Fig. S1

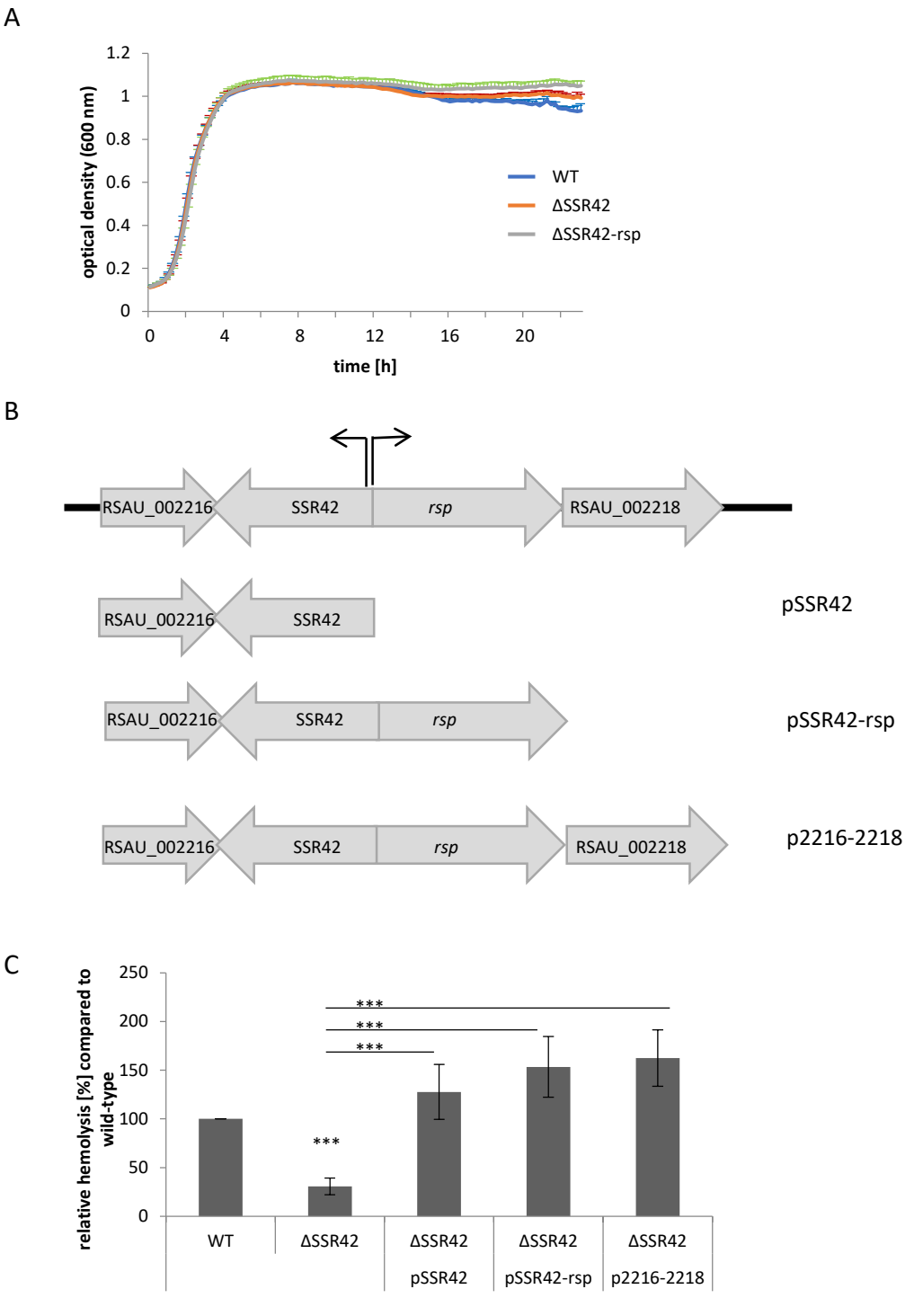
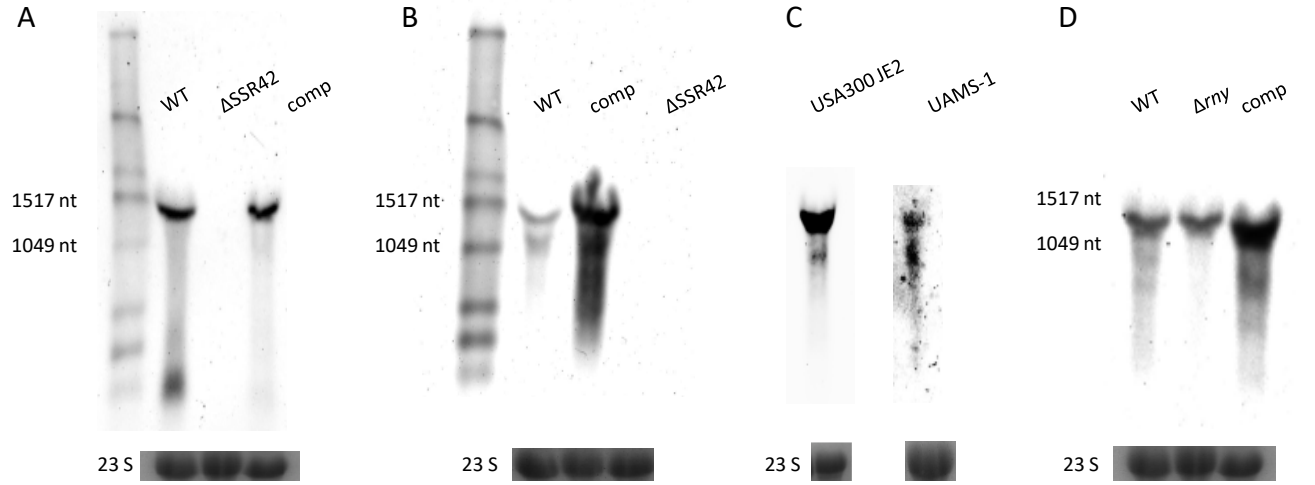


Fig. S1: Full length SSR42 is essential for wild-type levels of erythrocyte lysis but dispensable for growth. **A)** Deletion of SSR42 (ΔSSR42) or deletion of both, SSR42 and *rsp* (ΔSSR42-rsp), does not alter growth of *S. aureus* 6850 in TSB. Optical density of *S. aureus* cultures was recorded at 600 nm absorbance over 23 hours in a Tecan Infinite 200 plate reader. **B**) Complementation plasmids containing SSR42 and neighboring genes were constructed. The cartoon shows the genomic regions which were cloned into the complementation episomes. **C**) Decreased hemolysis in *S. aureus* ΔSSR42 culture supernatants is complemented by pSSR42, pSSR42-rsp and p2216-2218. For quantification of hemolysis sheep erythrocytes were exposed to stationary growth phase culture supernatants and absorbance of liberated hem at 405 nm was determined. Hemolytic level of wild-type supernatants (WT) was set to 100%. Statistical analysis was performed using Student´s t-test: ***: p< 0.001.

Fig. S2



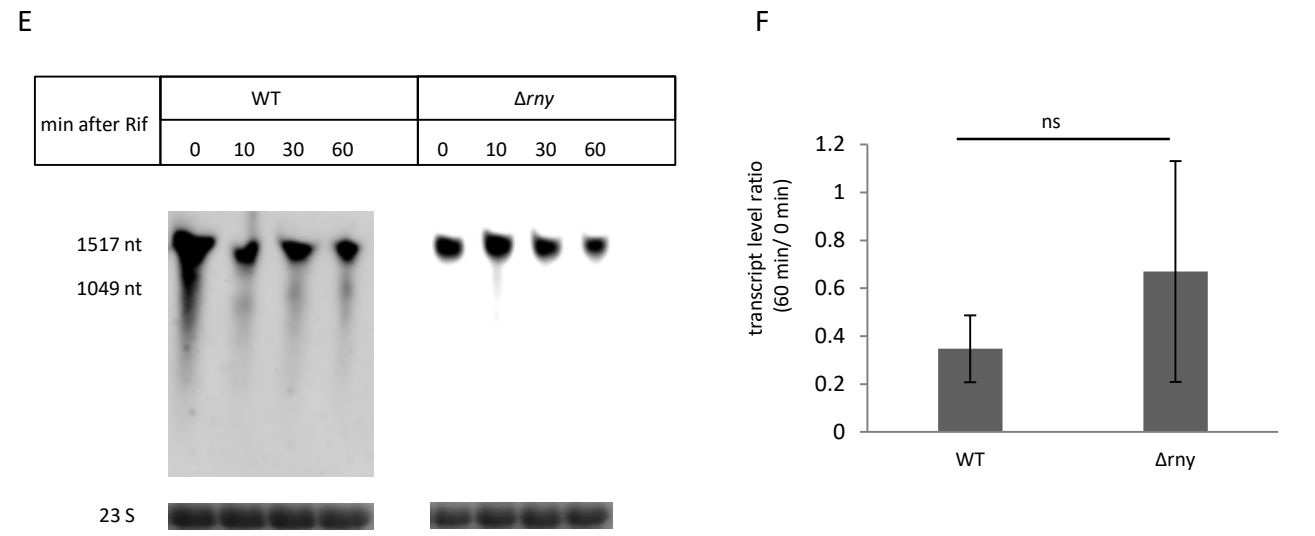
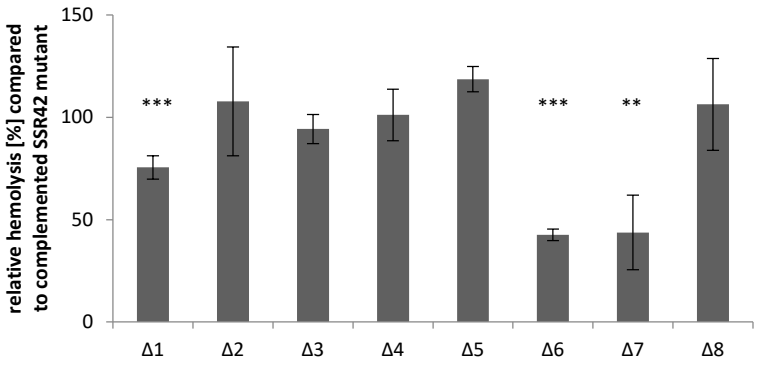


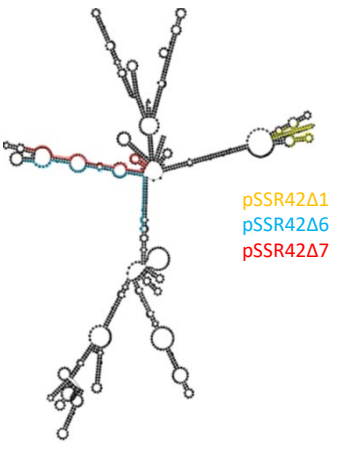
Fig. S2: SSR42 is a 1232 nt primary transcript and is processed. **Analysis of regions of SSR42 required for wild-type hemolysis**. Northern blots from stationary growth phase RNA of *S. aureus* 6850 (10 µg total RNA) were hybridized with **A**) probe 1 (5'-end of SSR42) and **B**) probe 2 (850-989 nt of SSR42). **C**) Northern blot of RNA extracted from *S. aureus* JE2 (10 µg) and UAMS-1 (40 µg) using probe 2 for detection of SSR42. **D**) RNase Y cleaves SSR42 producing an approximately 1050 nt product which is lost in a Δrny strain of *S. aureus* as demonstrated by Northern Blot in *S. aureus* JE2. **E)** Deletion of *rny* does not affect the stability of SSR42 transcript as shown by rifampicin assay followed by Northern blotting. **F**) Chemiluminescence signals were quantified using ImageJ. Loading controls (23S rRNA) were run on the same gel. Statistical analysis was performed using Student´s t-test.

Fig. S3

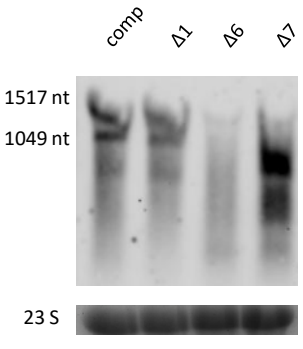
A



B



C



D

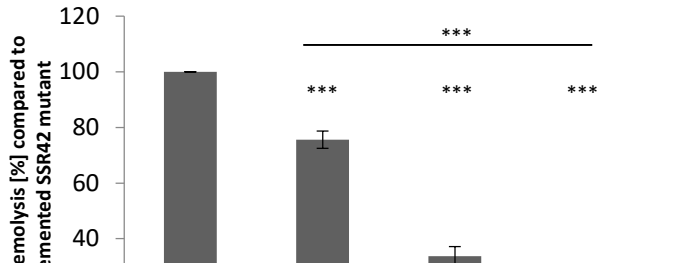
5´

ttaatatagatttcaaacctatgtatttcaaaaaacaatacatagccattttgaactaacttgctaatcttttattgatattcaactatgttataaaaaaagcaaat
tgaccaaaatggaagtttgaatcaaaaaacattaaatgtacccatgacttaaatttttatcgccattatttaagtttggacatacacttttgtcatcaaacgctgaa
aattagtacgattgaaatcacttcttgttataaaaatttcttagtagccctgttacaaaatattcgctacaaattattcaaatcaacaacaataaccatcaactaag
aaattttaaacaatccactaaaaaataaagcgtttaaaagttctgttagaaaacatcaagataagtataaacatctagaaaagtgaacatctaagaaaatcc
atctagttattgccgtaacaattagaataatcatttgaaaaagtgcattagacagcgaaattcaccatcaagataatgaagcaacaagaaacagcacacatca
atgaattaacaccttagattcatcatgagaaaatgcatggcacaactagatttaccatcaagataattcttaagcagcgaagaattaacatctagaaatgcata
aacatcttagattttacatcaagagatgcgataactataaacaactagaaacgtcaccaagatagtgcaacaacaaagaatcagcatcttgaaatcgcactaa
catctaagaatcaacatcaagtattatattaacatcatagataacttcatcaagacaggcatccacatctaagaaactcatctagaatagctaacacctaacat
caaagaatattcatcaagaacagtatctaacagaacttttgactatatatgaattaattatgttactagctaaatcatctattattaactttcacactcaatttcta
catctaatatcaatttattaacaatagaattagcagcaacaagatattacttaaaattactccccgaatttaaaatataaacaatgtaataattaagggtataa
attgatttatgctgtaactaattaaatgtgtatggtatcttctaacatcaagaaacgcattttaatatagatatgtacatttaaaccttgagagatagattatagta
aactttagtacaaagtatgaattgctgaactccaatgactactatttgtccgttacaactaaagtttcaatctatc 3´

