

Figure S1. Data for MIC determination of *S. aureus* growth in TSB supplemented with linoleic acid. Inoculum cultures were grown to mid-exponential phase in TSB containing antibiotics as required, and 20 ng/mL anhydrotetracyline for cultures with pALC2073 or pALC*farE*. Cultures were then inoculated into triplicate tubes of TSB containing varying concentrations of linoleic acid. Growth (OD₆₀₀) was quantified after 24h and is plotted as the mean \pm standard deviation of triplicate cultures.

				PAL1				PAL2	_
			•						
USA300	1	CAAAGTATATT-GCCTCCTTTTAAAAAT	CAACGTTA <u>TAGTTTA</u> AATATA	ACAGTG <u>TAGATTA</u> TTG	TTCGA-TTAT	AGTATCTATCCCCGAC	CTCTTAAAGAATCAATTGGA	AAATTTTGTATA-T <u>TAAACTA</u>	127
equorum	1	CAAACTATT-GCCTCCTAATTTAAT	CAACGTTATAGTTTATACAT	ATAGTGTAGATTATTT	ATATTA-ACAT	TTTA-CAAGCTAAGTCGTAT	TATAAAAAGAACTAATTATG	CCAATATGTCTATTTAAACTA	130
xylosus	1	CAAACTATT-GCCTCCTAAATTAAT	CAACGTTATAGTTTAAATAT	ATACTGTAGATTATTT	CTATCC-ACAT	ATTA-AATGCTTTACCCTTT	TATTAAAAGAATTAATTATT	ACAATTTGTATATTTAAACTA	130
argenteus	1	CAAAGTATT-GCCTCCTTTTAAAAT(CAACGTTATAGTTTAACTAT	ACAGTGTAGATTATTG	TTAGA-TTATA	ATTATCTATACCCTAC	CTCTTAAAGAATTAAATTTT	AAAATTTGTATA-CTAAACTA	125
saprophyticus	1	CAATCTATT-GCCTCCTCAGATAAT	CAACGTTATAGTTTAATTGT	ATAGTGTAGATTATTT	ATATTA-ACAC	AATA-AACGCTGTGTACAAT	TAATAAAAGAATTAAAAACT	GTAATATGCCTATTTAAACTA	130
carnosus	1	CAAAGTATT-GCCTCCTCCTAAAAT	ATACAGTATAGTTTA-TTCT	ACATAGTAGATTAATTA-	CGTATCACATAT(GCTA-CATGGTTAATTATTA	CCTGTACAGATATATTTCAA	GCCTAATATAAGGCTAAACTA	132
cohnii	1	CAAACTATT-GCCTCCTTAATTAAT	CAACGTTATAGTTTATACAT	ATACTGTAGATTATTT	CTATTG-ACAT	CTTATAATGATTTACCCTAT	TTAAAAAAGAACTAAATATC	CAATTCCGTATATTTAAACTA	131
succinus	1	CAAACTATT-GCCTCCTTATTTAAT(CAACGTTATAGTTTAAATGT	AATGTGTAGATTATCT	АТААТА-ТСАТ	AATAACCATGCTCCCCCTAA	TATATAAAGAATTAAATATA	CTAAAATGTATATTTAAACTA	131
schweizeri	1	CAAATATATT-GCCTCCCTTTAAAAT	CAACGTTATAGTTTAACTAT	ACAGTGTAGATTATTG	TTCGA-TTAT	AGTATCTATACCCTAC	CTCTTAAAGAATTAAATTTT	AAAATTTGTATA-CTAAACTA	126
simiae	1	CAAAGTATT-GCCTCCTCTTGATTT(CAACGCTATAGTTTAACTGT	ATAGTGTAGATTATTG	TGTGA-TTAT	AATATCTCTACCCTCT	TTTTAAAAGAAATAAATAAT	CGATTGTGTATT-TTAAACTA	125
gallinarum	1	CAAACTATTTGCCTCCCTATTTAAT	CAACGTTATA <u>t</u> TTTAAATATA	ATTATGTAGATTATCTGT	ATCTTACATATTCATAGTA-ACAT	ACTAATCAT-TTTTGATTCT	TTTATAAAGAATTAATAATA	TAGAACTGTATATTTAAACTA	146
