1 h post-infection. Vehicle and JSF-3151 were administered via intraperitoneal injection while vancomycin was administered by subcutaneous injection.

A)



Figure S4. IBDM studies of JSF-3151 and its metabolite JSF-3640. A) LC-MS data supporting the identification of amine metabolite JSF-3640 in JMB1100 treated with 10x MIC JSF-3151. B) Demonstration of the time-dependent accumulation of JSF-3640 within the WT JMB1100 strain treated with 12  $\mu$ M JSF-3640. p-values were determined by one-way ANOVA with Bonferroni post-hoc test. ns p>0.05, \* p<0.05, \*\* p<0.01, \*\*\* p<0.001.



**Figure S5. JSF-3151 neither depolarizes the membrane nor releases NO•.** A) Membrane depolarization assay was performed with *S. aureus* (ATCC 43300) cultures treated with DIBAC<sub>4</sub> in the presence or absence of JSF-3151 with sodium deoxycholate as a positive control. B) A) DAF-FM diacetate was used to assay for intrabacterial NO• production. A 2 h pre-incubation of bacteria with JSF-3151 or DEA/NONOate was utilized. The error bars were set as ± the standard error based on three independent experiments. C) The Griess reagent was also utilized to assay for NO• production by quantifying the nitrite concentration in the supernatant. A representative experiment, of two independent studies, is depicted.



**Figure S6. Increased transcription of** *ycel* **confers resistance to JSF-3151.** A) Growth inhibition assays of JSF-3151 versus MRSA strains with or without an additional copy of mutant *ycel*. Effect of drug concentration on optical density/growth of the parent (JMB1100) or *ycel::Tn* (JMB9587) with episomes. The data represent the average of three independent experiments and errors are shown as standard deviations. B) RT-qPCR with USA300\_FPR3757 and sequenced JSF-3151–resistant mutants. The mRNA were extracted from each mid-log phase *S. aureus* culture (OD<sub>600</sub> = 0.4), including the wild type strain and three JSF-3151 spontaneous resistant mutants, and the respective cDNA library was reverse transcribed. *ycel* in each of the four strains was amplified via qPCR and quantified with 16S rRNA as an internal reference. The *ycel* mRNA abundance in mutants was reported after normalization by the *ycel* level in the wild type strain (LAC). The Student's t-test was applied to compare statistical differences in the *ycel* transcription in three mutant strains as compared to the wild type. \*\* *p* < 0.01. \*\*\* *p* < 0.001.

#### Table S1. Internal Statistics for Bayesian Models.

| Model <sup>a</sup> | ROC    | Sensitivity <sup>c</sup> | Specificity <sup>d</sup> | Concordance <sup>e</sup> |
|--------------------|--------|--------------------------|--------------------------|--------------------------|
|                    | score⁵ | %                        | %                        | %                        |
| MRSA_1a            | 0.903  | 93.5                     | 81.7                     | 89.2                     |
| MRSA_1b            | 0.917  | 96.5                     | 81.0                     | 89.2                     |
| Broad_MSSA         | 0.901  | 85.5                     | 93.9                     | 93.8                     |

<sup>a</sup>All of these Bayesian models used FCFP\_6 fingerprints and 8 standard descriptors with 10 bins.

<sup>b</sup>ROC score is the area under the curve for the ROC plot.

<sup>c</sup>Sensitivity is the true positive rate and is indicated as the percent of correctly predicted active small molecules.

<sup>d</sup>Sensitivity is the true negative rate and is indicated as the percent of correctly predicted inactive small molecules.

<sup>e</sup>Concordance is a measure of overall accuracy and is calculated by the sum of true positives and true negatives divided by the total number of predictions.

