

1 **Table S1. List of strains used in this study.**

<b>Strain</b>	<b>Description</b>	<b>Designation</b>	<b>Source</b>
USA300 LAC	<i>S. aureus</i> USA300 Strain LAC (AH-1263). Plasmid cured.	AH-LAC (WT)	(1)
DH5 $\alpha$	<i>E. coli</i> strain for recombinant plasmids	DH5 $\alpha$	(2)
RN4220	Restriction-deficient <i>S. aureus</i>	RN4220	(3)
RN6390	<i>S. aureus</i> 8325 derivative	RN6390	(4, 5)
Newman	Osteomyelitis isolate - 1952	Newman	(6)
COL	Early MRSA isolate	COL	(7)
BK2395	USA500 clinical Isolate	USA500	(8, 9)
RN9011	RN4220 + pRN7203 expressing SaPI integrase	RN9011	(10)
FA-S1176	AH-LAC $\Delta$ lipL	$\Delta$ lipL	(11)
FA-S1178	AH-LAC $\Delta$ lipA $\Delta$ lipA1 $\Delta$ lipA2	$\Delta$ lipA $\Delta$ lipA1 $\Delta$ lipA2	(12)
FA-S973	AH-LAC $\Delta$ geh::kan	$\Delta$ geh	(13)
FA-S2192	AH-LAC $\Delta$ bmfBB::kan	$\Delta$ bmfBB::kan	This work
FA-S2143	AH-LAC $\Delta$ lipL $\Delta$ geh::kan	$\Delta$ lipL $\Delta$ geh	This work
FA-S2167	AH-LAC $\Delta$ lipL $\Delta$ geh::kan containing pJC1112-geh	$\Delta$ lipL $\Delta$ geh + geh	This work
FA-S2304	AH-LAC $\Delta$ bmfBB $\Delta$ pdhC::kan	$\Delta$ bmfBB $\Delta$ pdhC	This work
FA-S2230	AH-LAC $\Delta$ fakB1	$\Delta$ fakB1	This work
FA-S2232	AH-LAC $\Delta$ fakB2	$\Delta$ fakB2	This work
FA-S2242	AH-LAC $\Delta$ bmfBB::kan $\Delta$ fakB1	$\Delta$ bmfBB::kan $\Delta$ fakB1	This work
FA-S2244	AH-LAC $\Delta$ bmfBB::kan $\Delta$ fakB2	$\Delta$ bmfBB::kan $\Delta$ fakB2	This work
FA-S2260	AH-LAC $\Delta$ bmfBB::kan $\Delta$ fakB2 containing pJC1111-fakB2	$\Delta$ bmfBB::kan $\Delta$ fakB2 + fakB2	This work
FA-S2351	RN4220 $\Delta$ bmfBB::kan	RN4220 $\Delta$ bmfBB	This work
FA-S2358	RN6390 $\Delta$ bmfBB::kan	RN6390 $\Delta$ bmfBB	This work
FA-S2354	Newman $\Delta$ bmfBB::kan (geh disrupted)	Newman $\Delta$ bmfBB	This work
FA-S2355	COL $\Delta$ bmfBB::kan (geh disrupted)	COL $\Delta$ bmfBB	This work
FA-S2356	BK2395 $\Delta$ bmfBB::kan	USA500 $\Delta$ bmfBB	This work