arlettae	1	CAAGCTATT-GCCTCCTTATTTAAT(CAACGTTATAGT <u>C</u> TAAATATA	ATAGTGTAGATTATTT	ACAGTA-CTATA	ACTACTAGTAATTCCCCCAA	TTT-TAAAGAAATAATAATT	ATTATATGTATATTTAAACTA	130
lugdunensis	1	CAAAGTATT-GCCCCCTTCTAAAAT	CAACGTTATAGTTTAACTAT	ACATTGTAGATTATTTAA	CATGCCA-ATAT	GTTA-CATTCTTTTGCTCTG	TATTTAAAGTCATAATTAAT	GATATATGTATAGATAAACTA	133
stepanovicii	1	CAAACTATT-GCCTCCTCAATTAAT	CAACGTTATAGTTTAAATAT	ATAGTGTAGATTATTT	ATATTA-ACAC	ATTA-AACGCTAAACCCTAG	TATAAAAAGATTTAATTATT	GCAAAATGTATACTTAAACTA	130
condimenti	1	CAAAGTATT-GCCTCCTCTATAGAT	ATACAGTATAGTTTA-TTCT	ACATAGTAGATTAATTA-	CGTATCACATAT(GCTA-CATGGTTCATTATTA	CCTGTACAGATATATTTCAA	CCTATTTATAGGGCTAAACTA	132
piscifermentans	1	CAATTCTACATCTCCTC	CCTTAGTGTAGTTTAAACTT	ATAA-ATAGATTGAATTA	TATCTGTACAGGCAAT	AATT-AACCATGTAGCATAT	GTGATACGTAATTAATCTAC	TGTGTAGAATAAACTATACTg	129
		* *	* ** * ** **	* *****	*	*	* *	** ***	
		◆							
USA300	128	CACACAAAGGAGAAATGTAGATG	150						
equorum	131	CAAAAA-GGGAGAAGTGTAGAATG	153						
xylosus	131	CAAATA-AGGAGAAATGTATATTG	153						
argenteus	126	CACATTAAGGAGAAATGTAGATG	148						
saprophyticus	131	CAGATT-AGGAGAAATGTAGAATG	153						
carnosus	133	CACTAA-GGAGGAATTGTAGATTTG	156						
cohnii	132	CAGATT-AGGAGAATTGTAGAATG	154						
succinus	132	CAAATA-AGGAGAAATGTAGAATG	154						
schweizeri	127	CACATTAAGGAGAAATGTAGATG	149						
simiae	126	CATATTA-GGAGAAACGTAGATG	147						
gallinarum	147	CACATA-AGGAGAATTGTAGAATG	169						
arlettae	131	CAAAAACAGGAGGATTGTAGAATG	154						
lugdunensis	134	CGGTTTGGAGTGAATTGTATATG	156						
stepanovicii	131	CAGATT-AGGAGAAATGTAGAATG	153						
condimenti	133	CACTAA-GGAGGAATTGTAGATTTG	156						
piscifermentans	130	TATATTTAAAGAGGAGGCAATACTTTG	156						
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Figure S2. Alignment of *farER*^{IS} segment from *S. aureus* USA300 with the syntenic sequence in coagulase negative staphylococci. A conserved TAGWTTA motif is indicated by underlined bold font in *S. aureus farER*^{IS}, and this motif is shaded gray in all sequences. The span of nucleotides comprising PAL1 and PAL2 is demarcated by thick lines with arrows at each end. Nucleotides that are conserved in all sequences are indicated by asterisks below the bottom sequence.