### Table S2. External Statistics for Bayesian Models.

| Model <sup>a</sup> | ROC   | Sensitivity | Specificity | Concordance | Positive             |
|--------------------|-------|-------------|-------------|-------------|----------------------|
|                    | score | %           | %           | %           | Value <sup>b</sup> % |
| MRSA_1a            | 0.663 | 56.5        | 78.9        | 77.5        | 14.9                 |
| MRSA_1b            | 0.603 | 65.2        | 59.6        | 60.2        | 9.61                 |
| Broad_MSSA         | 0.534 | 21.7        | 67.2        | 64.4        | 4.16                 |

<sup>a</sup>All of these Bayesian models used FCFP\_6 and 8 standard descriptors with 10 bins.

<sup>b</sup>Positive Predictive Value (PPV) is also called the hit rate and is calculated by the number of true positives divided by the total number of positive calls (true positives + false positives)

| Compound ID<br>(JSF-#) | Chemical Structure  | Model (Score)      | MRSA ATCC<br>43300 MIC<br>in µg/mL | MSSA<br>ATCC 25923<br>MIC<br>in µg/mL |
|------------------------|---|--------------------|------------------------------------|---------------------------------------|
| Z296972134<br>(3151)   |   | MRSA_1b<br>(13.19) | 4.0                                | 1.6                                   |
| Z1542057255<br>(3157)  | -0 <sup>N0</sup> -1<br>N0-1<br>N0-1<br>N0N  | MRSA_1b<br>(13.37) | 12                                 | 6.3                                   |
| Z364195972<br>(3153)   | $\sum_{k=1}^{n} \sum_{i=1}^{n} \sum_{i$ | MRSA_1b<br>(14.22) | 12                                 | 6.3                                   |
| Z27692293<br>(3145)    |   | MRSA_1a<br>(7.80)  | 12                                 | 12                                    |
| Z27646636<br>(3144)    |   | MRSA_1a<br>(9.95)  | 25                                 | 25                                    |
| Z1024157644<br>(3154)  | °°°-<°°<br>°°-<°°<br>°°   | MRSA_1a<br>(7.81)  | 25                                 | 25                                    |
| Z31685691<br>(3147)    | $ \begin{array}{c}                                     $  | MRSA_1b<br>(13.24) | >50                                | >50                                   |
| Z1425795189<br>(3156)  |   | MRSA_1b<br>(13.20) | >50                                | >50                                   |

Table S3. *S. aureus* MIC values for top-scoring compounds from MRSA\_1a and MRSA\_1b predictions with the Enamine library.

| Z27403267<br>(3143)   | on the state of th | MRSA_1a<br>(8.64) | >50 | >50 |
|-----------------------|--|-------------------|-----|-----|
| Z1238474609<br>(3155) | $(\mathbf{x}_{n-n}^{N}) = (\mathbf{x}_{n-n}^{N})$  | MRSA_1a<br>(8.24) | >50 | >50 |
| Z56816307<br>(3148)   |  | MRSA_1a<br>(7.91) | >50 | >50 |
| Z29963029<br>(3146)   |  | MRSA_1a<br>(7.84) | >50 | >50 |
| Z303222454<br>(3152)  | o'N' N N N N N N N N N N N N N N N N N N   | MRSA_1a<br>(7.81) | >50 | >50 |
| Z166605150<br>(3150)  |  | MRSA_1a<br>(7.67) | >50 | >50 |
| Z58521079<br>(3149)   |  | MRSA_1a<br>(7.67) | >50 | >50 |

Table S4. MRSA MIC values for bottom-scoring compounds from MRSA\_1b predictions with the Enamine library.