Probe 1
Probe 2
pSSR42Δ1
pSSR42Δ6
pSSR42Δ7

RNase Y cleavage site
5´end Morrison et al., 2012

E



mini-1 mini-2

F

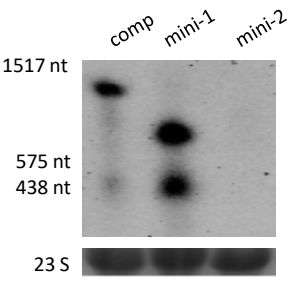
5´

ttaatatagatttcaaacctatgtatttcaaaaaacaatacatagccattttgaactaacttgctaatcttttattg
atattcaactatgttataaaaaaagcaaattgaccaaaatggaagtttgaatcaaaaaacattaaatgtaccc
atgacttaaatttttatcgccattatttaagtttggacatacacttttgtcatcaaacgctgaaaattagtacgatt
gaaatcacttcttgttataaaaatttcttagtagccctgttacaaaatattcgctacaaattattcaaatcaacaa
caataaccatcaactaagaaatttttaaacaatccactaaaaaataaagcgtttaaaagttctgtttagaaaac
atcaagataagtataaacatctagaaaagtgaacatctaagaaaatccatctagttattgccgtaacaattag
aataatcatttgaaaaagtgcattagacagcgaaattcaccatcaagataatgaagcaacaagaaacagcac
acatcaatgaattaacaccttagattcatcatgagaaaatgcatggcacaactagatttaccatcaagataatt
cttaagcagcgaagaattaacatctagaaatgcataaacatcttagattttacatcaagagatgcgataactat
aaacaactagaaacgtcaccaagatagtgcaacaacaaagaatcagcatcttgaaatcgcactaacatctaa
gaatcaacatcaagtattatattaacatcatagataacttcatcaagacaggcatccacatctaagaaactcat
ctagaatagctaacacctaacatcaaagaatattcatcaagaacagtatctaacagaacttttgactatatat
gaattaattatgttactagctaaatcatctattattaactttcacactcaatttctacatctaatatcaatttatta
acaatagaattagcagcaacaagatattacttaaaattactccccgaatttaaaatataaacaatgtaataat
taagggtataaattgatttatgctgtaactaattaaatgtgtatggtatcttctaacatcaagaaacgcattttaa
tatagatatgtacatttaaaccttgagagatagattatagtaaactttagtacaaagtatgaattgctgaactcc
aatgactactatttgtccgttacaactaaagtttcaatctatc 3´

Deletion mini-1
Additional deletion mini-2
Stem Loop

G

H



I

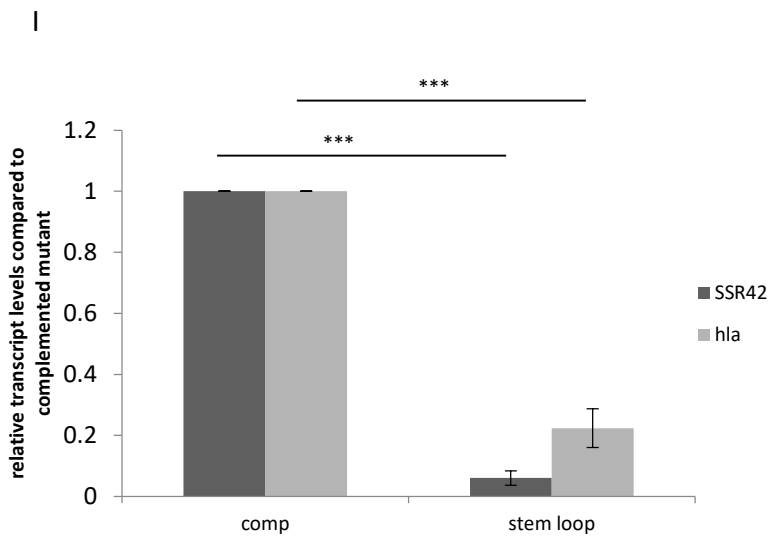


Fig. S3: Structural analysis of SSR42 reveals three domains implicated in stabilization of SSR42 and hemolysis regulation. A) A certain structural domain is required for full hemolytic capacity of *S. aureus* 6850. Comparison of erythrocyte lysis induced by culture supernatants *S. aureus* 6850 ΔSSR42, which was complemented with an SSR42 expression plasmid containing small sequence deletions Δ1-Δ8. **B**) Secondary structure of SSR42 predicted by RNA-fold. Highlighted are the regions Δ1, Δ6, and Δ7, whose deletion results in reduced hemolysis. **C**) These regions are essential for stabilization of SSR42 as shown by Northern blot. **D**) SSR42 sequence with highlighted deleted regions, position of SSR42 Northern Blot probes, RNase Y cleavage site and the 5´-end identified by Morrison et al, 2012. **E**) Secondary structure of minimal version 1 and 2 of SSR42 predicted by RNA-fold. **F**) SSR42 sequence with highlighted deleted regions for creation of minimal versions of SSR42. **G**) Hemolysis is partially restored by introducing minimal SSR42 version 1 but not version 2 into *S. aureus* 6850 ΔSSR42. Comparison of erythrocyte lysis induced by culture supernatants of *S. aureus* 6850 ΔSSR42, complemented for full-length SSR42 and 795 nt or 668 nt long minimal versions of SSR42. Statistical analysis was performed using student´s t-test: *: p< 0.05; ***: p< 0.001. **H**) Additional deletion in minimal SSR42 version 2 is required for stabilization of SSR42 as shown by Northern blot. **I**) Expression of only a stem loop structure of SSR42 encompassing the previous identified region Δ1 is not sufficient for complementation of SSR42 and *hla* expression.

Fig. S4

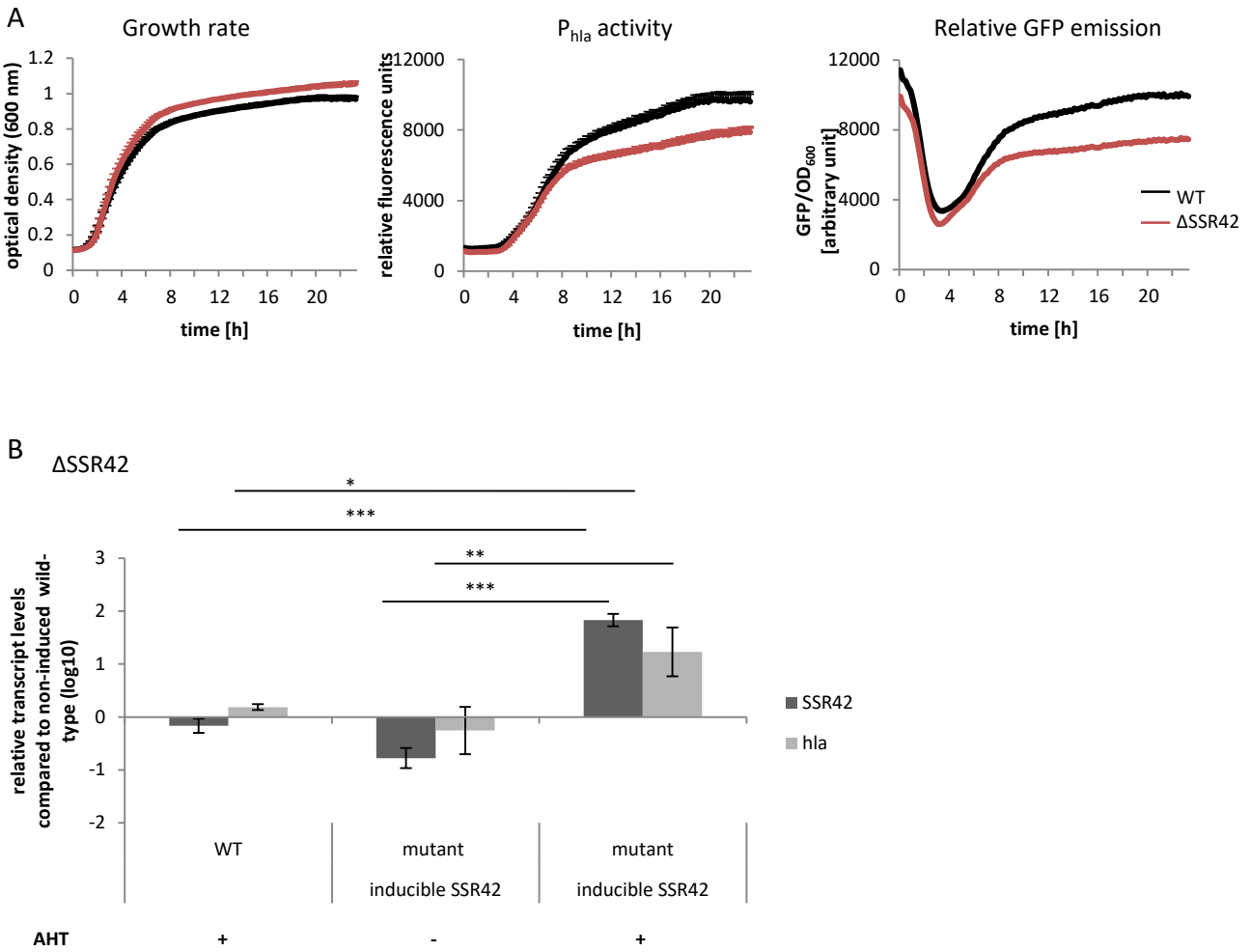
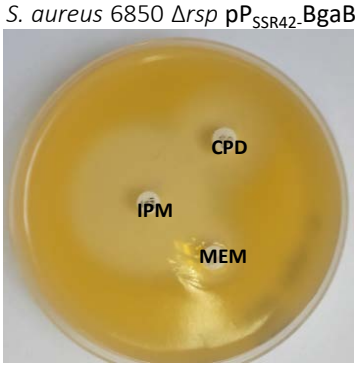


Fig. S4: SSR42 is required for *hla* transcription at wild-type expression levels. A) Whereas planktonic growth in TSB of *S. aureus* 6850 ΔSSR42 was enhanced when compared to wild-type (WT; upper left panel), *hla* promotor (P_{hla}) activity was significantly reduced in the deletion mutant starting 9 hours post inoculation ($p=7.34 \times 10^{-5}$; upper right panel). Lower Panel: GFP emission was normalized to bacterial growth (GFP/OD_{600}). Promotor activity of *hla* was measured using transcriptional fusion of P_{hla} and a promotorless GFP. OD_{600} as well as GFP emission was measured over 22 h in a TECAN Infinite 200 plate reader. **B)** *S. aureus* 6850 ΔSSR42 was transformed with pAHT-SSR42 enabling AHT-inducible expression of SSR42. qPCR demonstrates that addition of AHT to exponential growth phase bacteria (1.5 h after inoculation) not only led to transcription of SSR42, but also *hla* message when compared to non-induced samples or AHT-treated wild-type bacteria (WT). Statistical analysis was performed using Student´s t-test: *: p< 0.05; **: p<0.01; ***: p< 0.001.