Figure S3. Sequence chromatograms from cloned 5'-RACE products for determination of the +1 transcription start site of P_{farR} . The nucleotide sequence of P_{farR} and downstream sequence is shown above each chromatogram, and underlined nucleotides identify the -35 and -10 motifs of P_{farR} . The transcription start site at two adjacent nucleotides is labelled as +1 above the sequence, and the +1 nucleotide is indicated in large bold font. Arrows point to the +1 nucleotide on the chromatogram.



Figure S4. Growth of USA300 Δ *farER* complemented with pLI50 or pLI*farER* and derivatives in TSB. Each data point represents the mean and standard deviation of triplicate cultures.

Supplementary Table S1

Strain or plasmid	Description	Source or reference
Strains:		
S. aureus:		
USA300 LAC	Community associated MRSA; wild type strain cured of resistance plasmids	(Arsic <i>et al.</i> , 2012)
RN4220	$r_{K}^{-}m_{K}^{+}$; capable of accepting foreign DNA	(Novick,
USA300 <i>farE::</i> ФNE	USA300 LAC with transposon insertion in <i>farE</i> (SAUSA300_2489); Erm ^r .	(Alnaseri <i>et al.</i> , 2015)
USA300∆farER	USA300 with <i>farER</i> deletion; constructed using pKOR∆ <i>farER</i>	This study
USA300∆fakA	USA300 with <i>fakA</i> deletion; constructed using pKOR∆ <i>fakA</i>	This study
USA300∆farERfakA	USA300 with deletion of <i>fakA</i> and <i>farER</i>	This study
USA300∆ <i>farER</i> pLI50	USA300∆ <i>farER</i> with pLI50; Cm ^r	This study
USA300∆ <i>farER</i> pLI <i>farE</i>	USA300∆ <i>farER</i> complemented with pLI <i>farE</i> ; Cm ^r	This study
USA300∆farERpLIfarER	USA300 Δ <i>farER</i> complemented with pLI <i>farER</i> ; Cm ^r	This study
USA300∆ <i>farER</i> pLI <i>farER</i> 1	USA300∆ <i>farER</i> complemented with pLI <i>farER</i> 1; Cm ^r	This study
USA300∆ <i>farER</i> pLI <i>farR</i>	USA300∆ <i>farE</i> R complemented with pLI <i>farR</i> ; Cm ^r	This study
USA300∆ <i>farER</i> pLI <i>farR</i> 1	USA300∆ <i>farE</i> R complemented with pLIf <i>arR</i> 1; Cm ^r	This study
USA300∆ <i>fakA</i> pALC2073	USA300 $\Delta fakA$ with empty pALC2073 complementation vector; Cm ^r .	This study
USA300∆ <i>fakA</i> pAL <i>fak</i> ^{ON}	USA300 Δ <i>fakA</i> complemented with <i>fakA</i> cloned in plus orientation in pALC2073; Cm ^r	This study