| Compound ID<br>(JSF <i>-</i> #) | Chemical Structure   | Model (Score)      | MRSA ATCC<br>43300 MIC<br>in µg/mL |
|---------------------------------|--|--------------------|------------------------------------|
| Z16202993<br>(3328)             | CT & CO CO   | MRSA_1b<br>(-55.5) | >50                                |
| Z32362018<br>(3329)             |  | MRSA_1b<br>(-61.4) | >50                                |
| Z32890122<br>(3330)             |  | MRSA_1b<br>(-63.1) | >50                                |
| Z33297746<br>(3331)             |  | MRSA_1b<br>(-62.4) | >50                                |
| Z85932430<br>(3332)             |  | MRSA_1b<br>(-64.0) | >50                                |
| Z1051114684<br>(3333)           |  | MRSA_1b<br>(-53.8) | >50                                |
| Z1532850977<br>(3334)           |  | MRSA_1b<br>(-58.5) | >50                                |
| EN300-122381<br>(3335)          | STNH2<br>O.N.J.H.J.S.J.S.N.N<br>O.N.J.H.J.N.J.O.H<br>O.N.J.O.H | MRSA_1b<br>(-70.3) | >50                                |

**MIC JSF-3151 MIC Vancomycin MIC Rifampicin** Strain in  $\mu g/mL (\mu M)$ in  $\mu g/mL (\mu M)$ in  $\mu g/mL (\mu M)$ 3.1 MRSA ATCC 4.0 < 0.078 43300 (12) (2.1)(<0.095) **VRSA Strains VRSA 22522** 1.6 - 3.1 (4.8 - 9.3) >200 (>140) >10 (>12) **VRSA 22523** 1.6 - 3.1 (4.8 - 9.3) 100 - 200 (69 - 140) <0.078 (<0.095) **VRSA 22524** 1.6 - 3.1 (4.8 - 9.3) 200 (140) <0.078 (<0.095) 100 (69) <0.078 (<0.095) **VRSA 22525** 0.78 (2.3) 0.78 - 1.6 (2.3 - 4.8)>200 (>140) <0.078 (<0.095) **VRSA 22528 VISA Strains VISA 21143** 0.39 - 0.78(1.8 - 2.3)12 (8.3) <0.078 (<0.095) VISA 21157 <0.078 (<0.095) 1.6 (4.8) 12 (8.3) **VISA 21161** 6.2 (19) 6.2 (4.2) <0.078 (<0.095) **VISA 21178** 1.6 (4.8) 6.2 (4.2) <0.078 (<0.095) **VISA 21186** 6.2 (4.2) 1.6 (4.8) >10 (>12) **VISA 21352** 1.6 - 3.1 (4.8 - 9.3)6.2 (4.2) >10 (>12)

Table S5. MIC of JSF-3151 versus VRSA and VISA strains.

Table S6. Primers used in this study.

| Primer name         | Sequence  |
|---------------------|---|
| 2620upBamHI         | GGGGGATCCGTACGAGTCACTTGATGCAAAGTCAATAG                    |
| 2620downSall        | GGGGTCGACGAAAGGCGTCTAAAATTAGGGTGAACAG                     |
| 2620uppEPBamHI      | GGGGGATCCTTAGGAGGATGATTATTTATGACTAACTTTACTTTTGATGGTGCACA  |
|                     | C   |
| SCV8                | GCACATAATTGCTCACAGCCA                                     |
| SCV9                | GCTGATCTAACAATCCAATCCA                                    |
| qRT-PCR forward     | GGATGGTTCTCAAGTAACAGGTAT                                  |
| qRT-PCR reverse     | TAGCATTACGCCACCAGTTT                                      |
| 16srRNA- forward    | CGCTTTAGCGGTGTGGGAT                                       |
| 16srRNA- reverse    | GGCCGGCTACCCGTCGTC  |
| pEPSA5-0206 EcoRI   | TGTAGAATTCGAGCTCGGTACCCATACTTAAGAAAATAACAGAAACAGAGGAGAA   |
| for                 |   |
| pEPSA5-0206         | GGTCGACTCTAGAGGATCCCCTCAAAATACTTCATTAACACTATAATAATATATTTT |
| BamHlrev            | GAAAAATATTTAC   |
| pCM28-0206 BamHI    | GGATCCTCTAGAGTCTCAAAATACTTCATTAACACTATAATAATATATTTTGAAAAA |
| rev                 | TATTTAC   |
| pCM28-0206 Pstl for | TACAGAAGCTTGCATGCCTGCAGGTCAGTAGTACTGCCAAATATAGATGAAGAAA   |
|                     | ATGATG  |