2

3

4

5

6

7

8 **Table S2. List of oligonucleotides used in this study.**

Name	Sequence
bmfBBSOE1-KpnI	CCC <b>GGTAC</b> C GCTAAAGGGCTTTATTATCA
bmfBBSOE2-KasI	ATAGATGCATCTATGTTATCA <b>GGCGCC</b> ACTTCCTCCCTAGAATT
bmfBBSOE3-KasI	AATTCTAGGGAGGGAAAGT <b>GGCGC</b> TGATAACATAGATGCATCTAT
bmfBBSOE4-SacI	CCC <b>GAGCT</b> C GCTGGCTGGCGATACCA
kanF-KasI	TCCC <b>GGCGCC</b> CTCGACGATAAACCCAGCGAAC
kanR-KasI	TCCC <b>GGCGCC</b> CTTTTAGACATCTAAATCTAGGTAC
fakB1SOE1-KpnI	ATAT <b>GGTAC</b> C AGCACCGCTATAGGCG
fakB1SOE2	CGAGAGATAATTCCAATTACAATATAGACTCTTTATTAAAATTTATAA AGTCGTCCCTCTAAATATACTCATAGC
fakB1SOE3	GCTATGAGTATATTAGGAGGACGACTTATAAAATTAAATAAAAAGAG TCTATATTGTAATTGAAATTATCTCTCG
fakB1SOE4-SacI	ATAT <b>GAGCT</b> C TGTCTCTTCTAATGGCTCGAAC
fakB2SOE1-KpnI	ATAT <b>GGTAC</b> C ATAATTATGACTTTATCCATTCTAGTTGCACATG
fakB2SOE2	TGAAATGGAAGTAATTAACACTGAAAAGATTAAATGGTCGTTCCCCCT TATTTTTAC
fakB2SOE3	GTAAAAAATAAGGGGGAAACGACCATTAAATCTTCAGTGTAAATTAC TTCCATTCA
fakB2SOE4-SacI	ATAT <b>GAGCT</b> C GATAGAAATTATGGTTAATAGGTGTAATCACTGG
UniCompSOE1-PstI	ATAT <b>CTGCAG</b> ATCCCATTATGCTTGCGA
fakB2CompSOE2	GAGTCTGTTACTATAATCTGTTTGTATGGTTCACTCTCCTCTAC
fakB2CompSOE3	GTAGAAGGAGAGTGAACCCATGACAAAACAGATTATAGAACAGACTC
fakB2CompSOE4-SacI	ATAT <b>GAGCT</b> C GATGTAATGGTTAACCATGCAC

9

10

11

12

13

14

15

16

17

18

19

20      **References**

- 21      1. B. R. Boles, M. Thoendel, A. J. Roth, A. R. Horswill, Identification of genes involved  
22      in polysaccharide-independent *Staphylococcus aureus* biofilm formation. *PLOS ONE*  
23      **5**, e10146 (2010).
- 24      2. M. R. Green, J. Sambrook, J. Sambrook, *Molecular cloning: a laboratory manual*, 4th  
25      ed (Cold Spring Harbor Laboratory Press, 2012).
- 26      3. R. P. Novick, “[27] Genetic systems in staphylococci” in *Methods in enzymology*,  
27      bacterial genetic systems., (Academic Press, 1991), pp. 587–636.
- 28      4. H. L. Peng, R. P. Novick, B. Kreiswirth, J. Kornblum, P. Schlievert, Cloning,  
29      characterization, and sequencing of an accessory gene regulator (agr) in  
30      *Staphylococcus aureus*. *J Bacteriol* **170**, 4365–4372 (1988).
- 31      5. K. T. Bæk, D. Frees, A. Renzoni, C. Barras, N. Rodriguez, C. Manzano, W. L. Kelley,  
32      Genetic variation in the *Staphylococcus aureus* 8325 strain lineage revealed by  
33      whole-genome sequencing. *PLoS One* **8**, e77122 (2013).
- 34      6. E. S. Duthie, L. L. Lorenz, Staphylococcal coagulase; mode of action and antigenicity.  
35      *J Gen Microbiol* **6**, 95–107 (1952).
- 36      7. H. de Lencastre, A. Tomasz, Reassessment of the number of auxiliary genes  
37      essential for expression of high-level methicillin resistance in *Staphylococcus aureus*.  
38      *Antimicrob Agents Chemother* **38**, 2590–2598 (1994).
- 39      8. K. G. Dyke, Penicillinase production and intrinsic resistance to penicillins in methicillin-  
40      resistant cultures of *Staphylococcus aureus*. *J Med Microbiol* **2**, 261–278 (1969).
- 41      9. R. B. Roberts, A. de Lencastre, W. Eisner, E. P. Severina, B. Shopsin, B. N. Kreiswirth,  
42      A. Tomasz, Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in  
43      12 New York hospitals. MRSA Collaborative Study Group. *J Infect Dis* **178**, 164–171  
44      (1998).
- 45      10. J. Chen, P. Yoong, G. Ram, V. J. Torres, R. P. Novick, Single-copy vectors for  
46      integration at the SaPI1 attachment site for *Staphylococcus aureus*. *Plasmid* **76**, 1–7  
47      (2014).
- 48      11. W. P. Teoh, Z. J. Resko, S. Flury, F. Alonzo 3rd, Dynamic relay of protein-bound lipoic  
49      acid in *Staphylococcus aureus*. *Journal of Bacteriology* **201** (2019).
- 50      12. A. Zorzoli, J. P. Grayczyk, F. Alonzo 3rd, *Staphylococcus aureus* tissue infection  
51      during sepsis is supported by differential use of bacterial or host-derived lipoic acid.  
52      *PLOS Pathogens* **12**, e1005933 (2016).

53 13. X. Chen, F. Alonzo 3rd, Bacterial lipolysis of immune-activating ligands promotes  
54 evasion of innate defenses. *PNAS* **116**, 3764–3773 (2019).

55