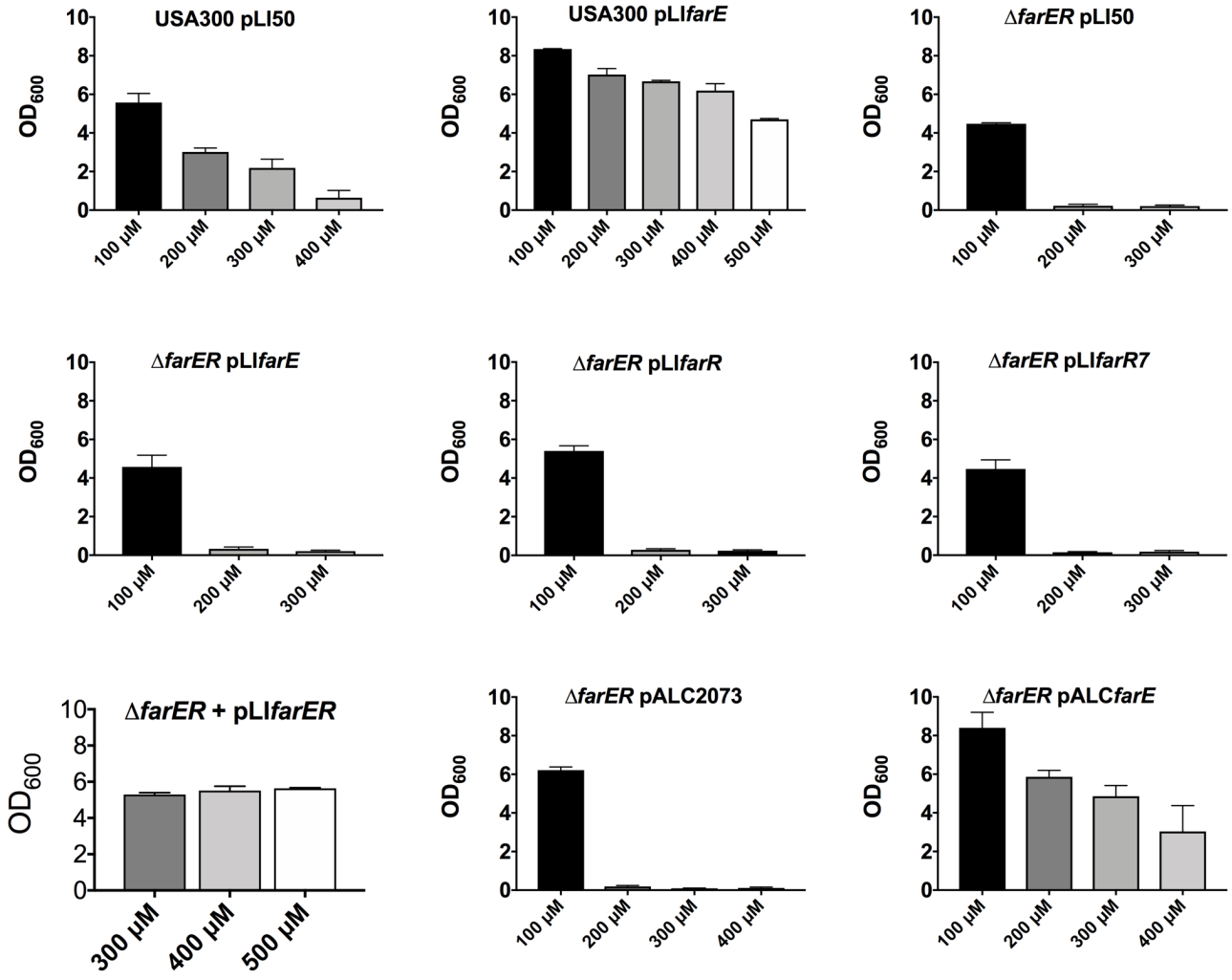

Figure S1. Data for MIC determination of *S. aureus* growth in TSB supplemented with linoleic acid. Inoculum cultures were grown to mid-exponential phase in TSB containing antibiotics as required, and 20 ng/mL anhydrotetracyline for cultures with pALC2073 or pALC*farE*. Cultures were then inoculated into triplicate tubes of TSB containing varying concentrations of linoleic acid. Growth (OD_{600}) was quantified after 24h and is plotted as the mean ± standard deviation of triplicate cultures.

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.