USA300∆ <i>fakA</i> pAL <i>fak</i> ^{OFF}	³ USA300 ^{<i>fakA</i>} complemented with <i>fakA</i> cloned in minus orientation in pALC2073; Cm ^r	This study
E. coli		
DH5a	$F^{-} \Phi 80 lac Z\Delta M15 \ recA1 \ endA1 \ gyrA96 \ thi-1 \ hsdR17 \ (r_{K}^{-}m_{K}^{+}) \ supE44 \ relA1 \ deoR \ \Delta(lacZYA-argF)U169 \ phoA$	Invitrogen
M15[pREP4]	F-, $\Phi 80lacZ\Delta M15$ <i>thi lac mtl recA</i> ⁺ ; Host strain for pQE30. Contains pREP4 (Km ^r) with constitutive <i>lacI</i> repressor	Qiagen
Plasmids:		
pLI50	E. coli-S. aureus shuttle vector	(Lee and Iandolo, 1986)
pQE30	<i>E. coli</i> vector for expression of N-terminal 6His- tagged fusion proteins	Qiagen
pALC2073	Shuttle vector used for expression of genes under control of tetracycline-inducible $P_{xyl/tetO}$ promoter in <i>S. aureus</i> ; genes are expressed at a basal level in absence of induction	(Bateman <i>et al.</i> , 2001)
pQE-FarR	<i>farR</i> open reading frame amplified from USA300 with primers 6H <i>farR</i> -F and 6H <i>farR</i> -R, cloned at <i>Sac</i> I and <i>Hind</i> III sites of pQE30.	This study
pQE- ^{H121Y} FarR	As for pQE-FarR, except that template DNA was from <i>S. aureus</i> FAR7	This study
pLI <i>farR</i>	pLI50 with <i>farR</i> expressed from native promoter	(Alnaseri <i>et al.</i> , 2015)
pLI <i>farR</i> 1	pLI <i>farR</i> after mutagenesis with <i>farR</i> 1-P and <i>farR</i> 1-M primers; G>A substitution in -10 motif of P_{farR} .	This study
pLIfarR2	Mutagenesis of pLI <i>farR</i> with primers <i>farR</i> 2-P and <i>farR</i> 2-M; nucleotide substitutions in PAL1.	This study

pLIfarR3	Mutagenesis of pLI <i>farR</i> 1 with primers <i>farR</i> 3-P and <i>farR</i> 3-M	This study
pLI <i>farE</i>	pLI50 with <i>farE</i> expressed from native promoter	(Alnaseri <i>et al.</i> , 2015)
pLI <i>farER</i>	<i>farE</i> excised from pLI <i>farE</i> with <i>KpnI-SacII</i> , and ligated into <i>KpnI-SacII</i> digested pLI <i>farR</i> .	This study
pLIfarER1	As for pLI <i>farER</i> , except that the <i>KpnI-SacII</i> fragment was ligated into pLI <i>farR</i> 1	This study
pLI <i>farER</i> 2	As for pLI <i>farER</i> , except that <i>KpnI-SacII</i> fragment was ligated into pLI <i>farR</i> 2	This study
pLIfarER3	As for pLI <i>farER</i> , except that <i>KpnI-SacII</i> fragment was ligated into pLI <i>farR</i> 3	This study
pGY <i>lux</i>	<i>E. coli-S. aureus</i> shuttle vector harboring promoterless <i>luxABCDE</i> operon	(Mesak <i>et al.</i> , 2009)
pGY <i>farE::lux</i>	farE promoter segment cloned in pGYlux	(Alnaseri <i>et al.</i> , 2015)
pGY <i>farR::lux</i>	<i>farR</i> promoter segment amplified with primers <i>farR::lux</i> -F and <i>farR::lux</i> -R, cloned in pGY <i>lux</i>	This study
pGY <i>farR</i> 1::lux	As for pGY <i>farR::lux</i> , using plasmid pLl <i>farR</i> 1 as template for PCR	This study
pKOR-1	<i>E. coli-S. aureus</i> shuttle vector (Cm ^r); contains $P_{xyl/tetO}$; antisense <i>secY</i> RNA expression	(Bae and Schneewind, 2006)
pKOR∆ <i>farER</i>	pKOR-1 containing ligated PCR products generated with primer pairs <i>farE</i> -UP- <i>attB1/farE</i> -UP- <i>SacII</i> and <i>far</i> R-DW- <i>SacII/farR</i> -DW- <i>attB2</i> .	This study
pKOR∆ <i>fakA</i>	pKOR-1 containing ligated PCR products generated with primer pairs <i>fakA</i> -UP- <i>attB1/fakA</i> -UP- <i>SacII</i> , and <i>fakA</i> -DW- <i>SacII</i> /fakA-DW- <i>attB2</i>	This study
pALC <i>fakA</i> ^{ON}	Promoterless <i>fakA</i> gene amplified by PCR with primers <i>fakA</i> -pALC-F and <i>fakA</i> -pALC-R, cloned in plus orientation (<i>KpnI</i>) with respect to P _{xyl/tetO} in pALC2073	This study

pALC <i>fakA</i> ^{OFF}	As above, but gene segment cloned in minus orientation	This study
pALC <i>farE</i>	Promoterless <i>farE</i> amplified with primers pALC- <i>farE</i> -F and pALC- <i>farE</i> -R, cloned in to <i>SacI</i> site of pALC2073	Thus study