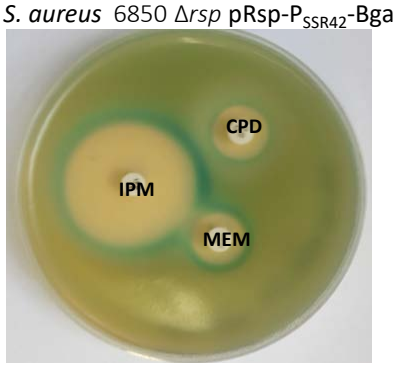
Fig. S5

A

S. aureus 6850 Δ*rsp* pP$_{SSR42}$-BgaB

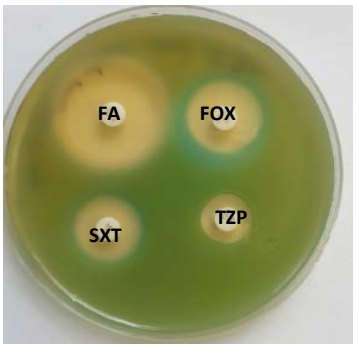


S. aureus 6850 Δ*rsp* pRsp-P$_{SSR42}$-BgaB

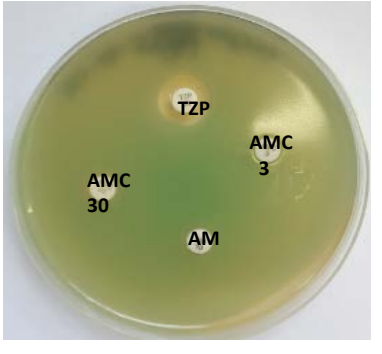


B

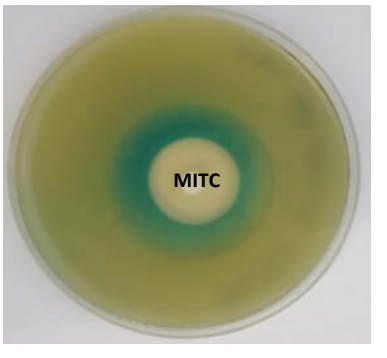
S. aureus 6850 pP$_{SSR42}$-BgaB



S. aureus 6850 pP$_{SSR42}$-BgaB

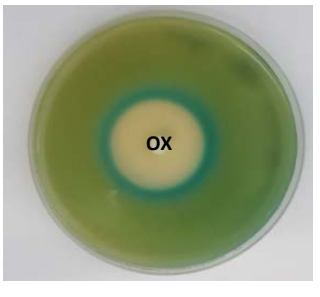


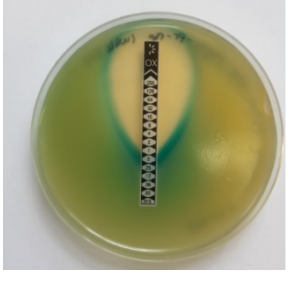
S. aureus 6850 pP$_{SSR42}$-BgaB



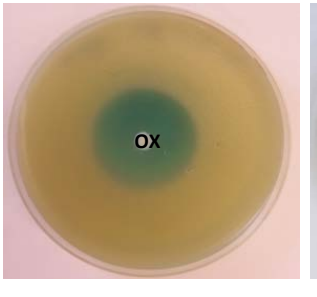
C

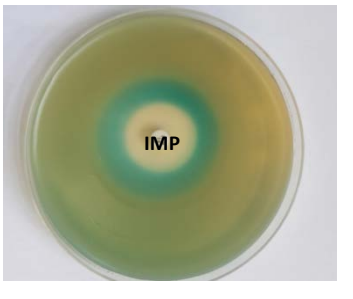
S. aureus 6850 pP$_{SSR42}$-BgaB





S. aureus JE2 pP$_{SSR42}$-BgaB





D

S. aureus HG001 pP$_{SSR42}$-BgaB

S. aureus HG001 lexAG94E pP$_{SSR42}$-BgaB

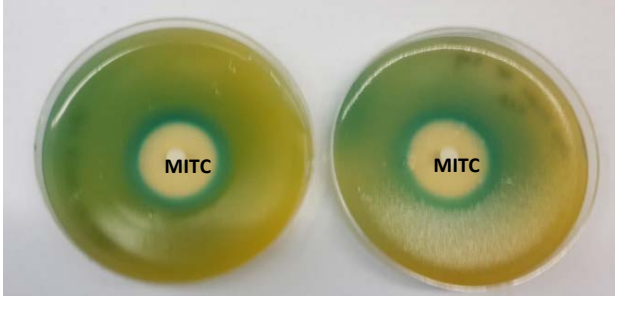


Fig. S5: Disk diffusion assays demonstrate SSR42 promotor activation by antibiotics. The plasmid vector, pP$_{SSR42}$-BgaB, expressing the β-galactosidase BgaB under control of P$_{SSR42}$ was transformed in *S. aureus* strains 6850, 6850 Δ*rsp*, or JE2 where indicated. Bacteria were plated on TSB/X-Gal and disks with various antibiotics were applied followed by overnight incubation at 37 °C. Promoter activation is indicated by formation of blue dye. **A**) Application of imipenem (IPM), and meropenem (MEM), and cefpodoxim (CPD) resulted in P$_{SSR42}$ activation. This was strongly dependent on the presence of Rsp, since no blue dye is formed in *S. aureus* 6850. **B**) *S. aureus* 6850 fusidic acid (FA), cefoxitin (FOX), trimethoprim-sulphamethoxazole (SXT), piperacillin-tazobactam (TZP), ampicillin (AC) and amoxicillin-clavulanate (AMC). **C**) Oxacillin applied to *S. aureus* 6850 as diffusion disk or via MIC strip resulted in activation of P$_{SSR42}$ beyond the inhibition zone, whereas the MRSA strain JE2 is resistant to oxacillin and hence the whole area demonstrates P$_{SSR42}$ activation. By contrast, imipenem, to which JE2 is sensitive, shows blue coloration beyond the inhibition zone. **D**) Mitomycin C (MITC) induces P$_{SSR42}$ activity, but this activation is independent of *lex*A- mediated SOS-response since blue dye is formed in *S. aureus* HG001 as well as an isogenic *lex*A-G94E mutant.

Fig. S6

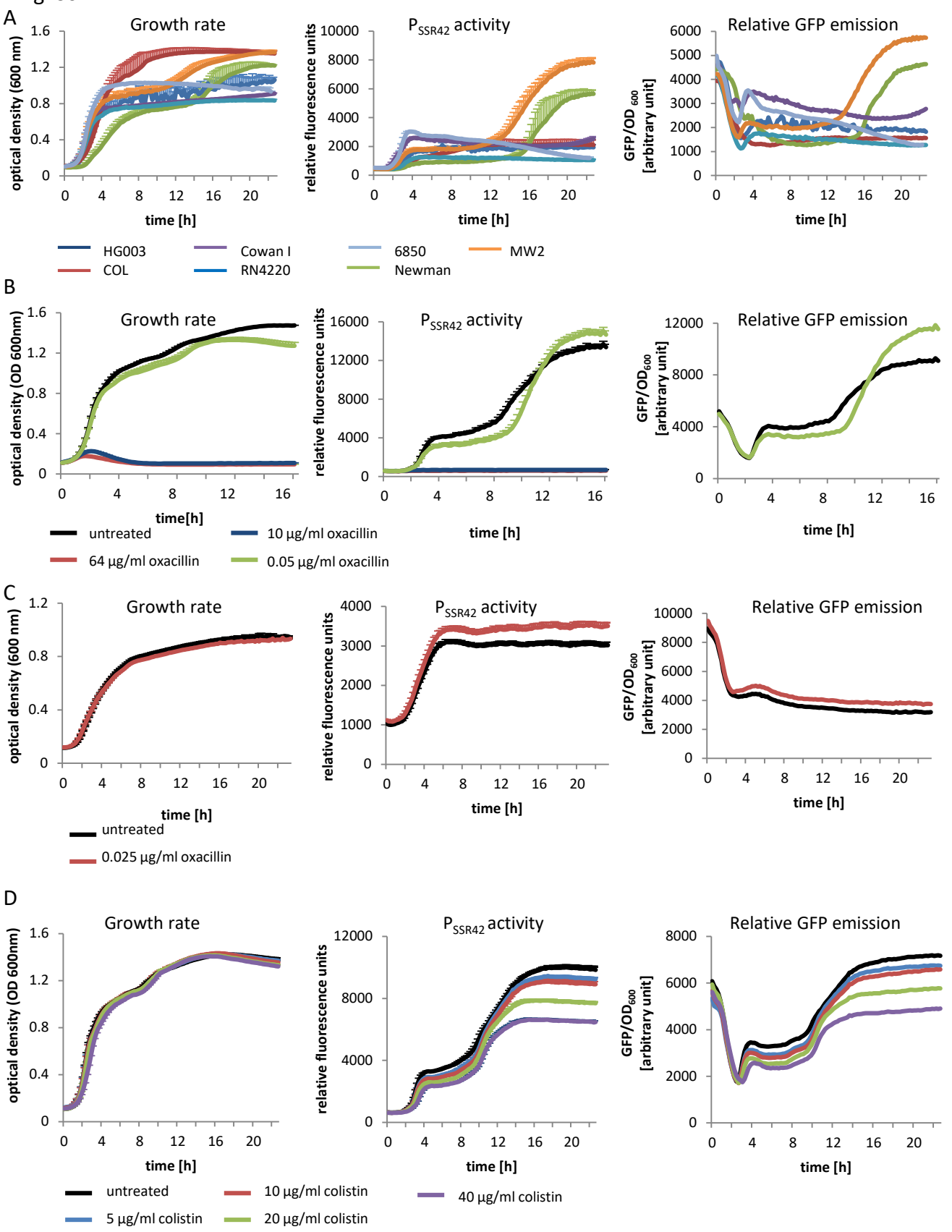
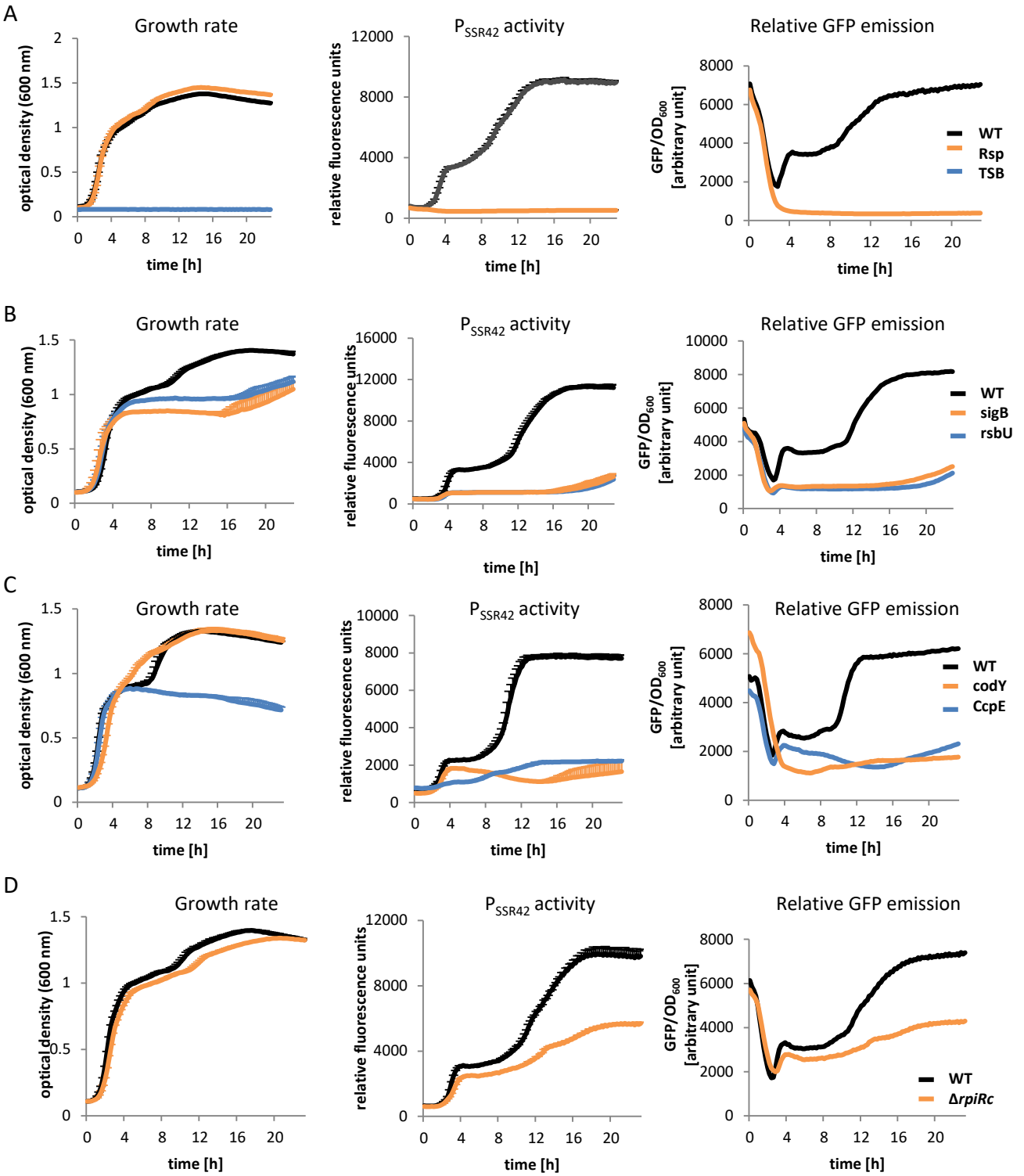
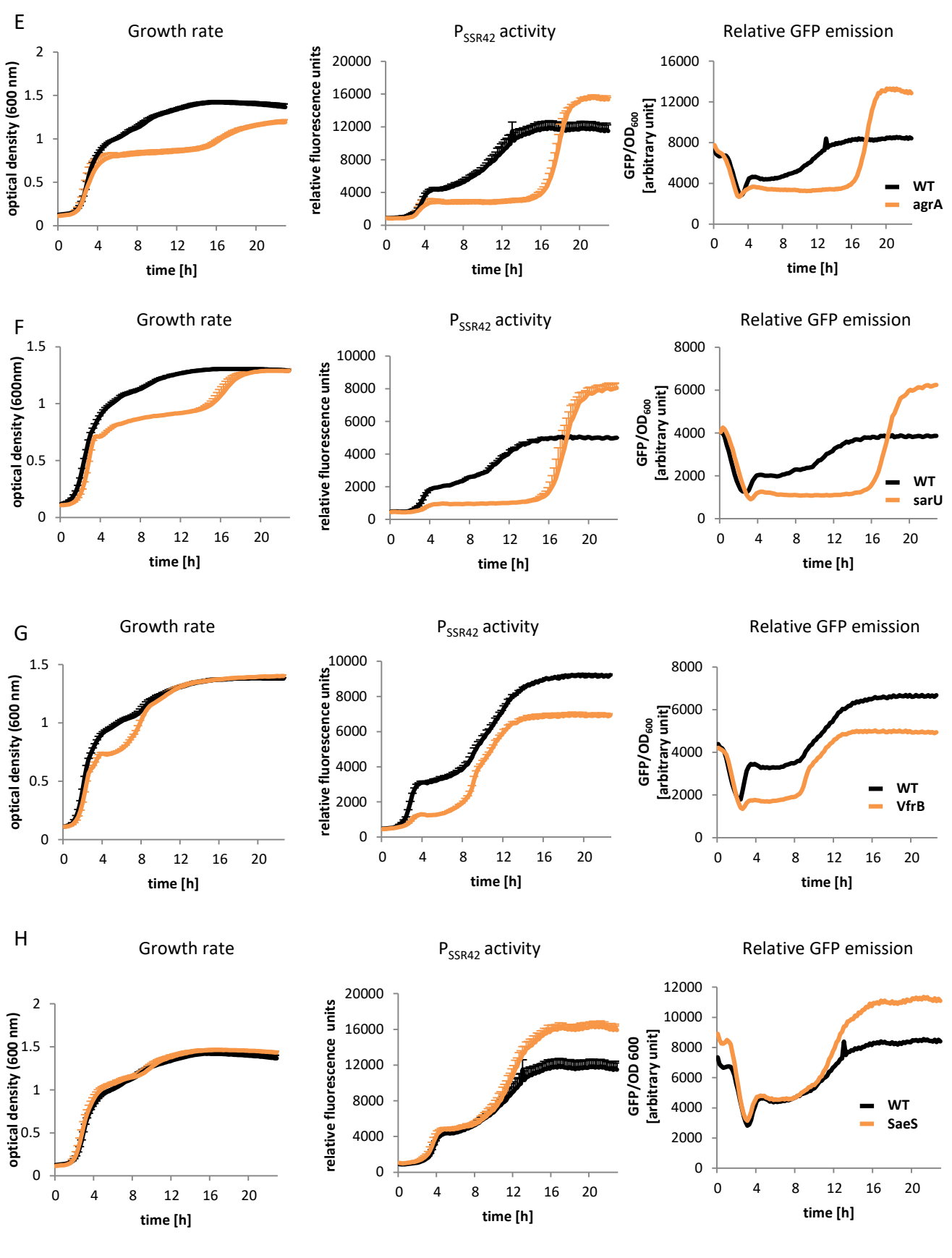
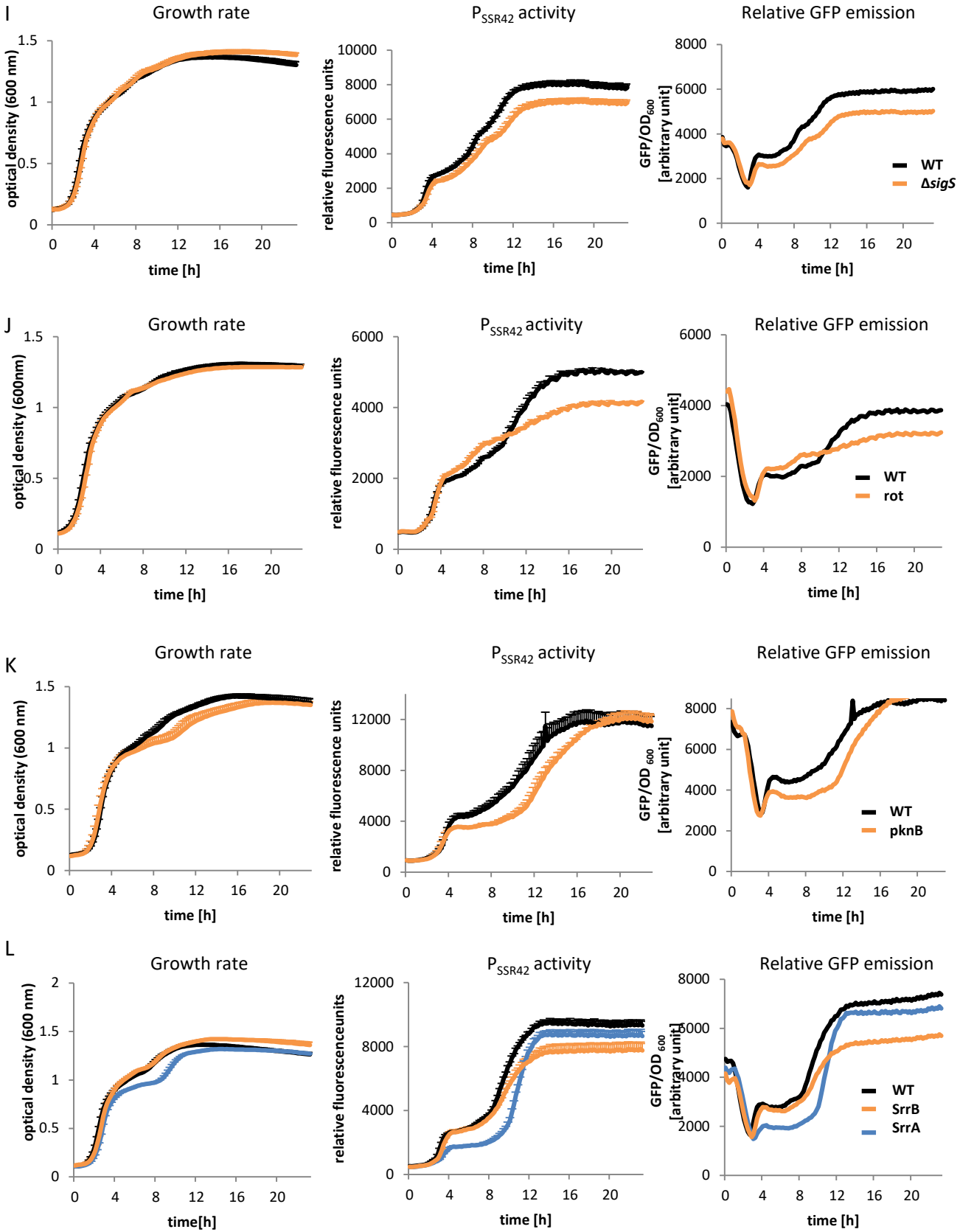