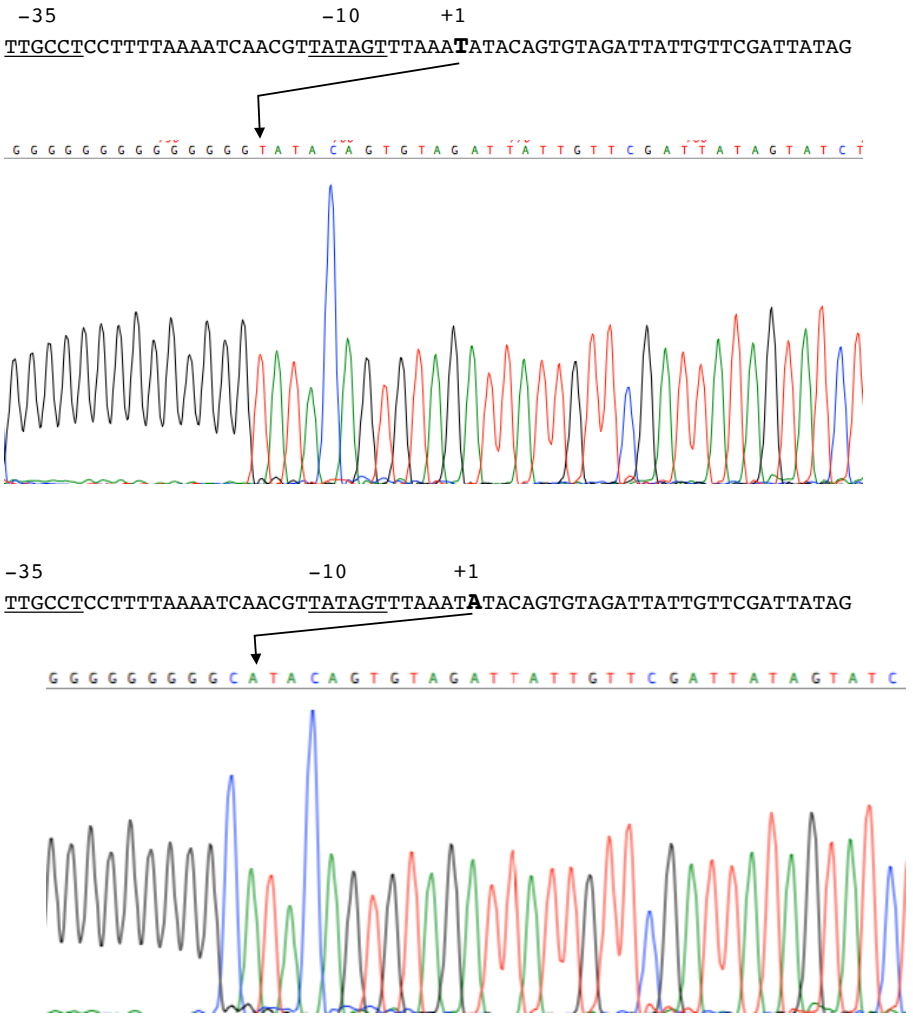

Figure S3. Sequence chromatograms from cloned 5′-RACE products for determination of the +1 transcription start site of P_{farR}. The nucleotide sequence of P_{farR} and downstream sequence is shown above each chromatogram, and underlined nucleotides identify the -35 and -10 motifs of P_{farR}. The transcription 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.

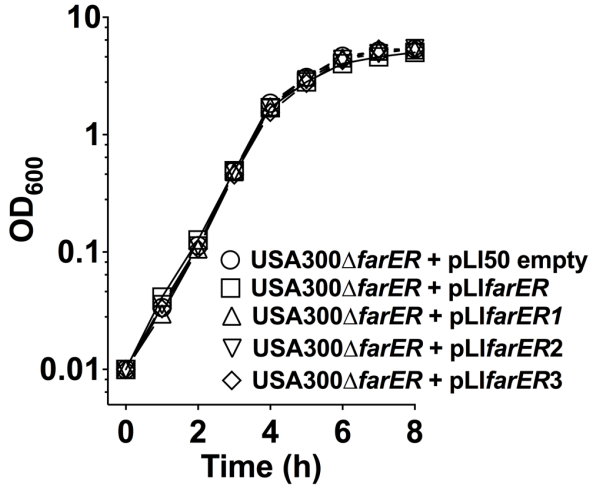

Figure S4. Growth of USA300Δ*farER* complemented with pLI50 or pLI*farER* and derivatives in TSB. Each data point represents the mean and standard deviation of triplicate cultures.