# Table S7. Strains and plasmids used in this study.

| Strain name                          | Notes   |
|--------------------------------------|---|
| USA300_FPR3757                       | Parental strain   |
| JMB1100                              | USA300_LAC that has been cured of the pUSA03 <sup>67</sup>                |
| JMB9587 ycel::Tn                     | JMB1100 with <i>ycel::Tn</i>  |
| USA300_FPR3757                       | $T \rightarrow G$ mutation at position -43 in <i>ycel</i>                 |
| USA300_FPR3757                       | adenosine base insertion at -37 in ycel                                   |
| USA300_FPR3757                       | adenosine base deletion at position -41 in ycel                           |
| RN4220                               | Restriction minus S. aureus strain <sup>66(Kreiswirth et al., 1983)</sup> |
| JMB1100 pLL39                        | Contains empty vector   |
| JMB1100 pLL39_ <i>ycel</i>           | Expression of single copy of <i>ycel</i>                                  |
| JMB1100 pLL39_ <i>ycel1</i>          | Expression of single copy of <i>ycel1</i>                                 |
| JMB1100 pLL39_ <i>ycel2</i>          | Expression of single copy of <i>ycel2</i>                                 |
| JMB1100 pLL39_ <i>ycel3</i>          | Expression of single copy of <i>ycel3</i>                                 |
| JMB9587 <i>ycel::Tn</i> pLL39 *      | Contains empty vector   |
| JMB9587 ycel::Tn pLL39_ycel          | Over-expression of <i>ycel</i>  |
| JMB9587 ycel::Tn pLL39_ycel1         | Over-expression of <i>ycel1</i>   |
| JMB9587 ycel::Tn pLL39_ycel2         | Over-expression of yce/2  |
| JMB9587 ycel::Tn pLL39_ycel3         | Over-expression of <i>yce13</i>   |
| JMB9587 ycel::Tn azoR1               | azoR Trp100stop   |
| JMB9587 ycel::Tn azoR2               | azoR Trp <sub>60</sub> stop   |
| JMB9587 ycel::Tn azoR3               | azoR Thr <sub>121</sub> lle   |
| JMB9587 ycel::Tn azoR4               | azoR Trp₄₀stop  |
| JMB9769 <i>azoR</i> ::Tn             | JMB1100 with <i>azoR</i> ::Tn   |
| Plasmids utilized                    |   |
| pLL2787                              | Contains an IPTG inducible int  |
| pLL39                                | Single copy expression construct for select gene                          |
| pEPSA5                               | Over-expression construct for select gene                                 |
| pCM28                                | Gene expression from native promoter                                      |
| * 9587 is shown here as its full des | cription ( <i>ycel</i> :: <i>Tn(ermR)</i> ). Tn represents transposon.    |

# Table S8. Accumulation metrics for JSF-3151 and JSF-3640 in select strains.

|        | JSF-3151 accumulation          |                   | JSF-3640 a                     | ccumulation       | Biotransformation |
|--------|--------------------------------|-------------------|--------------------------------|-------------------|-------------------|
| MRSA   | Average AUC <sub>0-90min</sub> | Relative level of | Average AUC <sub>0-90min</sub> | Relative level of | from JSF-3151 to  |
| strain | (nmol*min/OD <sub>600</sub> )  | accumulation (%)  | (nmol*min/OD <sub>600</sub> )  | accumulation (%)  | JSF-3640 (%)      |
| WT     | 2010 ± 70                      | 100               | 150 ± 10                       | 100               | 7.3               |
| azoR   | 1530 ± 30                      | 76                | 80 ± 2                         | 55                | 5.2               |
| ycel2  | 860 ± 40                       | 43                | 77 ± 9                         | 52                | 9.0               |