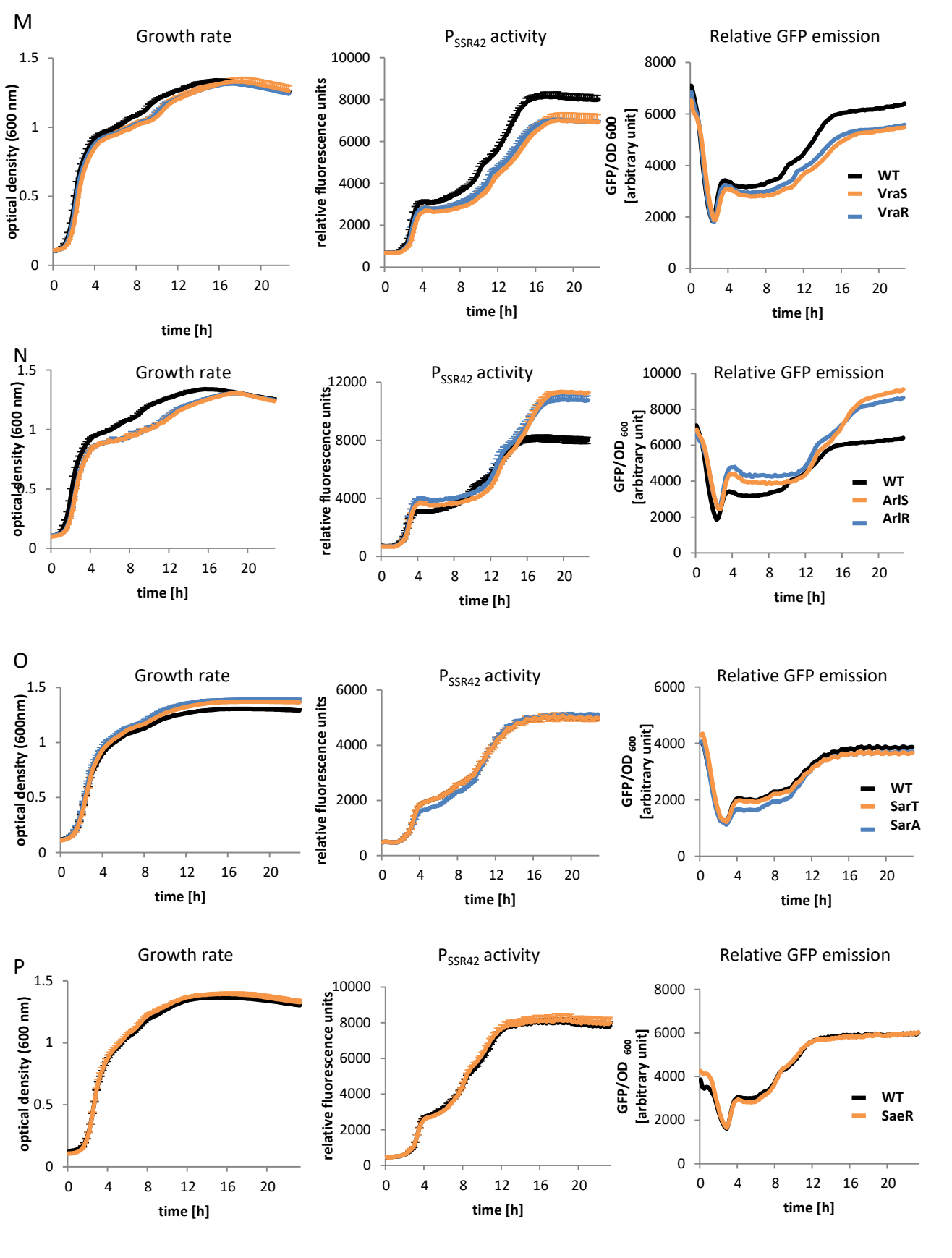
Fig. S6: The promoter of SSR42 is highly active in *S. aureus* JE2. Sub-inhibitory concentration of oxacillin increases and colistin decreases SSR42 promotor activity. P_{SSR42} activity was measured by using a transcriptional fusion of the promoter and GFP. OD_{600} as well as GFP emission was measured over 22 h in a TECAN Infinite 200 plate reader. **A**) P_{SSR42} promotor activity profiles in *S. aureus* strains JE2 and 6850 differ significantly with JE2 demonstrating a prominent second activation phase after 9 hours post inoculation. P_{SSR42} activity profiles in other strains demonstrate that only strains MW2 and Newman share a similar profile with JE2, whereas other strains do not show a secondary activation peak. The plasmid vector, pP_{SSR42}-GFP, expressing the GFP under control of P_{SSR42} was transformed in various *S. aureus* strains. OD_{600} as well as GFP emission was measured over 23 hours in a TECAN Infinite 200 plate reader and a normalized signal (GFP/OD) was calculated. **B**) Treatment of *S. aureus* JE2 pP_{SSR42}-GFP with sub-inhibitory concentration of 0.05 µg/ml oxacillin resulted in alteration of SSR42 promotor activity depending on the growth phase, whereas higher concentrations resulted in inhibition of bacterial growth. **C**) Similarly, treatment of the MSSA 6850 with 0.025 µg/ml oxacillin resulted in enhanced SSR42 promotor activity. **D**) Treatment of *S. aureus* JE2 with colistin decreased SSR42 promotor activity in a dose-dependent manner.

Fig. S7


A
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
Rsp
TSB
B
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
sigB
rsbU
C
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
codY
CcpE
D
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
ΔrpiRc


E
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
agrA
F
Growth rate
optical density (600nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
sarU
G
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
VfrB
H
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD 600 [arbitrary unit]
time [h]
WT
SaeS


I
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
ΔsigS
J
Growth rate
optical density (600nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
rot
K
Growth rate
optical density (600 nm)
time [h]
P SSR42 activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD 600 [arbitrary unit]
time [h]
WT
pknB
L
Growth rate
optical density (600 nm)
time[h]
P SSR42 activity
relative fluorescenceunits
time [h]
Relative GFP emission
GFP/OD 600 [arbitrary unit]
time [h]
WT
SrrB
SrrA


M
Growth rate
optical density (600 nm)
time [h]
P$_{SSR42}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD 600 [arbitrary unit]
time [h]
WT
VraS
VraR
N
Growth rate
optical density (600 nm)
time [h]
P$_{SSR42}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD $_{600}$ [arbitrary unit]
time [h]
WT
ArlS
ArlR
O
Growth rate
optical density (600nm)
time [h]
P$_{SSR42}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD $_{600}$ [arbitrary unit]
time [h]
WT
SarT
SarA
P
Growth rate
optical density (600 nm)
time [h]
P$_{SSR42}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD $_{600}$ [arbitrary unit]
time [h]
WT
SaeR

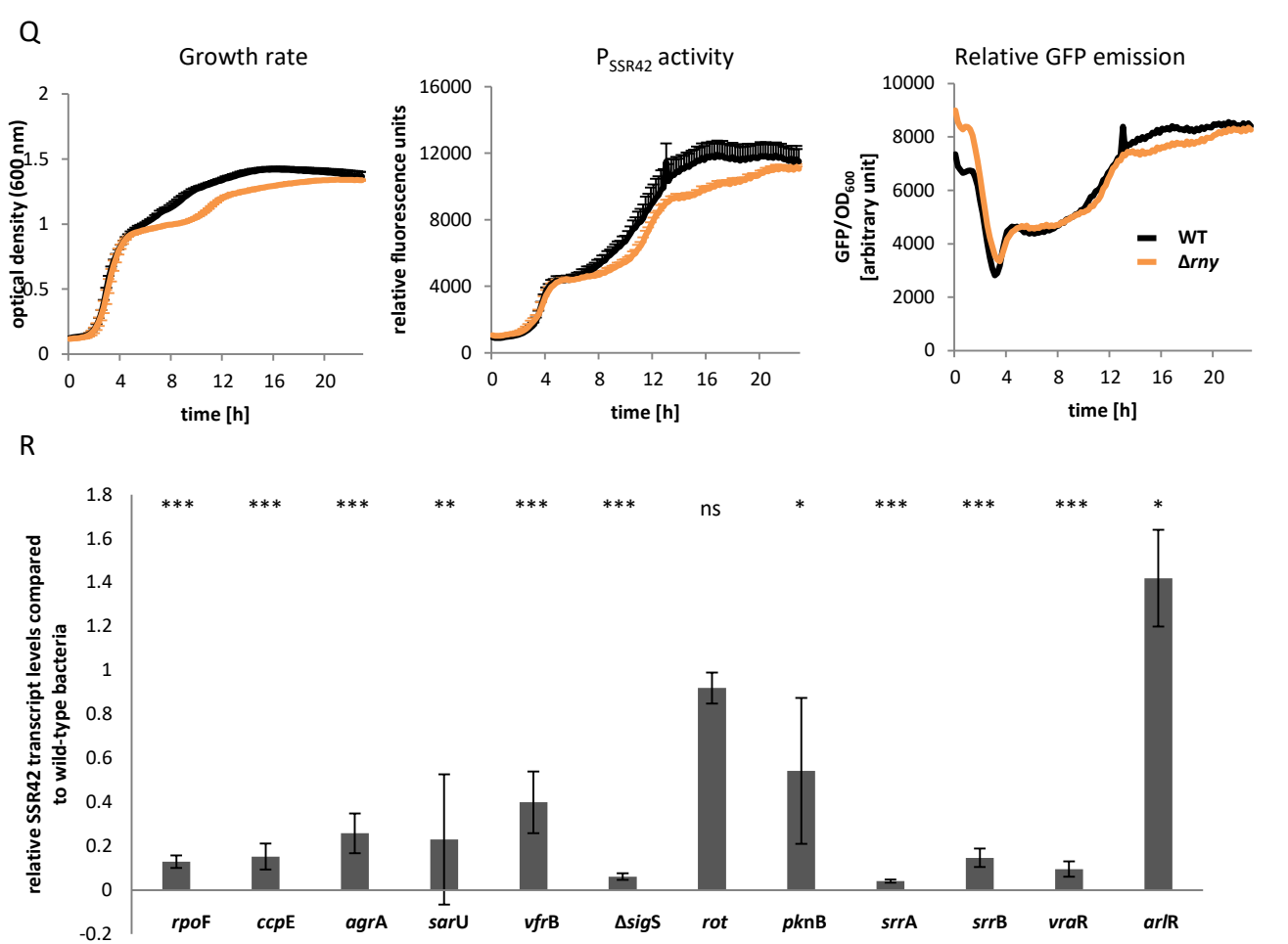
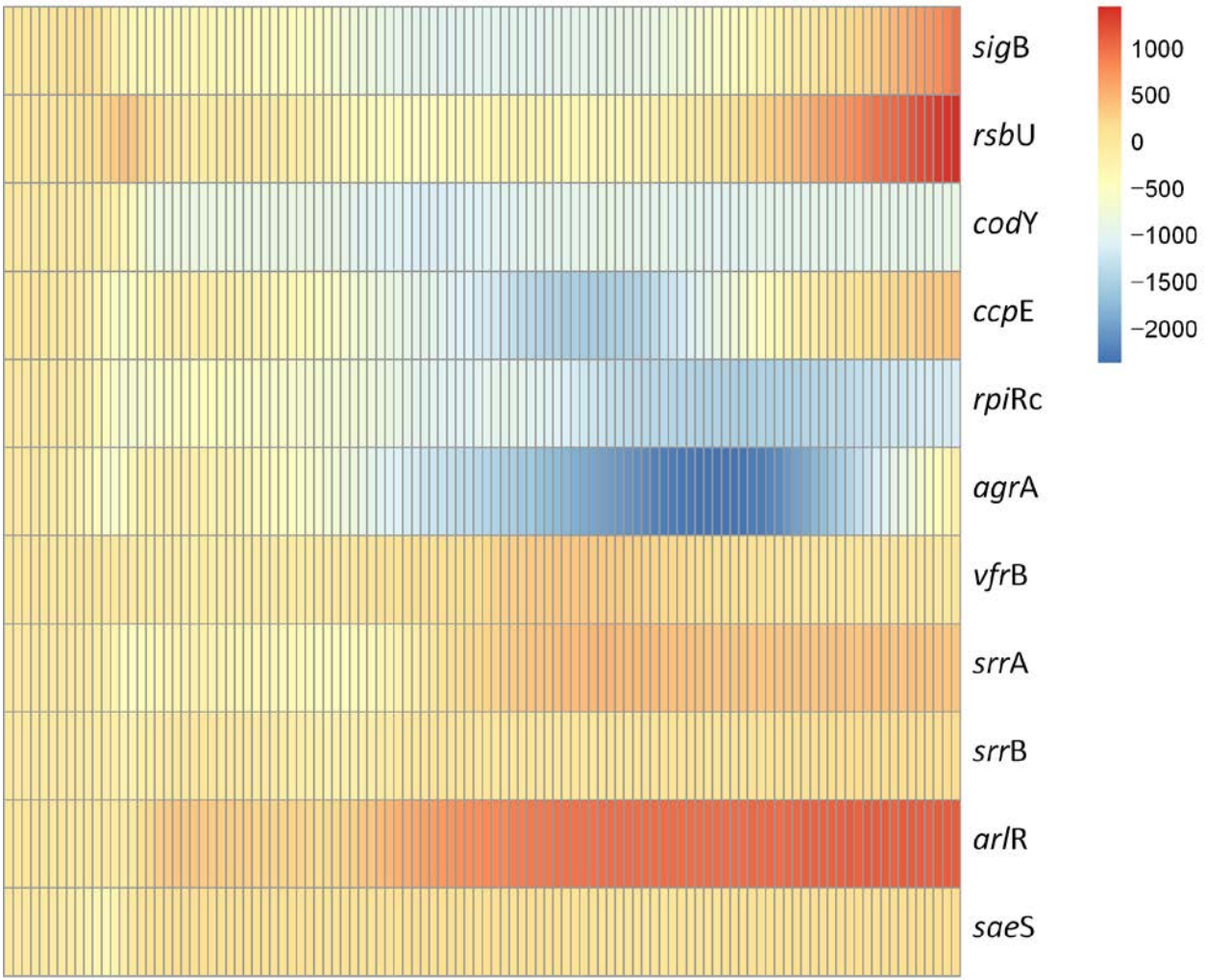