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 *et al.*, 2012)
RN4220	r_K^- m_K^+; capable of accepting foreign DNA	(Novick, 1991)
USA300*farE::*ΦNE	USA300 LAC with transposon insertion in *farE* (SAUSA300_2489); Erm^r.	(Alnaseri *et al.*, 2015)
USA300Δ*farER*	USA300 with *farER* deletion; constructed using pKORΔ*farER*	This study
USA300Δ*fakA*	USA300 with *fakA* deletion; constructed using pKORΔ*fakA*	This study
USA300Δ*farERfakA*	USA300 with deletion of *fakA* and *farER*	This study
USA300Δ*farER*pLI50	USA300Δ*farER* with pLI50; Cm^r	This study
USA300Δ*farER*pLI*farE*	USA300Δ*farER* complemented with pLI*farE*; Cm^r	This study
USA300Δ*farER*pLI*farER*	USA300Δ*farER* complemented with pLI*farER*; Cm^r	This study
USA300Δ*farER*pLI*farER*1	USA300Δ*farER* complemented with pLI*farER*1; Cm^r	This study
USA300Δ*farER*pLI*farR*	USA300Δ*farE*R complemented with pLI*farR*; Cm^r	This study
USA300Δ*farER*pLI*farR*1	USA300Δ*farE*R complemented with pLIf*arR*1; Cm^r	This study
USA300Δ*fakA*pALC2073	USA300Δ*fakA* with empty pALC2073 complementation vector; Cm^r.	This study
USA300Δ*fakA*pAL*fak*^{ON}	USA300Δ*fakA* complemented with *fakA* cloned in plus orientation in pALC2073; Cm^r	This study

USA300Δ*fakA*pAL*fak*^{OFF}	USA300^{fakA} complemented with *fakA* cloned in minus orientation in pALC2073; Cm^r	This study

E. coli

DH5α	F$^-$ Φ80*lacZ*ΔM15 *recA1 endA1 gyrA96 thi-1 hsdR17* ($r_K^- m_K^+$) *supE44 relA1 deoR* Δ(*lacZYA-argF*)*U169 phoA*	Invitrogen
M15[pREP4]	F-, Φ80*lac*ZΔM15 *thi lac mtl recA*$^+$; Host strain for pQE30. Contains pREP4 (Km^r) with constitutive *lacI* repressor	Qiagen

Plasmids:

pLI50	*E. coli-S. aureus* shuttle vector	(Lee and Iandolo, 1986)
pQE30	*E. coli* 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 *S. aureus*; genes are expressed at a basal level in absence of induction	(Bateman *et al.*, 2001)
pQE-FarR	*farR* open reading frame amplified from USA300 with primers 6H*farR*-F and 6H*farR*-R, cloned at *Sac*I and *Hind*III sites of pQE30.	This study
pQE-^{H121Y}FarR	As for pQE-FarR, except that template DNA was from *S. aureus* FAR7	This study
pLI*farR*	pLI50 with *farR* expressed from native promoter	(Alnaseri *et al.*, 2015)
pLI*farR*1	pLI*farR* after mutagenesis with *farR*1-P and *farR*1-M primers; G>A substitution in -10 motif of P_{farR}.	This study
pLI*farR*2	Mutagenesis of pLI*farR* with primers *farR*2-P and *farR*2-M; nucleotide substitutions in PAL1.	This study

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

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

Supplementary Table S2

Oligonucleotide	Description[a]
farE-UP-*attB1*[b]	*attB1*-TTCCTTTGCCTGTACGTGC
farE-UP-*SacII*[c]	ggacct**ccgcgg**AACGATGGCATTGTACCAAG
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]	ccc**ggatcc**TGCAGCTACAATCACTATCCATGC
farR::lux-R[f]	ccc**gtcgac**TAAATCAGTCTCTTTCATCTACATTTCTCC
farE-pALC-F[g]	CACTGTATATTTAAACTATAA**gagctc**TTTTAAAAGGAGGCAATATACTTGGT
farE-pALC-R[g]	CACTTCCATGCAAAAACCCT**gagctc**CAAATGTCATTGATAGAC
6H*farR*-F[g]	CTACACACAAAGGAGAAATGTA**gagctc**ATGAAAGAGACTGATTTACGAG
6H*farR*-R[h]	GGTAACGCTCATGAGTTTCT**aagctt**CTATTTAATCTTAATATTGATTAATCTATGG

