

Fig. S1. The effect of the *ypfP*-deletion on the LTA expression in three different *S. aureus* strains. -, wild-type; Δ , a *ypfP*-transposon insertion mutant. USA, USA300.

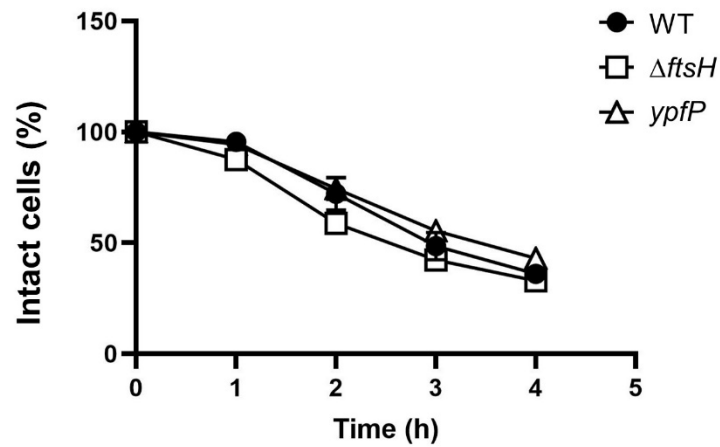


Fig. S2. The effect of the *ftsH* and the *ypfP* mutation on the autolysis activity of *S. aureus* USA300. WT, wild-type; $\Delta ftsH$, *ftsH*-deletion mutant; *ypfP*, a *ypfP* transposon insertion mutant.

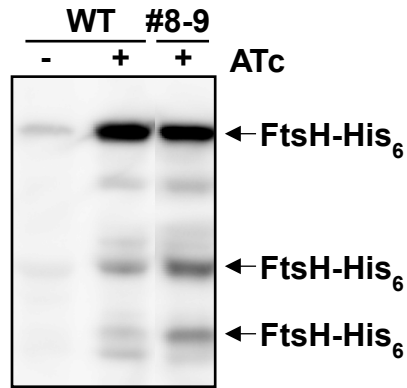


Fig. S3. FtsH expression in the wild-type and the suppressor mutant #8-9. All the strains contain the FtsH-expression plasmid pYJ-ftsH-His₆. FtsH expression was examined with an anti-His₆ antibody. ATc, anhydrotetracycline (100 ng/ml). Note that FtsH appears to be self-processed, as described previously {Akiyama, 1999 #6524}.

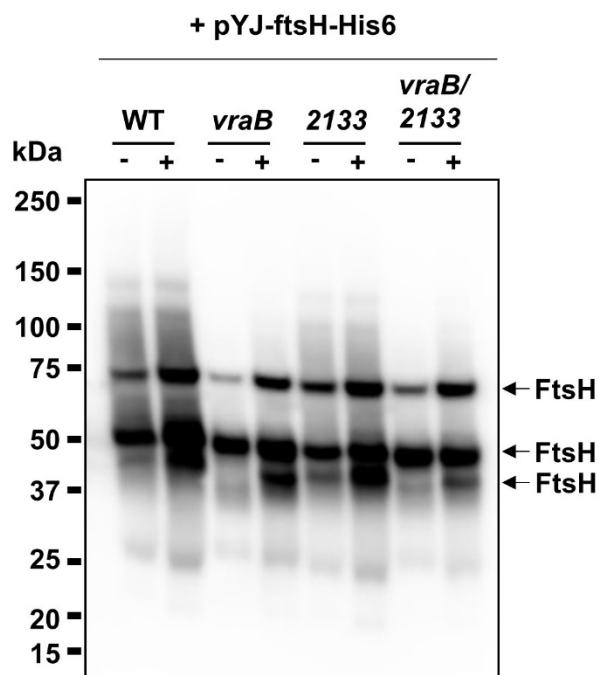


Fig. S4 FtsH expressions in the wild-type, *vraB*, 2133, and the *vraB*/2133 double mutant containing pYJ-ftsH-His6. FtsH expression was examined with an anti-FtsH antibody. The FtsH protein and its fragment species are indicated with arrows. -, no anhydrotetracycline; +, the addition of anhydrotetracycline. WT, USA300 wild-type; *vraB*, USA300::*vraB*(V144S); 2133, USA300::2133(V220L)

Table S1. Oxacillin MICs of FtsH-target gene mutants of USA300.