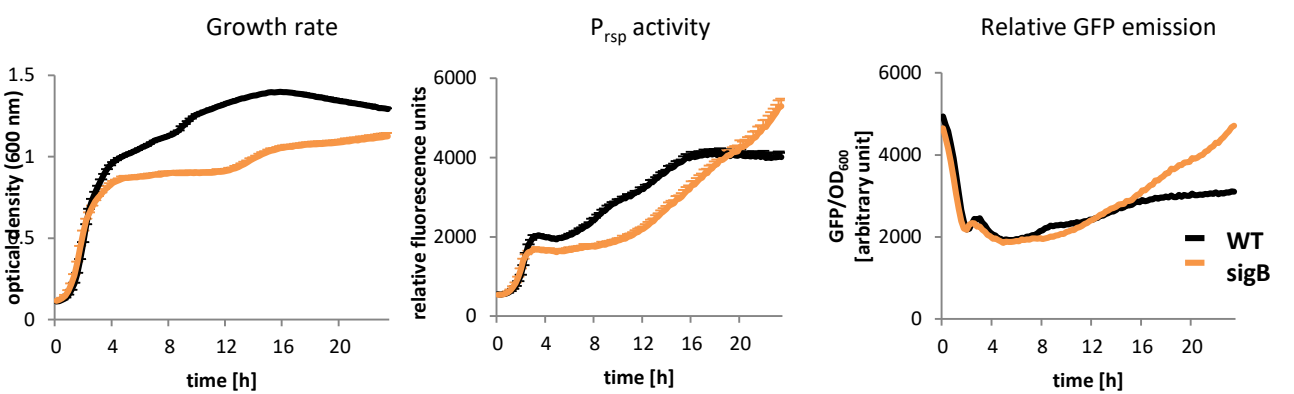
Fig. S7: Regulation of SSR42 promotor activity by global regulators in *S. aureus* JE2. If not indicated otherwise, insertional mutations in a variety of insertional mutants available through NTML (Fey et al., 2013) were transduced in a wild-type *S. aureus* JE2 background and a plasmid harboring a transcriptional fusion of the SSR42 promoter region, $P_{SSR42,}$ and GFP was introduced. OD_{600} as well as GFP emission was measured over 23 hours in a TECAN Infinite 200 plate reader and a normalized signal (GFP/OD) was calculated. **A**) Inactivation of *rsp* (NE1304) completely abolished P_{SSR42} activity indicating the requirement of Rsp for SSR42 transcription. **B**) A profound decrease in P_{SSR42} activity was also identified in mutants within *rpoF* (NE1109) and *rsbU* (NE1607) as well as in mutants in *cod*Y (NE1555), *ccp*E (NE1560) (**C**) and Δ*rpi*Rc (**D**). **E**) A mutant in *agrA* (NE1532), as well as sarU (NE96) (**F**) displayed a strongly altered promotor activity profile, whereby P_{SSR42} was inactive during the first 16 hours, but later strongly increased eventually exceeding promoter activity of the wild-type. **G**) A mutant in *vfrB* (NE229) displayed an overall decrease in P_{SSR42} activity, whereas insertional disruption of histidine kinase SaeS (NE1296) (**H**) resulted in higher activity starting at 11 h of growth. **I**) Deletion of ECF sigma-factor σS (Δ*sigS*), insertional disruption of *rot* (NE386) (**J**) and *pknB* (NE217) (**K**) reduced the activity of SSR42 promotor. **L**) Two-component system SrrAB displayed a regulatory impact on transcriptional regulation of ncRNA SSR42. Analysis of promotor activity in mutants in *srrA* (NE1309) and *srrB* (NE588) revealed lower overall activity in P_{SSR42} activity. **M**) Insertional disruption of *vraR* (NE554) and *vraS* (NE823) resulted in a lower activity of P_{SSR42}. **N**) By contrast disruption of *arlR* (NE1684) resulted in a profound increase in P_{SSR42} activity. **O**) Mutants in transcriptional regulators SarA (NE1193) and SarT (NE514) displayed similar P_{SSR42} activity as wild-type bacteria. **P**) Insertional disruption of response regulator SaeR (NE1622) as well as knockout of RNase Y (Δ*rny*) (**Q**) had no effect on the activity of $P_{SSR42.}$ **R)** Analysis of SSR42 transcript levels via qRT-PCR of stationary growth phase bacteria (15 h) confirms influence of analyzed regulators on transcription of SSR42. Statistical analysis was performed using Student´s t-test: *: p< 0.05; **: p<0.01; ***: p< 0.001.

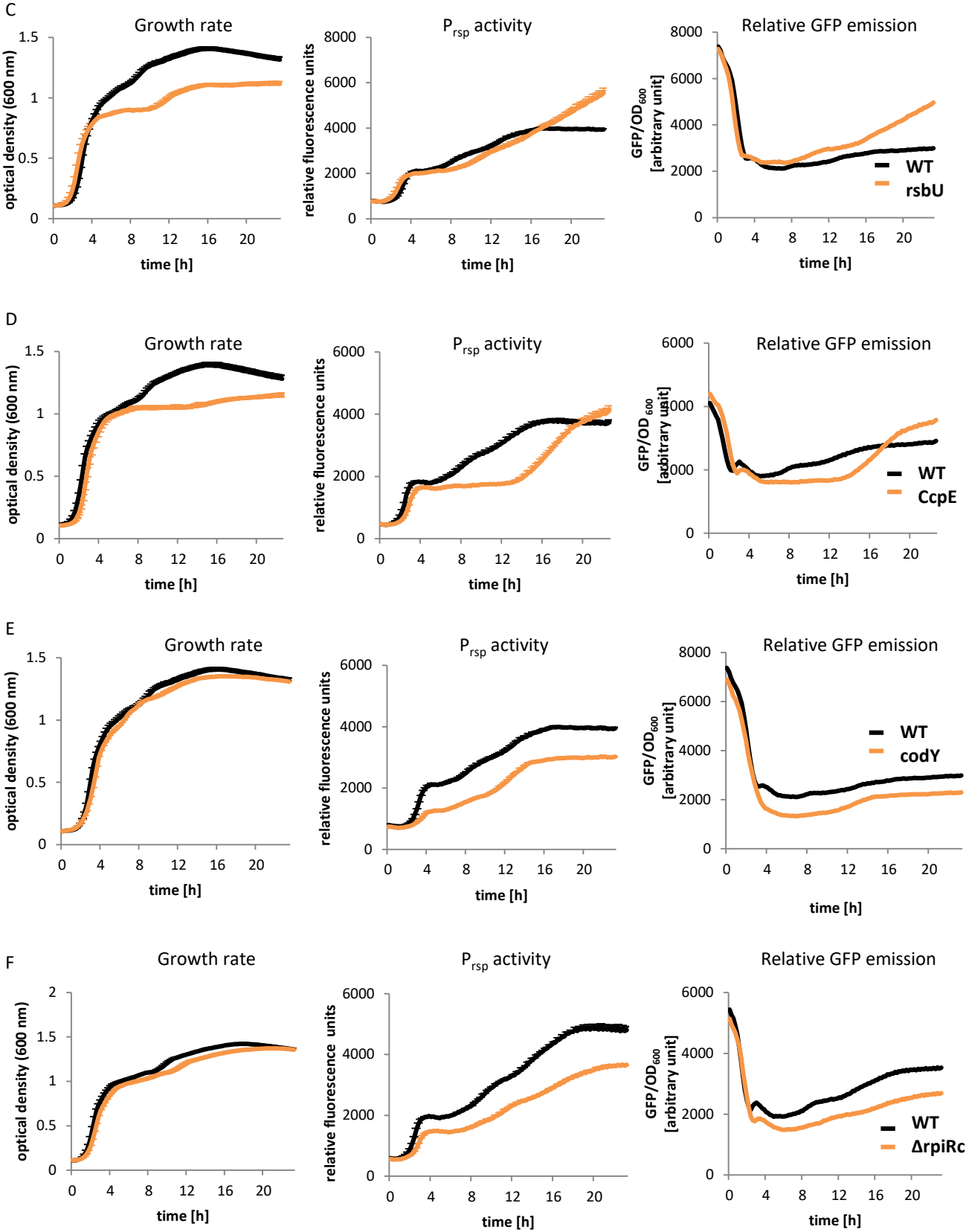
Fig. S8

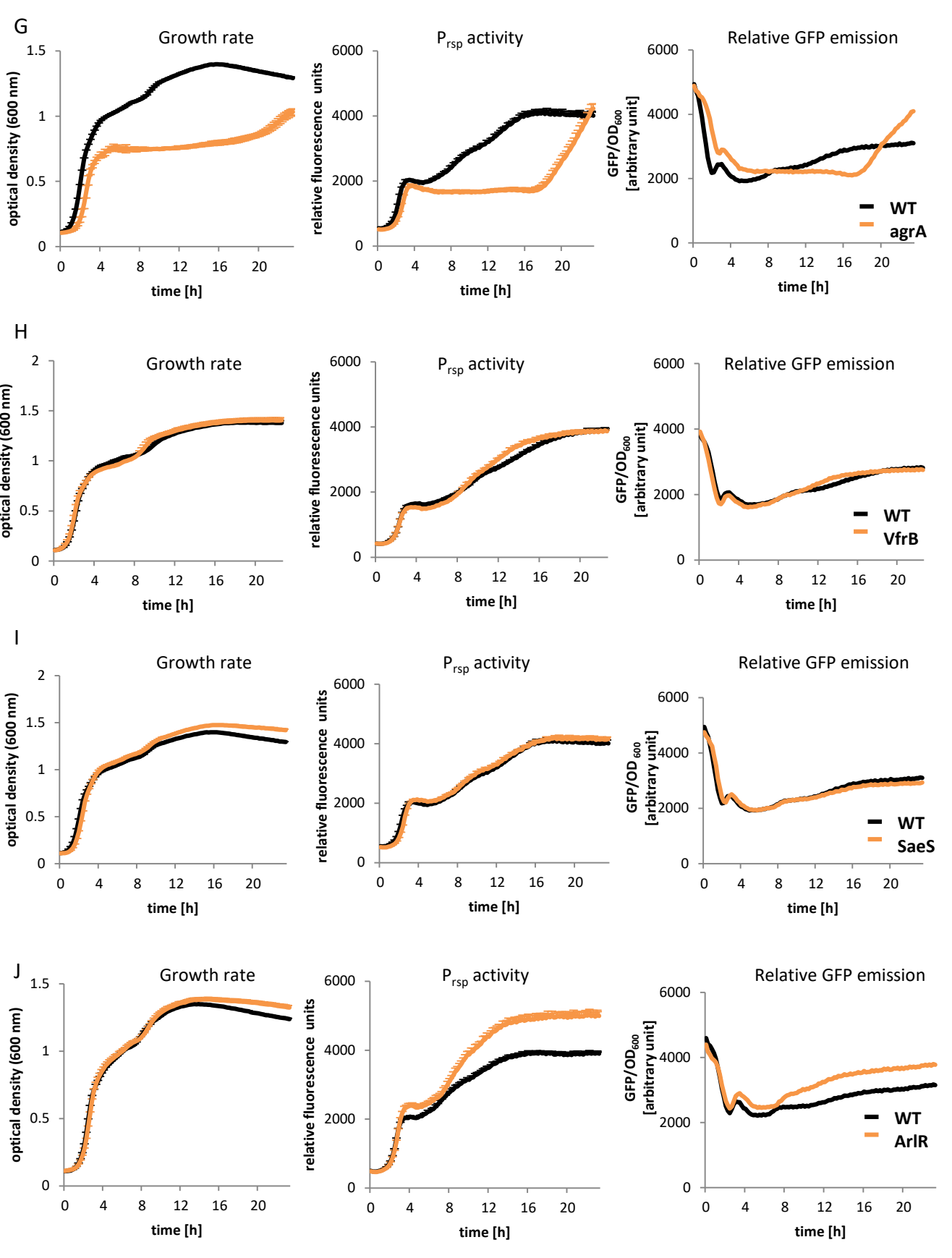
A


sigB
rsbU
codY
ccpE
rpiRc
agrA
vfrB
srrA
srrB
arlR
saeS
1000
500
0
−500
−1000
−1500
−2000