fakA-UP-*attB1*[b]	*attB1*-GCGTGTGAACGTCTGTTACCAGTCGAAGC
fakA-UP-*SacII*[c]	ggacct**ccgcgg**CATTTCAAGTTGTCCTCCTAAGCTTTCTTGC
fakA-DW-*SacII*[c]	ggacct**ccgcgg**GTTCATGAAGGTGGACAACCAATTTATC
fakA-DW-*attB2*[d]	*attB2*-GATGACTTTTCTAATCTATTTAGCCATTGC
*farR*1-P[i]	CCTTTTAAAATCAACGTTATAaTTTAAATATACAGTGTAG
*farR*1-M[i]	CTACACTGTATATTTAAAtTATAACGTTGATTTTAAAAGG
*farR*2-P[j]	ATCAACGTTATAGTTTAAATATttAGatTAGATTATTGTTCGATTATAGTATC
*farR*2-M[j]	GATACTATAATCGAACAATAATCTAatCTaaATATTTAAACTATAACGTTGAT
*farR*3-P[j]	ATCAACGTTATAATTTAAATATttAGatTAGATTATTGTTCGATTATAGTATC
*farR*3-M[j]	GATACTATAATCGAACAATAATCTAatCTaaATATTTAAATTATAACGTTGAT
fakA-pALC-F[k]	ttt**ggtacc**ACAGGCAAGAAAGCTTAGGAGGAC
fakA-pALC-R[k]	ttt**ggtacc**GCAACTCGAGAACGATACTTTTAACC
farER[IS]-F1[l]	/5IRD800/CCCCATCTTATATAAAAATTTTGCC
farER[IS]-R1	CTACATTTCTCCTTTGTGTGTAG
farER[IS]-F2	ATGACCGCGGACCATTTATGT
farER[IS]-R2	GTACGGTGTACGAGTGCGTT
farER[UP]-F[l]	/5IRD800/GTTTTTCAATCTTTTTATTCGTATCTAACG
farER[UP]-R	GATGGGGACATTCATCGC
farER[DW]-F[l]	/5IRD800/GATGAAAGAGACTGATTTACGAG
farER[DW]-R	CATATTTATCATAAAAATGTTTATAAAATGTTGTACGG

^{IRD800}OP1 [l]	/5IRD800/GTATATTGCCTCCTTTTAAAATCAACGTTATAGTTTAAATATA
^{IRD800}OP2 [l]	/5IRD800/GTTATAGTTTAAATATACAGTGTAGATTATTGTTCGATTATAG
^{IRD800}OP3 [l]	/5IRD800/TTATTGTTCGATTATAGTATCTATCCCCGACCTCTTAAAGAAT
^{IRD800}OP4 [l]	/5IRD800/GAATCAATTGGAAAATTTTGTATATTAAACTACACACAAAGGAGAAATGTAG
^{IRD800}OP4.1[l]	/5IRD800/TGTGTGTAGTTTAATATACAAAAT
^{IRD800}OP5 [l]	/5IRD800/AGTTTAAATATACAGTGTAGATTATTGTT
^{IRD800}OP5.1[l]	/5IRD800/TTTAAATATACAGTGTAGATTATTG
^{IRD800}OP5.2[li]	/5IRD800/TTTAAATATACAGTGTAaATTATTG
OP1.1	GTATATTGCCTCCTTTTAAAATCAACGTTA
OP1.2	GCCTCCTTTTAAAATCAACGTTATAGTTTA
OP1.3	TTTTAAAATCAACGTTATAGTTTAAATATA
[i]OP1.3$_{G>A}$	TTTTAAAATCAACGTTATA<u>a</u>TTTAAATATA
^{IRD800}OP1.3[l]	/5IRD800/TTTTAAAATCAACGTTATAGTTTAAATATA

[a]Additions to or modification of primer sequences include addition of [b]*attB1* GGGGACAAGTTTGTACAAAAAAGCAGGCT or [d]*attB2* GGGGACCACTTTGTACAAGAAAGCTGGGT; restriction sites (lower case bold) [c]*SacII*, [e]*Bam*HI, [f]*Sal*I, [g]*Sac*I, [h]*Hind*III, [k]*Kpn*I; [l]IRD800 fluorophore for EMSA (complementary non-labelled strand is not shown); substitutions in mutagenic oligonucleotides (lower case underlined) [i]G>A substitution in $^{-10}P_{farR}$, [j]substitutions in IR1 and IR2-octamers of PAL1, or [m]substitutions in both $^{-10}P_{farR}$ and PAL1

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