Mutated gene	Gene product	MIC (µg/ml)
-*		8
<i>lrgB</i>	antiholin-like protein LrgB	4
0310	hypothetical protein	8
0579	hypothetical protein	8
<i>saeQ</i>	SaeS regulatory protein SaeQ	8
<i>ypfP</i>	diacylglycerol glucosyltransferase	0.5
<i>cydA</i>	cytochrome D ubiquinol oxidase, subunit I	8
<i>cyoE</i>	protoheme IX farnesyltransferase	8
<i>hemA</i>	glutamyl-tRNA reductase	8
<i>htrA</i>	putative serine protease	8
<i>mbtS</i>	membrane-bound transcription factor	8
<i>hslO</i>	Hsp33-like chaperonin	8
<i>hrtA</i>	ATP-binding protein	8
<i>hrtB</i>	ABC transporter permease protein	8
2637	conserved hypothetical protein	8

* -, no mutation

Table S2. β-lactam resistance of *ypfP* mutants in three MRSA strain backgrounds.

Strains	MIC (µg/ml)		
	Oxacillin	Cefotaxime	Cefazolin
USA300	8	32	32
USA300:: <i>ypfP</i>	0.5	2	0.5
COL	256	128	32
COL:: <i>ypfP</i>	0.125	2	0.5
MW2	8	32	128
MW2:: <i>ypfP</i>	0.125	2	0.5

Table S3. The effect of the *ypfP*-deletion and the core suppressor mutations on the oxacillin MIC of the USA300.

Strains	MIC (μg/ml)
WT	8
<i>ΔftsH</i>	64
<i>ΔypfP</i>	0.5
<i>ΔftsHΔypfP</i>	0.5
WT (pYJ-ftsH-His ₆)	0.5
#8-9*	32
#8-13*	32
#8-9C**	32
#8-9C:: <i>ypfP</i>	0.125

* These strains contain pYJ-ftsH-His₆

** #8-9 without pYJ-ftsH-His₆

Table S4. Bacterial strains and plasmids used in this study

Strain/ plasmid	Relevant characteristic	Source
<i>E. coli</i>		
DH5 α	Plasmid free, Lac-	New England Biolabs
<i>S. aureus</i>		
RN4220	Restriction deficient, prophage cured	[48]
USA300-0114	Clinical isolate	NARSA*
COL	Clinical isolate	[33]
MW2	Clinical isolate	[34]
USA300-P23	USA300-0114 without plasmid 2 and 3	[49]
USA300 Δ <i>ftsH</i>	<i>ftsH</i> -deletion mutant of USA300-P23	[21]
USA300:: <i>mecA</i>	Transposon insertion in <i>mecA</i> in NE1868 transduced into USA300-P23	This study
USA300 Δ <i>ftsH</i> :: <i>mecA</i>	Transposon insertion in <i>mecA</i> in NE1868 transduced into USA300 Δ <i>ftsH</i>	This study
USA300:: <i>ypfP</i>	Transposon insertion in <i>ypfP</i> in NE1663 was transduced into USA300-P23	This study

USA300:: <i>lrgB</i>	Transposon insertion in <i>lrgB</i> in NE1726 transduced into USA300-P23	This study
USA300::0310	Transposon insertion in SAUSA300_0310 in NE1254 transduced into USA300-P23	This study
USA300::0579	Transposon insertion in SAUSA300_0579 in NE219 transduced into USA300-P23	This study
USA300:: <i>saeQ</i>	Transposon insertion in <i>saeQ</i> in NE1549 transduced into USA300-P23	This study
USA300:: <i>cydA</i>	Transposon insertion in <i>cydA</i> in NE117 transduced into USA300-P23	This study
USA300:: <i>cyoE</i>	Transposon insertion in <i>cyoE</i> in NE1434 transduced into USA300-P23	This study
USA300:: <i>hemA</i>	Transposon insertion in <i>hemA</i> in NE1742 transduced into USA300-P23	This study
USA300:: <i>htrA</i>	Transposon insertion in <i>htrA</i> in NE1141 transduced into USA300-P23	This study
USA300:: <i>mbtS</i>	Transposon insertion in <i>mbtS</i> in NE132 transduced into USA300-P23	This study
USA300:: <i>hslO</i>	Transposon insertion in <i>hslO</i> in NE1128 transduced into USA300-P23	This study