B


Growth rate
optical density (600 nm)
time [h]
P rsp activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
time [h]
WT
sigB


C
Growth rate
optical density (600 nm)
time [h]
P rsp activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
WT
rsbU
time [h]
D
Growth rate
optical density (600 nm)
time [h]
P rsp activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD 600 [arbitrary unit]
WT
CcpE
time [h]
E
Growth rate
optical density (600 nm)
time [h]
P rsp activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
WT
codY
time [h]
F
Growth rate
optical density (600 nm)
time [h]
P rsp activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD600 [arbitrary unit]
WT
ΔrpiRc
time [h]


G
Growth rate
optical density (600 nm)
time [h]
P$_{rsp}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD$_{600}$ [arbitrary unit]
time [h]
WT
agrA
H
Growth rate
optical density (600 nm)
time [h]
P$_{rsp}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD$_{600}$ [arbitrary unit]
time [h]
WT
VfrB
I
Growth rate
optical density (600 nm)
time [h]
P$_{rsp}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD$_{600}$ [arbitrary unit]
time [h]
WT
SaeS
J
Growth rate
optical density (600 nm)
time [h]
P$_{rsp}$ activity
relative fluorescence units
time [h]
Relative GFP emission
GFP/OD$_{600}$ [arbitrary unit]
time [h]
WT
ArlR

K

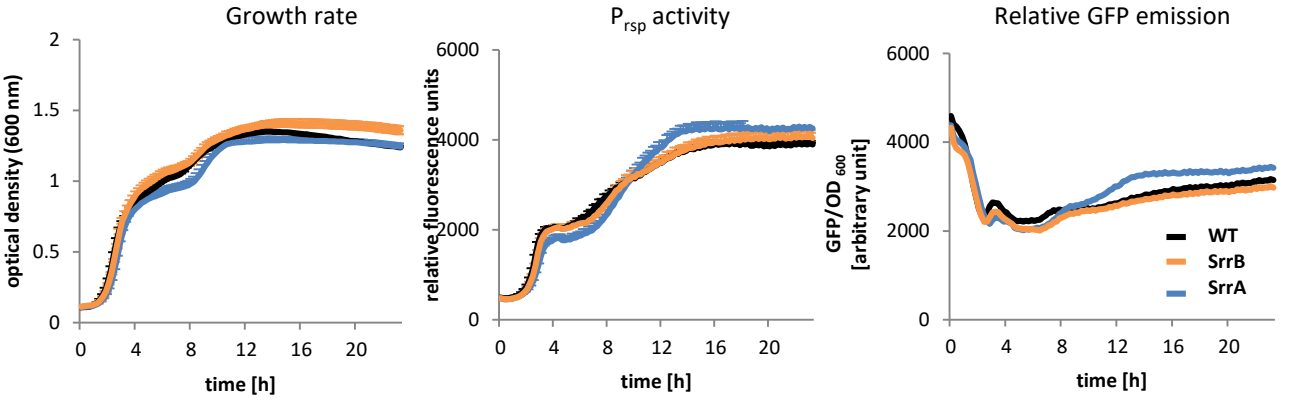


Fig. S8: Dependency of *rsp* promotor activity by global regulators. If not indicated otherwise, insertional mutations in a variety of insertional mutants available through NTML (Fey t al., 2013) were transduced in a wild-type *S. aureus* JE2 background and a plasmid harboring a transcriptional fusion of the *rsp* promoter region, $P_{rsp,}$ and GFP was introduced. OD_{600} as well as GFP emission was measured over 23 hours in a TECAN Infinite 200 plate reader. **A)** Heatmap of differentially activities of P_{rsp} in mutants from the NTML (Fey et al., 2013) compared to the activity in wild-type *S. aureus* JE2. **B**) A slight increase in P_{rsp} activity was identified in mutants within *rpoF* (NE1109) and *rsbU* (NE1607) (**C**) starting 15 h of growth. **D**) Mutants in *ccp*E (NE1560) displayed an altered profile with initial less P_{rsp} activity catching up to even higher activity starting at 17 h of growth. **E**) Insertional disruption of *cod*Y (NE1555) and deletion of *rpi*Rc (Δ*rpi*Rc) (**F**) resulted in overall lower activity of P_{rsp}. **G**) A mutant in *agrA* (NE1532) displayed an altered promotor activity profile, whereby P_{rsp} activity was reduced during the first 16 hours, but later strongly increased eventually exceeding promoter activity of the wild-type. **H**) Mutation of *vfrB* (NE229) and *saeS* (NE1296) (**I**) had no effect on the promotor activity of *rsp*. **J**) Similar, insertional disruption of *srrB* (NE588) had no effect on the activity of P_{rsp} while disruption of *srrA* (NE1309) resulted in a higher promotor activity. **K**) Likewise, mutation in *arlR* (NE1684) resulted in enhanced activity of P_{rsp}.

Fig. S9

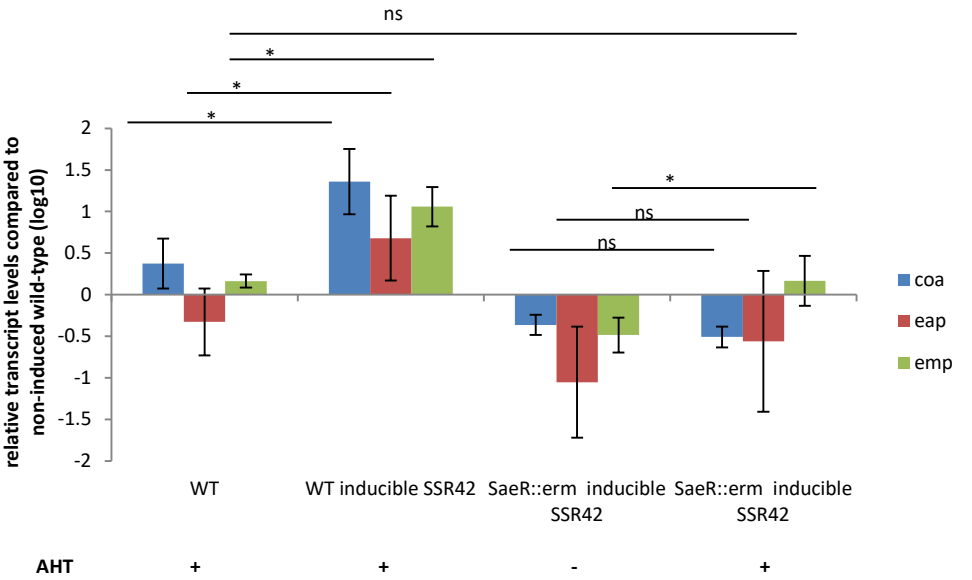


Fig. S9: Upregulation of *hla* transcription is dependent on functional SaeRS system. Comparison of *coa*, *eap* and *emp* transcript levels upon induction of SSR42 via an AHT-inducible complementation plasmid in *S. aureus* JE2, and in insertial mutant in SaeR (SaeR::erm, NE1622) obtained from Nebraska library. No significant upregulation of *coa*, *eap* and *emp* transcription in the SaeR mutant upon induction of SSR42 overexpression reveal dependence on functional SaeR.

Elaborated materials and methods

Oligonucleotides used in this study

Name	Sequence	purpose
RT-gyrB-F	CGACTTTGATCTAGCGAAAG	*gyr*B qPCR
RT-gyrB-R	ATAGCCTGCTTCAATTAACG	*gyr*B qPCR
RT agrB F	GCAGGTCTTAGCTGTAAATATAGG	*agr*B qPCR
RT agrB R	CACCATGTGCATGTCTTC	*agr*B qPCR
RT-RNAIII-F	ACATAGCACTGAGTCCAAGG	RNAIII qPCR
RT-RNAIII-R	TCGACACAGTGAACAAATTC	RNAIII qPCR
RT-saeS-F	TTGCAACCATATGAGCAAC	*sae*S qPCR, Northern Blot
RT-saeS-R	AATATCAATGCGACTACCAAC	*sae*S qPCR, Northern Blot
RT-SSR42-F	TATCTTGTTGCTGCTAATTCTATTG	SSR42 qPCR, Northern Blot Probe 2
RT-SSR42-R	CACCTAACATCAAAGAATATTCATC	SSR42 qPCR, Northern Blot Probe 2
RT-2217-F	TTCACTCGTTTCCAAGATTAC	*rsp* qPCR
RT-2217-R	GACTTCGATCTTTCGGATT	*rsp* qPCR
RT-hla-F	CAATTTGTTGAAGTCCAATG	*hla* qPCR
RT-hla-R	GATCCTAACAAAGCAAGTTCTC	*hla* qPCR
coa RT F	AGTGACGAATCAACGTACTG	*coa* qPCR
coa RT R	AGCATCAAATCCATCTTTATG	*coa* qPCR
eap RT F	CCCAGTAACAATAAATAAATTTGA	*eap* qPCR
eap RT R	ACACTTTATACACTGCGCG	*eap* qPCR
emp RT F	GTACAAAGAAATTAATAATCGCG	*emp* qPCR
emp RT R	CTTTCTTGTAGTGGGTTTGC	*emp* qPCR
RT-sigB-F	CTTTGAACGGAAGTTTGAAG	*rpo*F qPCR
RT-sigB-R	GGCCCAATTTCTTTAATACG	*rpo*F qPCR
codY RT F	GGAAATCTTACGTGAGAAGC	*cod*Y qPCR
codY RT R	CAACTTTTGATGCGATTAATAG	*cod*Y qPCR
NB-SSR42-up-f	ATAGATTTCAAACCTATGTATTTC	SSR42 qPCR, Northern Blot Probe 1
NB-SSR42-up-r	ATAAAATTTAAGTCATGGGTAC	SSR42 qPCR, Northern Blot Probe 1
Af_base6	CCGGAGCTCGGTACCCAACACTCTATTATCATTTTAT	Construction of various deletion and complementation constructs
Ar_SacII	CTAGCCGCGGCCGCACTATCTCTTTCTTTTTGTGTTTA	Construction of ΔSSR42 and ΔSSR42-rsp
Br_Base6	GATCTGCGCGCTAGCCCGACGATCTAAATAACCAT	Construction of ΔSSR42
Bf_SacII	TGCGGCCGCGGCTAGCACCAAATAATTTAATTAGAC	Construction of ΔSSR42

Cr_BASE6	GATCTGCGCGCTAGCCCAGCTAACATGTTCAATTGCTGG	Construction of various deletion and complementation constructs
Cf_SacII	TGCGGCCGCGGCTAGTACTAACAGTCCTCTTGTGTTTAG	Construction of ΔSSR42-rsp
compvek-f	GGGCTAGCGCGCAGATCTGTCGACGACTGGCCGTCGTTTTACAAC	cloning pSSR42-rsp
compvek-r	GGTACCGAGCTCCGGAATTCGTCGAGTTATCGATACCGTC	cloning pSSR42-rsp
comp_no_rsp_rev	GATCTGCGCGCTAGCCCGTTGAATATTTTTGAAAAGGTAATAGTG	cloning pSSR42
comp_no2218_r	GATCTGCGCGCTAGCCCCTTATGATATAAATAGCCTTTACG	cloning pSSR42-rsp
ssr42-r	CGACGGCCAGTGAATTCAAGAAAGAGATAGATTG	cloning pAHT-SSR42
ssr42-f	TAACGCGTTTAATATAGATTTCAAACC	cloning pAHT-SSR42
2217ORF-PmeI-f	GACGTTTAAACATGACATGCCAACTTAAAATACATC	cloning pAHT-rsp
2127ORF_EcoRI_R	AATATAGAATTCCCTCATTAGCTAGGTTTAAAGCAAATATATTTAA	cloning pAHT-rsp
2217_ssrProm-SF-r	GATGAATTCATTAATTATGCACCAAATAATTTAATTAGAC	cloning pJL78-P_{rsp}-gfp
2217_ssrProm-SF-f	GATCGGTACCAGCATGCTTAGCTAGGTTTAAAGC	cloning pJL78-P_{rsp}-gfp
bgab-sf-f	CATCGAATTCTTAGGAGGTGATTATTTATGAATGTGTTATCCTCAATTTG	cloning pRsp-P_{SSR42}-BgaB
bgab-sf-r	CTATGGCGCGCCCTAAACCTTCCCCGGCTTCATCATGC	cloning pRsp-P_{SSR42}-BgaB
hla-Inf-Prom R	CATCCTCCTAAGAATTCTATTTTTTAAAACGATTTGAGGAAACAATAA	cloning pP_{hla}-GFP
hla prom F	CTAGCATGCTCCTGAATTTTTCGAAATTTTATAG	cloning pP_{hla}-GFP
sigS-Uf-Base6	CCGGAGCTCGGTACCCGATGCATCTCTCCATGCATC	Construction of Δ*sigS*
sigS-Ur-SacII	CTAGCCGCGGCCGCAGTGTTAAATATAAGACATAAG	Construction of Δ*sigS*
sigS-Df-SacII	TGCGGCCGCGGCTAGGTGAAATTTCGGTCGATGTG	Construction of Δ*sigS*
sig-S-Dr-Base6	GATCTGCGCGCTAGCCCGCCTGTTACCTTGCTAATGC	Construction of Δ*sigS*
SSR42 trunc 2 fwd	CCCTTAATTATTACATTGTTTAGTAGAAATTGAGTGTGAAAGT	Construction of pSSR42Δ1
SSR42 trunc 2 rev	TAAACAATGTAATAATTAAGGGTATAAATTG	Construction of pSSR42Δ1
SSR42 trunc 3 fwd	CTAGTAACATAATTAATTCGCTATTCTAGATGAGTTTCTTAG	Construction of pSSR42Δ2
SSR42 trunc 3 rev	GAATTAATTATGTTACTAGCTAAATCATC	Construction of pSSR42Δ2
SSR42 trunc 4 fwd	ATGAAGTTATCTATGATGTTACTATCTTGGTGACGTTTCTAG	Construction of pSSR42Δ3
SSR42 trunc 4 rev	TAACATCATAGATAACTTCATCAAGACAGG	Construction of pSSR42Δ3
SSR42 trunc 5 fwd	TGATGTAAAATCTAAGATGTTGCATTTTCTCATGATGAATC	Construction of pSSR42Δ4