Supplementary Table S2

Oligonucleotide	Description ^a
free LID and D th	
<i>JarE</i> -UP- <i>attB1</i> °	
farE-UP-SacII ^c	ggacctccgcggAACGATGGCATTGTACCAAG
farR-DW-SacII ^c	ggacct ccgcgg GGCGAAGATATTGATAACATTTTCC
farR-DW-attB2 ^d	attB2-GGTAAATTAGAACAAGGTGGCG
farR-GSP1	TTATCTGGGATGTCGCTG
farR-GSP2	CCCGTCGACTCAGCGTCTTCTTCTTGG
farR-GSP3	ATTGTCGACACTCATCGTTTGGAATGG
farR::lux-F ^e	cccggatccTGCAGCTACAATCACTATCCATGC
<i>farR::lux</i> -R ^f	cccgtcgacTAAATCAGTCTCTTTCATCTACATTTCTCC
farE-pALC-F ^g	CACTGTATATTTAAACTATAA gagete TTTTAAAAGGAGGCAAT ATACTTGGT
farE-pALC-R ^g	CACTTCCATGCAAAAACCCTgagctcCAAATGTCATTGATAGAC
6HfarR-F ^g	CTACACACAAAGGAGAAATGTA gagete ATGAAAGAGACTGAT TTACGAG
6HfarR-R ^h	GGTAACGCTCATGAGTTTCT aagctt CTATTTAATCTTAATATTG ATTAATCTATGG

fakA-UP-attB1 ^b	attB1-GCGTGTGAACGTCTGTTACCAGTCGAAGC
fakA-UP-SacII ^c	ggacctccgcggCATTTCAAGTTGTCCTCCTAAGCTTTCTTGC
fakA-DW-SacII ^c	ggacctccgcggGTTCATGAAGGTGGACAACCAATTTATC
fakA-DW-attB2 ^d	attB2-GATGACTTTTCTAATCTATTTAGCCATTGC
farR1-P ⁱ	CCTTTTAAAATCAACGTTATA <u>a</u> TTTAAATATACAGTGTAG
farR1-M ⁱ	CTACACTGTATATTTAAA <u>t</u> TATAACGTTGATTTTAAAAGG
farR2-P ^j	ATCAACGTTATAGTTTAAATAT <u>tt</u> AG <u>at</u> TAGATTATTGTTCGATT ATAGTATC
farR2-M ^j	GATACTATAATCGAACAATAATCTA <u>at</u> CT <u>aa</u> ATATTTAAACTAT AACGTTGAT
farR3-P ^j	ATCAACGTTATAATTTAAATAT <u>tt</u> AG <u>at</u> TAGATTATTGTTCGATT ATAGTATC
farR3-M ^j	GATACTATAATCGAACAATAATCTA <u>at</u> CT <u>aa</u> ATATTTAAATTAT AACGTTGAT
fakA-pALC-F ^k	tttggtaccACAGGCAAGAAAGCTTAGGAGGAC
fakA-pALC-R ^k	tttggtaccGCAACTCGAGAACGATACTTTTAACC
farER ^{IS} -F1 ¹	/5IRD800/CCCCATCTTATATAAAAATTTTGCC
farER ^{IS} -R1	CTACATTTCTCCTTTGTGTGTGTAG
<i>farER</i> ^{IS} -F2	ATGACCGCGGACCATTTATGT
<i>farER</i> ^{IS} -F2 <i>farER</i> ^{IS} -R2	ATGACCGCGGACCATTTATGT GTACGGTGTACGAGTGCGTT
<i>farER</i> ^{IS} -F2 <i>farER</i> ^{IS} -R2 <i>farER</i> ^{UP} -F ¹	ATGACCGCGGACCATTTATGT GTACGGTGTACGAGTGCGTT /5IRD800/GTTTTTCAATCTTTTTTTTCGTATCTAACG
<i>farER</i> ^{IS} -F2 <i>farER</i> ^{IS} -R2 <i>farER</i> ^{UP} -F ¹ <i>farER</i> ^{UP} -R	ATGACCGCGGACCATTTATGT GTACGGTGTACGAGTGCGTT /5IRD800/GTTTTTCAATCTTTTATTCGTATCTAACG GATGGGGACATTCATCGC
farER ^{IS} -F2 farER ^{IS} -R2 farER ^{UP} -F ¹ farER ^{UP} -R farER ^{DW} -F ¹	ATGACCGCGGACCATTTATGT GTACGGTGTACGAGTGCGTT /5IRD800/GTTTTTCAATCTTTTATTCGTATCTAACG GATGGGGACATTCATCGC /5IRD800/GATGAAAGAGACTGATTTACGAG
farER ^{IS} -F2 farER ^{IS} -R2 farER ^{UP} -F ¹ farER ^{UP} -R farER ^{DW} -F ¹ farER ^{DW} -R	ATGACCGCGGACCATTTATGT GTACGGTGTACGAGTGCGTT /5IRD800/GTTTTTCAATCTTTTATTCGTATCTAACG GATGGGGACATTCATCGC /5IRD800/GATGAAAGAGACTGATTTACGAG CATATTTATCATAAAATGTTTATAAAATGTTGTACGG