USA300:: <i>hrtA</i>	Transposon insertion in <i>hrtA</i> in NE1489 transduced into USA300-P23	This study
USA300:: <i>hrtB</i>	Transposon insertion in <i>hrtB</i> in Φ NE-8752 transduced into USA300-P23	This study
USA300::2637	Transposon insertion in SAUSA300_2637 in NE1164 transduced into USA300-P23	This study
USA300 Δ <i>ftsH</i> :: <i>ypfP</i>	Transposon insertion in <i>ypfP</i> in NE1663 transduced into USA300 Δ <i>ftsH</i>	This study
COL:: <i>ypfP</i>	Transposon insertion in <i>ypfP</i> in NE1663 was transduced into COL	This study
MW2:: <i>ypfP</i>	Transposon insertion in <i>ypfP</i> in NE1663 was transduced into MW2	This study
#8-9	A suppressor mutant of USA300 Δ <i>ftsH</i> (pYJ- <i>ftsH</i> -His ₆) whose oxacillin MIC is not affected by FtsH overexpression	This study
#8-13	Another suppressor mutant of USA300 Δ <i>ftsH</i> (pYJ- <i>ftsH</i> -His ₆) whose oxacillin MIC is not affected by FtsH overexpression	This study
#8-9C	#8-9 without pYJ- <i>ftsH</i> -His ₆	This study
USA300:: <i>vraB</i> (V144S)	The <i>vraB</i> (V144S) mutation was introduced into USA300-P23	This study

USA300::2133(V220L)	The SAUSA300_2133 (V220L) mutation was introduced into USA300-P23	This study
USA300:: <i>vraB</i> (V144S) /2133(V220L)	The <i>vraB</i> (V144S) and the SAUSA300_2133 (V220L) mutation were introduced into USA300-P23	This study
#8-9C:: <i>ypfP</i>	Transposon insertion in <i>ypfP</i> in NE1663 was transduced into #8-9C	This study

Plasmids

pYJ335	An <i>E. coli</i> - <i>S. aureus</i> shuttle vector with anhydrotetracycline-inducible promoter	[50]
pYJ-ftsH-His ₆	pYJ335 containing the <i>ftsH</i> gene with His-tag sequence at the C-terminus	[20]
pKOR1	An allelic replacement plasmid for <i>S. aureus</i>	[40]
pKOR1- <i>vraB</i>	pKOR1 carrying a 2-kb fragment with the <i>vraB</i> (V144S) mutation	This study
pKOR1-2133	pKOR1 carrying a 2-kb fragment with the SAUSA300_2133 (V220L) mutation	This study

* Network on Antimicrobial Resistance in *Staphylococcus aureus*.

Table S5. Oligonucleotides used in this study

Name	Sequence (5' > 3')	Target
P3764	TAAAATAAGCTTGATATCGATGGGAAGTAGGAGGAAATG	<i>ftsH</i>
P3765	TGACATTAGAAAACCGACTGCGTACTTCCAATCCAATGC	<i>ftsH</i>
P3766	AGCATTGGATTGGAAGTACGCAGTCGGTTTTCTAATGTCAC	pYJ335
P3767	TCATTTCTCCTACTTCCCATCGATATCAAGC	pYJ335
P4043	CCAAGCCGGAGCTGGC	<i>vraB</i>
P4044	GGAATATGTGATTTTAGACTTTCATCAGTGTTG	<i>vraB</i>
P4045	GGCAATGCCTCAGTACGCG	2133
P4046	ATTGCCAGGACGTTTCGACGG	2133
P4203	CTGCTAGCTAGCTAGAGATGCAGTGGAGATAAGCTGCATTCTTC	<i>vraB</i>
P4204	AACCGGTACCAATGGATCATTAACTTCATCTCTACTTCATCGCC	<i>vraB</i>
P4205	CTGCTAGCTAGCTAGAGATGGATGTGTTTGAATAATGGCAATGACAG	2133
P4206	AACCGGTACCAATGGATGCCCCGTGGCATTTCATCCG	2133
P4246	ACGTTGAGCCTCGGAACCGG	pKOR1
P4248	GTTAGTTTACAACAATGAACATACAATCCAATC	<i>vraB</i>
P4249	GTATCACATAACTCTTGAAAACGATTACAAAATCG	2133