SSR42 trunc 5 rev	AACATCTTAGATTTTACATCAAGAGATGCG	Construction of pSSR42Δ4
SSR42 trunc 6 fwd	TCATTGATGTGTGCTGTTTCCGGCAATAACTAGATGGATT	Construction of pSSR42Δ5
SSR42 trunc 6 rev	GAAACAGCACACATCAATGAATTAACACC	Construction of pSSR42Δ5
SSR42 trunc 7 fwd	TAGATGTTTATACTTATCTTGGTTGTTGATTTGAATAATTTG	Construction of pSSR42Δ6
SSR42 trunc 7 rev	CAAGATAAGTATAAACATCTAGAAAAGTG	Construction of pSSR42Δ6
SSR42 trunc 8 fwd	TTGTAACAGGGCTACTAAGAACCAAACTTAAATAATGGCG	Construction of pSSR42Δ7
SSR42 trunc 8 rev	TTCTTAGTAGCCCTGTTACAAAATATTCG	Construction of pSSR42Δ7
SSR42 trunc 9 fwd	TACATTTAATGTTTTTTGATTGCAAGTTAGTTCAAAATGGC	Construction of pSSR42Δ8
SSR42 trunc 9 rev	AATCAAAAAACATTAAATGTACCCATGAC	Construction of pSSR42Δ8
miniSSR42_1J rev	CTTATCTTGATGTTTTCTAAACAGAACTTTTAAACG	Cloning of minimal SSR42 complementation plasmid pSSR42-mini-1
miniSSR42_1J fwd	GTTCTGTTTAGAAAACATCAAGATAAGTTCATCAAGAACAGTATCTAAC	Cloning of minimal SSR42 complementation plasmid pSSR42-mini-1
miniSSR42_2 rev	TTATGCACCAAATAATTTAATTAGACTC	Cloning of minimal SSR42 complementation plasmid pSSR42-mini-2
miniSSR42_2 fwd	TAAATTATTTGGTGCATAACATTAAATGTACCCATGACTTAA	Cloning of minimal SSR42 complementation plasmid pSSR42-mini-2
Vek Term F2	GCCAGCAGGACGACCTGCTGGTTTT	Cloning of plasmid pstemloop
Stem loop 2 neu R	CAGGTCGTCCTGCTGGCAGATACCATACACATTTAATTAGTTACAGC	Cloning of plasmid pstemloop
Stem loop 2 neu F	GTATAATTAAAATAACGTGACTATATATGAATTAATTATGTTACTAG	Cloning of plasmid pstemloop
Vek Term R	CGTTATTTTAATTATACTCTATCAATGATAGAGTGTC	Cloning of plasmid pstemloop

Plasmids used in this study

Name	Description/ purpose	source
p2085	Derivative of pALC2084 (Bateman, 2001 #854), shuttle vector with pC194 and pUC19 backbones, containing *tet*R and Pxyl/tet promoter driving GFPuvr expression, Cm^R	[1]
pJL78	vector expressing gfp in *S. aureus* under control of the constitutive sarAP1 promoter	[2]
pSSR42	complementation of SSR42; native promotor	This study
pSSR42-rsp	complementation of SSR42; native promotor	This study
p2216-2218	complementation of SSR42, upstream (RSAU_002216) and downstream genes (RSAU_002218)	This study
prsp	*rsp* complementation plasmid, originally designated as pS2217*;* native promotor of *rsp*	[3]
pAHT-SSR42	AHT-inducible expression of SSR42	This study
pAHT-rsp	AHT-inducible expression of *rsp*	This study
pP_{SSR42}-GFP	GFP transcription under control of SSR42 Promotor	This study
pP_{hla}-GFP	GFP transcription under control of *hla* Promotor	This study
pP_{SSR42}-BgaB	β-galactosidase BgaB transcription under control of SSR42 Promotor	This study
pRsp-P_{SSR42}-BgaB	β-galactosidase BgaB transcription under control of SSR42 Promotor; including *rsp*	This study
pSSR42Δ1	complementation of SSR42 with small deletion in 2,352,023-2,352,099 bp; native promotor	This study
pSSR42Δ2	complementation of SSR42 with small deletion in 2,352,157-2,352,221bp; native promotor	This study
pSSR42Δ3	complementation of SSR42 with small deletion in 2,352,282-2,352,348 bp; native promotor	This study
pSSR42Δ4	complementation of SSR42 with small deletion in 2,352,415-2,352,483 bp; native promotor	This study
pSSR42Δ5	complementation of SSR42 with small deletion in 2,352,535-2,352,607 bp; native promotor	This study
pSSR42Δ6	complementation of SSR42 with small deletion in 2,352,688-2,352,742 bp; native promotor	This study
pSSR42Δ7	complementation of SSR42 with small deletion in 2,352,858-2,352,794 bp; native promotor	This study
pSSR42Δ8	complementation of SSR42 with small deletion in 2,352,917-2,352,978 bp; native promotor	This study

pSSR42-mini-1	complementaion of SSR42 with deletion in bp 2,352,219-2,352,658; native promotor	This study
pSSR42-mini-1	complementaion of SSR42 with deletion in bp 2,352,219-2,352,658 and in bp 2,352,916-2,353044; native promotor	This study
pstemloop	complementation of bp 2,351,984-2,352,137 encompassing astem loop structure of SSR42	This study
pCG296	Complementation of RNase Y	[4]

Bacterial strains used in this study

Strain	Description	Source
Staphylococcus aureus		
UAMS-1	Osteomyelitis isolate, Little Rock, AR, USA	[5]
HG003	NCTC8325, *rsb*U^{+}, *tca*R^{+}	[6]
N315	pharyngeal smear isolate, Japan, 1985, ORSA,	[7]
COL	Archaic MRSA strain from UK	[8]
MW2	Community-acquired isolate causing septicemia and septic arthritis, North Dakota	[9]
Cowan I	non-haemolytic, protein A overproducer	ATCC 12598
Newman	strong producer of clumping factor	[10]
RN4220	derivative of NCTC8325-4 accepting foreign DNA	[11]
JE2	USA300 LAC MRSA, plasmid-cured; erm^{S}	[12]
NE1622	JE2, *sae*R::Bursa (SAUSA300_0691)	[12]
NE1532	JE2, *agrA*::Bursa (SAUSA300_1992)	[12]
NE217	JE2, *pknB* ::Bursa (SAUSA300_1113)	[12]
NE1296	JE2, *saeS*::Bursa (SAUSA300_0690)	[12]
NE1304	JE2, *rsp*::Bursa (SAUSA300_2326)	[12]
NE1109	JE2, *rpoF*::Bursa (SAUSA300_2022)	[12]
NE1607	JE2, *rsbU*::Bursa (SAUSA300_2025)	[12]
NE1560	JE2, *ccpE*::Bursa (SAUSA300_0658)	[12]
NE1555	JE2, *codY*::Bursa (SAUSA300_1148)	[12]
NE554	JE2, *vraR*::Bursa (SAUSA300_1865)	[12]
NE823	JE2, *vraS*::Bursa (SAUSA300_1866)	[12]

NE229	JE2, *vfrB*::Bursa (SAUSA300_1119)	[12]
NE1684	JE2, *arlR*::Bursa (SAUSA300_1308)	[12]
NE1183	JE2, *arlS*::Bursa (SAUSA300_1307)	[12]
NE1309	JE2, *srrA*::Bursa (SAUSA300_1442)	[12]
NE588	JE2, *srrB*::Bursa (SAUSA300_1441)	[12]
JE2-217	JE2 Δ*rny* , deletion of gene *rny* encoding RNAse Y (SAUSA300_1179)	[4]
JE2 Δ*rpi*Rc	JE2, deletion of gene *RpiRc* (SAUSA300_2264)	[13]
JE2 ΔsigS	JE2, markerless deletion of gene *sigS* encoding σS	This publication
6850	MSSA, Isolated from a patient with a skin abscess, progressed to bacteremia, osteomyelitis, septic arthritis, and multiple systemic abscesses	[14]
6850 Δ*rsp*	6850 with targeted deletion of gene locus RSAU_002217 (*rsp* gene)	[3]
6850 ΔSSR42	6850 with targeted deletion of ncRNA SSR42	This study
6850 ΔSSR42-rsp	6850 with targeted deletion of ncRNA SSR42 and gene locus RSAU_002217 (*rsp* gene)	This study
HG001 lexAG94E	HG001, LexAG94E	[15]
Escherichia coli		
DH5α	*fhuA2 lac(del)U169 phoA glnV44 Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Douglas Hanahan, BRL Life Technology

Construction of *S. aureus* knockout strains. Markerless targeted gene deletions were generated using the vector pBASE6 [16]. pBASE6 was linearized with SmaI. Approximately 500 bp regions flanking the target gene were amplified by PCR. The fragments contained 19 bp overlaps to either each other or the vector allowing for cloning by the Infusion cloning system (Takara). After Infusion Cloning the assembled vector was transformed into *E. coli* DH5α. Recombinant plasmids were confirmed via Sanger sequencing (SeqLab, Göttingen), and subsequently transformed via electroporation into *S. aureus* 6850. Gene deletions were performed as described previously [17]. Briefly, recombinant *S. aureus* was plated on TSA containing 10 µg/ml chloramphenicol (Cam) and incubated overnight at a permissive temperature of 30 °C. Bacteria first were grown in TSB/ 5 µg/ml Cam overnight at 42°C and then were plated on TSA with 5 µg/ml Cam and incubated at the restrictive temperature. Resulting colonies were grown overnight in TSB without any antibiotic thereby allowing co-integrate resolution. Cultures were diluted 1:10,000 and plated on TSA containing 100 ng/ml anhydrous tetracycline (AHT). The plates were incubated overnight at 37°C and resulting colonies were tested for successful deletion via PCR using genomic DNA.

All *S. aureus* insertional transposon mutants available through the NTML Library (Table S1) were transduced via phage 11 into the genetic background of wild-type *S. aureus* JE2 in order to avoid secondary site mutations.

Construction of complementation and reporter plasmids: Complementation plasmids were constructed using the InFusion cloning system (Takara) according to manufacturer´s instruction.

Construction of complementation plasmid pSSR42: The linearized vector was amplified from plasmid p2085 using oligonucleotides compvek-rev and compvek-fwd. SSR42 sequence and ORF of RSAU_002216 was amplified from genomic DNA of *S. aureus* 6850 using

oligonucleotides Af_base6 and comp_no_rsp_rev. This 1937 bp fragment was cloned using infusion cloning in the linearized vector.

Construction of complementation plasmid pSSR42-rsp: The linearized vector was amplified from plasmid p2085 using oligonucleotides compvek-rev and compvek-fwd. SSR42 sequence and ORF of RSAU_002216 and *rsp* (RSAU_002216) were amplified from genomic DNA of *S. aureus* 6850 using oligonucleotides Af_base6 and comp_no2218_rev. This 4193 bp fragment was cloned using infusion cloning in the linearized vector.

Construction of complementation plasmid p2216-2218: The linearized vector was amplified from plasmid p2085 using oligonucleotides compvek-rev and compvek-fwd. SSR42 sequence, ORF of RSAU_002216, *rsp* (RSAU_002216) and downstream gene RSAU_002218 were amplified from genomic DNA of *S. aureus* 6850 using oligonucleotides Af_base6 and Cr_BASE6. This 4678 bp fragment was cloned using infusion cloning in the linearized vector.

Construction of pRsp-P_{SSR42}-BgaB: SSR42 promotor sequence and the *rsp* encoding ORF were amplified from genomic DNA of *S. aureus* 6850 using primers 2217_ssrProm-SF-rev and 2217_ssrProm-SF-fwd and cloned via SphI and EcoRI in pJL78 containing a GFPmut2 sequence thus resulting in plasmid pJL78-Pr-rsp-gfp. β-galactosidase encoding ORF (*bga*B) was amplified from pMAD [18] using primers bgab-sf-fwd and bgab-sf-rev and cloned via EcoRI and AscI sites into an accordingly restricted pJL78-Pr-rsp-gfp, thereby replacing *gfp* with *bga*B. The resulting plasmid was restricted with EcoRI and KasI and the fragment encompassing promotor, *rsp* and *bga*B encoding was cloned into p2085 [19], resulting in pRsp-P_{SSR42}-BgaB.

Construction of pP_{SSR42}-BgaB: Plasmid prsp-P_{SSR42}-BgaB was restricted with PacI and XhoI and religated in order to remove the ORF encoding *rsp*.

Construction of pP_{SSR42}-GFP: Plasmid pJL78-Pr-rsp-gfp was restricted with KasI and SphI, thus the promotor, *rsp* and *gfpmut2* encoding sequences were cloned into vector p2085. The resulting plasmid was restricted with PacI and XhoI and religated in order to remove the *rsp* encoding ORF.