IRD800OP1 1	/5IRD800/GTATATTGCCTCCTTTTAAAAATCAACGTTATAGTTTA AATATA
IRD800OP21	/5IRD800/GTTATAGTTTAAATATACAGTGTAGATTATTGTTCG ATTATAG
IRD800OP31	/5IRD800/TTATTGTTCGATTATAGTATCTATCCCCGACCTCTTA AAGAAT
IRD800OP41	/5IRD800/GAATCAATTGGAAAAATTTTGTATATTAAACTACACA CAAAGGAGAAATGTAG
IRD800OP4.11	/5IRD800/TGTGTGTGTAGTTTAATATACAAAAT
IRD800OP51	/5IRD800/AGTTTAAATATACAGTGTAGATTATTGTT
IRD800OP5.11	/5IRD800/TTTAAATATACAGTGTAGATTATTG
IRD800OP5.2li	/5IRD800/TTTAAATATACAGTGTAaATTATTG
OP1.1	GTATATTGCCTCCTTTTAAAATCAACGTTA
OP1.2	GCCTCCTTTTAAAATCAACGTTATAGTTTA
OP1.3	TTTTAAAATCAACGTTATAGTTTAAATATA
ⁱ OP1.3 _{G>A}	TTTTAAAATCAACGTTATAaTTTAAATATA
IRD800OP1.31	/5IRD800/TTTTAAAATCAACGTTATAGTTTAAATATA

^aAdditions to or modification of primer sequences include addition of ^b*attB1* GGGGACAAGTTTGTACAAAAAGCAGGCT or ^d*attB2* GGGGACCACTTTGTACAAGAAAGCTGGGT; restriction sites (lower case bold) ^cSacII, ^eBamHI, ^fSalI, ^gSacI, ^hHindIII, ^kKpnI; ^lIRD800 fluorophore for EMSA (complementary nonlabelled strand is not shown); substitutions in mutagenic oligonucleotides (lower case underlined) ⁱG>A substitution in ⁻¹⁰P_{farR}, ^jsubstitutions in IR1 and IR2-octamers of PAL1, or ^msubstitutions in both ⁻¹⁰P_{farR} and PAL1

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