

**Supplemental Table S1.** Daptomycin (DAP) MICs, *mprF* single nucleotide polymorphisms (SNPs), surface charge, host defense peptide susceptibilities, and cell wall thickness profiles of the study strains.

Strains	DAP MICs ( $\mu\text{G}/\text{ML}$ )	<i>mprF</i> SNPs <sup>#</sup>	Surface charge <sup>§</sup>	% survival against tPMPs <sup>‡</sup>		% survival against hNP-1 <sup>‡</sup>		Cell wall thickness (nm) <sup>†</sup>
				2 $\mu\text{g}/\text{ml}$	1 $\mu\text{g}/\text{ml}$	20 $\mu\text{g}/\text{ml}$	10 $\mu\text{g}/\text{ml}$	
G1	$\leq 0.5$	-	87 $\pm$ 13	12	34	9	21	19
G10	$\leq 0.5$	-	78 $\pm$ 10	33	41	8	27	23
G12	$\leq 0.5$	-	132 $\pm$ 13	28	34	28	49	24
G14	$\leq 0.5$	-	129 $\pm$ 17	42	60	47	68	27
G16	$\leq 0.5$	-	174 $\pm$ 18	13	17	32	51	25
G34	$\leq 0.5$	-	113 $\pm$ 26	10	14	13	30	25
G40	$\leq 0.5$	-	148 $\pm$ 16	1	14	26	39	25
G70	$\leq 0.5$	-	152 $\pm$ 26	1	0	1	11	24
G71	$\leq 0.5$	-	116 $\pm$ 2.5	4	10	1	4	25
G72	$\leq 0.5$	-	138 $\pm$ 22	3	8	8	16	27
G89	$\leq 0.5$	-	194 $\pm$ 13	2	1	0	2	22
G80	$\leq 0.5$	I498N	105 $\pm$ 8	2	16	1	11	25
G86	$\leq 0.5$	I498N	93 $\pm$ 15	3	11	21	34	28
G87	$\leq 0.5$	I498N	208 $\pm$ 15	1	11	58	79	26
G88	$\leq 0.5$	I498N	141 $\pm$ 30	5	15	11	34	29
G90	$\leq 0.5$	I498N	105 $\pm$ 18	2	19	11	31	26
G9	1	-	86 $\pm$ 18	29	41	23	51	25
G35	1	-	89 $\pm$ 22	22	26	40	29	26
G38	1	-	138 $\pm$ 28	36	40	50	67	26
G69	1	-	127 $\pm$ 24	55	78	58	71	27
G74	1	-	171 $\pm$ 18	8	21	14	16	23
G94	1	-	180 $\pm$ 18	1	2	0	0	25
G47	1	Q692E	190 $\pm$ 35	20	44	8	24	23
G53	1	Q692E	190 $\pm$ 33	55	78	15	42	31
G56	1	Q692E	115 $\pm$ 23	76	85	47	64	28
G51	1	> 30 SNPs	180 $\pm$ 23	23	48	12	38	24
G66	1	> 30 SNPs	87 $\pm$ 32	63	63	18	36	25

<b>161</b>	<b>4</b>	<b>L826F</b>	<b>90 ± 14</b>	<b>24</b>	<b>52</b>	<b>71</b>	<b>73</b>	<b>22</b>
<b>165</b>	<b>4</b>	<b>L341S</b>	<b>103 ± 25</b>	<b>16</b>	<b>18</b>	<b>17</b>	<b>39</b>	<b>19</b>
<b>167</b>	<b>4</b>	<b>L826F</b>	<b>100 ± 10</b>	<b>83</b>	<b>99</b>	<b>100</b>	<b>86</b>	<b>22</b>
<b>169</b>	<b>4</b>	<b>L826F</b>	<b>105 ± 9</b>	<b>97</b>	<b>97</b>	<b>56</b>	<b>80</b>	<b>29</b>
<b>212</b>	<b>2</b>	<b>L826F</b>	<b>89 ± 24</b>	<b>20</b>	<b>45</b>	<b>41</b>	<b>64</b>	<b>28</b>
<b>234</b>	<b>2</b>	<b>L826F</b>	<b>90 ± 22</b>	<b>31</b>	<b>59</b>	<b>86</b>	<b>91</b>	<b>25</b>
<b>235</b>	<b>3</b>	<b>L341S</b>	<b>77 ± 7</b>	<b>41</b>	<b>63</b>	<b>38</b>	<b>48</b>	<b>29</b>
<b>333</b>	<b>2</b>	<b>L826F</b>	<b>64 ± 14</b>	<b>33</b>	<b>39</b>	<b>27</b>	<b>43</b>	<b>24</b>

<sup>#</sup>*mprF* SNPs compared to the *mprF* sequences from *S. aureus* Mu50 (CC5).

<sup>§</sup>% cytochrome *c* bound vs *S. aureus* MU50. The higher the amounts of bound cytochrome *c*, the less relative positive surface charge exists.

<sup>¥</sup>Mean % survival of the strains after 2h exposure to host defense peptides (three experimental runs).

<sup>‡</sup>Mean of 100 measurements for each strain.