Construction of pP_{hla}-GFP: The linearized vector was amplified from plasmid p2085 using oligonucleotides pGFP-Inf-Prom F and pGFP vec R. P_{hla} sequence was amplified from genomic DNA of *S. aureus* 6850 using oligonucleotides hla-Inf-Prom R and hla prom F. This 898 bp fragment was cloned using infusion cloning in the linearized vector.

Construction of a vector for AHT-induced transcription of SSR42 or rsp: The plasmids pAHT-SSR42 and pAHT-rsp were constructed for inducible complementation of SSR42 and *rsp*, respectively. The linearized vector containing AHT-inducible promotor sequence was amplified from plasmid p2085 using oligonucleotides ssr-vec-f_vec1-f and ssr-vec-r_vec1-r. The open reading frame containing SSR42 was amplified from genomic DNA of *S. aureus* 6850 using oligonucleotides ssr42-r and ssr42-f. The 1238 bp fragment was cloned via InFusion cloning into the linearized vector. Sequences were verified by Sanger sequencing (SeqLab, Göttingen).

For inducible complementation of *rsp*, the open reading frame of *rsp* was amplified from genomic DNA using oligonucleotides 2217ORF-PmeI-f and 2127ORF_EcoRI_Reverse. The 2134 kb insert initially was TA-cloned in pCR2.1 TOPO TA (Invitrogen), transformed in *E. coli* DH5α. and the sequence of *rsp* was verified by Sanger sequencing (SeqLab, Göttingen). A 2116 bp DNA fragment was prepared by restriction of the vector with PmeI and EcoRI, purified and ligated into accordingly treated p2085. For induction of gene transcriptions under control of an AHT-inducible promotor 200 ng/ml AHT was added to a *S. aureus* liquid culture. Bacteria

were further grown for 1.5 h at 37°C. If not stated otherwise transcription was induced at exponential phase culture (OD_{600} 0.4) of *S. aureus*.

Construction of complementation plasmids containing small deletions in SSR42

Complementation plasmids containing small deletion mutants in SSR42 were constructed by amplifying the sequence of SSR42 from plasmid pSSR42 using primer SSR42 trunc 2 fwd and SSR42 trunc 2 rev for creation of pSSR42 Δ1, SSR42 trunc 3 fwd and SSR42 trunc 3 rev for creation of pSSR42 Δ2, SSR42 trunc 4 fwd and SSR42 trunc 4 rev for creation of pSSR42 Δ3, SSR42 trunc 5 fwd and SSR42 trunc 5 rev for creation of pSSR42 Δ4, SSR42 trunc 6 fwd and SSR42 trunc 6 rev for creation of pSSR42 Δ5, SSR42 trunc 7 fwd and SSR42 trunc 7 rev for creation of pSSR42 Δ6, SSR42 trunc 8 fwd and SSR42 trunc 8 rev for creation of pSSR42 Δ7 and SSR42 trunc 9 fwd and SSR42 trunc 9 rev for creation of pSSR42 Δ8, via mutagenesis PCR respectively. Remaining wild-type pSSR42 plasmid was subsequently digested with DpnI.

RNA Isolation: Bacterial RNA was extracted using the previously described TRIzol method [20]. Shortly, bacteria were harvested at desired optical densities, flash-frozen in liquid nitrogen and stored at -80°C. For isolation of RNA, frozen pellets were thawed on ice with addition of 0.5 M EDTA to a final concentration of 65 mM. Bacterial pellets were resuspended in Buffer A (glucose 10%, Tris12.5 mM, pH 7.6, EDTA 10 mM) and transferred to Lysing matrix B tubes (MP Biomedicals) containing 500 µl of acidic phenol. Bacteria were lysed using a FastPrep (MP Biomedicals) at a setting of 6 m/s for 45 seconds. Phases were separated at 10,000 x g for 10 min at 4°C. The aqueous layer was added to 1 ml TRI reagent and incubated for 5 min at room temperature, followed by addition of 100 µl chloroform and incubation for additional 3 minutes. Phases were separated by centrifugation at 17,900 x g for 10 min. The upper phase was collected, mixed with 200 µl chloroform and incubated at room temperature for 5 min.

Phases were separated by centrifugation at 17,900 x g, the aqueous phase was collected and RNA was precipitated for 15 min by addition of isopropanol. RNA was precipitated for at least 30 min at 17,900 x g. The resulting pellet was washed with 75% Ethanol, air-dried, and dissolved in DEPC-treated water. RNA was stored at -80°C. Genomic DNA was removed using Turbo DNA-free Kit (Invitrogen) according to manufacturer´s protocol.

Quantitative Real time PCR (qRT-PCR): Reverse transcription was performed using RevertAID reverse transcriptase (Thermo Scientific) according to manufacturer´s protocol. Real time PCR was performed in 96 well format using SybrGreen Master Mix (Genaxxon, 2x) on a StepOne® Plus Real Time PCR system (Applied Biosystems). Cycle settings and composition of reaction mixture were chosen according to manufacturer´s recommendation. The SybrGreen Master Mix was diluted to 1x in dH_2O and mixed with 0.9 µM each of forward and reverse primer (Table S1) and 10 ng of template cDNA in a total volume of 20 µl per well. Reactions were performed in technical triplicates. Reactions were run for 40 cycles with an initial 10 min denaturation at 95°C. Each cycle consisted of 15 sec at 95°C followed by 60°C for 1 min. Analysis of relative transcription was performed according to the $2^{-\Delta\Delta CT}$ method [21]. Relative gene expression was normalized to expression of the housekeeping gene of gyrase subunit B (*gyr*B) and to the corresponding expression in wild-type cells.

Northern Blot: Northern Blotting of RNA was performed as previously described [22] using digoxigenin-labeled probes. For Northern Blot 10 µg of DNase I-treated RNA was mixed with three volumes of RNA loading buffer before heating at 65°C for 15 min. After heating, 1/6 volumes of Blue Juice (Thermo Scientific) were added to the samples and RNA was loaded onto a 1% formaldehyde agarose gel. RNA was separated in MOPS running buffer at 50 V for approximately 3.5 h. RNA was visualized under UV light to check the quality of RNA and the

gel was washed three times in DEPC-treated water. RNA was transferred for 2.5 h to a nylon membrane using alkaline transfer, shortly incubated in 1x phosphate buffer and UV-cross-linked to the membrane by Stratalinker according to manufacturer´s recommendation. The membrane was pre-hybridized at 64°C for 30 min before digoxigenin-labelled probe was added. The Digoxigenin-labelled DNA-fragments were produced using PCR DIG Labelling kit (Roche Digoxigenin Detection kit) according to manufacturer´s protocol and used as probes. DNA probe was heated for 10 min at 95°C and added to hybridization buffer. The Northern blot was hybridized overnight at 64°C. The blot was washed twice with 2x SSC containing 0.1% SDS for 5 min at room temperature and twice with 0.2x SSC containing 0.1% SDS for 15 min at 64°C. To detect the digoxigenin-labelled probes the membrane was equilibrated in maleic acid washing buffer and blocked in blocking buffer (Roche Digoxigenin Detection kit). Anti-Digoxigenin antibody (Roche) was used in a 1:10,000 fold dilution and added to the membrane. After washing the membrane was washed twice with maleic acid washing buffer for 20 min and once with buffer 3 for 3 min, the signal was developed using CSPD readymade solution (Roche) according to manufacturer´s recommendation. Chemiluminescence was recorded using INTAS Imager.

References

1. Giese B, Dittmann S, Paprotka K, Levin K, Weltrowski A, Biehler D, Lâm T-T, Sinha B, Fraunholz MJ. 2009. Staphylococcal Alpha-Toxin Is Not Sufficient To Mediate Escape from Phagolysosomes in Upper-Airway Epithelial Cells. Infection and Immunity 77:3611-3625.
2. Liese J, Rooijakkers SH, van Strijp JA, Novick RP, Dustin ML. 2013. Intravital two-photon microscopy of host-pathogen interactions in a mouse model of Staphylococcus aureus skin abscess formation. Cell Microbiol 15:891-909.
3. Das S, Lindemann C, Young BC, Muller J, Österreich B, Ternette N, Winkler A-C, Paprotka K, Reinhardt R, Förstner KU, Allen E, Flaxman A, Yamaguchi Y, Rollier CS, van Diemen P, Blättner S, Remmele CW, Selle M, Dittrich M, Müller T, Vogel J, Ohlsen K, Crook DW, Massey R, Wilson DJ, Rudel T, Wyllie DH, Fraunholz MJ. 2016. Natural mutations in a Staphylococcus aureus virulence regulator attenuate cytotoxicity but permit bacteremia and abscess formation. Proceedings of the National Academy of Sciences of the United States of America 113:E3101-E3110.
4. Marincola G, Schafer T, Behler J, Bernhardt J, Ohlsen K, Goerke C, Wolz C. 2012. RNase Y of Staphylococcus aureus and its role in the activation of virulence genes. Mol Microbiol 85:817-32.
5. Gillaspy AF, Hickmon SG, Skinner RA, Thomas JR, Nelson CL, Smeltzer MS. 1995. Role of the accessory gene regulator (agr) in pathogenesis of staphylococcal osteomyelitis. Infection and Immunity 63:3373-3380.
6. Herbert S, Ziebandt AK, Ohlsen K, Schafer T, Hecker M, Albrecht D, Novick R, Gotz F. 2010. Repair of global regulators in Staphylococcus aureus 8325 and comparative analysis with other clinical isolates. Infect Immun 78:2877-89.
7. Kuroda M, Ohta T, Uchiyama I, Baba T, Yuzawa H, Kobayashi I, Cui L, Oguchi A, Aoki K, Nagai Y, Lian J, Ito T, Kanamori M, Matsumaru H, Maruyama A, Murakami H, Hosoyama A, Mizutani-Ui Y, Takahashi NK, Sawano T, Inoue R, Kaito C, Sekimizu K, Hirakawa H, Kuhara S, Goto S, Yabuzaki J, Kanehisa M, Yamashita A, Oshima K, Furuya K, Yoshino C, Shiba T, Hattori M, Ogasawara N, Hayashi H, Hiramatsu K. 2001. Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet 357:1225-40.
8. Gill SR, Fouts DE, Archer GL, Mongodin EF, DeBoy RT, Ravel J, Paulsen IT, Kolonay JF, Brinkac L, Beanan M, Dodson RJ, Daugherty SC, Madupu R, Angiuoli SV, Durkin AS, Haft DH, Vamathevan J, Khouri H, Utterback T, Lee C, Dimitrov G, Jiang L, Qin H, Weidman J, Tran K, Kang K, Hance IR, Nelson KE, Fraser CM. 2005. Insights on Evolution of Virulence and Resistance from the Complete Genome Analysis of an Early Methicillin-Resistant Staphylococcus aureus Strain and a Biofilm-Producing Methicillin-Resistant Staphylococcus epidermidis Strain. Journal of Bacteriology 187:2426-2438.
9. Baba T, Takeuchi F, Kuroda M, Yuzawa H, Aoki K, Oguchi A, Nagai Y, Iwama N, Asano K, Naimi T, Kuroda H, Cui L, Yamamoto K, Hiramatsu K. 2002. Genome and virulence determinants of high virulence community-acquired MRSA. Lancet 359:1819-27.
10. Duthie ES, Lorenz LL. 1952. Staphylococcal coagulase; mode of action and antigenicity. J Gen Microbiol 6:95-107.
11. Kreiswirth BN, Lofdahl S, Betley MJ, O'Reilly M, Schlievert PM, Bergdoll MS, Novick RP. 1983. The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature 305:709-12.

12. Fey PD, Endres JL, Yajjala VK, Widhelm TJ, Boissy RJ, Bose JL, Bayles KW. 2013. A Genetic Resource for Rapid and Comprehensive Phenotype Screening of Nonessential Staphylococcus aureus Genes. mBio 4:e00537-12.
13. Gaupp R, Wirf J, Wonnenberg B, Biegel T, Eisenbeis J, Graham J, Herrmann M, Lee CY, Beisswenger C, Wolz C, Tschernig T, Bischoff M, Somerville GA. 2016. RpiRc Is a Pleiotropic Effector of Virulence Determinant Synthesis and Attenuates Pathogenicity in Staphylococcus aureus. Infect Immun 84:2031-41.
14. Vann JM, Proctor RA. 1987. Ingestion of Staphylococcus aureus by bovine endothelial cells results in time- and inoculum-dependent damage to endothelial cell monolayers. Infect Immun 55:2155-63.
15. Schroder W, Goerke C, Wolz C. 2013. Opposing effects of aminocoumarins and fluoroquinolones on the SOS response and adaptability in Staphylococcus aureus. J Antimicrob Chemother 68:529-38.
16. Geiger T, Goerke C, Mainiero M, Kraus D, Wolz C. 2008. The Virulence Regulator Sae of Staphylococcus aureus: Promoter Activities and Response to Phagocytosis-Related Signals. Journal of Bacteriology 190:3419-3428.
17. Bae T, Schneewind O. 2006. Allelic replacement in Staphylococcus aureus with inducible counter-selection. Plasmid 55:58-63.
18. Arnaud M, Chastanet A, Débarbouillé M. 2004. New Vector for Efficient Allelic Replacement in Naturally Nontransformable, Low-GC-Content, Gram-Positive Bacteria. Applied and Environmental Microbiology 70:6887-6891.
19. Giese B, Dittmann S, Paprotka K, Levin K, Weltrowski A, Biehler D, Lâm T-T, Sinha B, Fraunholz MJ. 2009. Staphylococcal Alpha-Toxin Is Not Sufficient To Mediate Escape from Phagolysosomes in Upper-Airway Epithelial Cells. Infection and Immunity 77:3611-3625.
20. Lasa I, Toledo-Arana A, Dobin A, Villanueva M, de los Mozos IR, Vergara-Irigaray M, Segura V, Fagegaltier D, Penades JR, Valle J, Solano C, Gingeras TR. 2011. Genome-wide antisense transcription drives mRNA processing in bacteria. Proc Natl Acad Sci U S A 108:20172-7.
21. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-8.
22. Goerke C, Campana S, Bayer MG, Doring G, Botzenhart K, Wolz C. 2000. Direct quantitative transcript analysis of the agr regulon of Staphylococcus aureus during human infection in comparison to the expression profile in vitro. Infect Immun 68:1304-11.