FIG S1

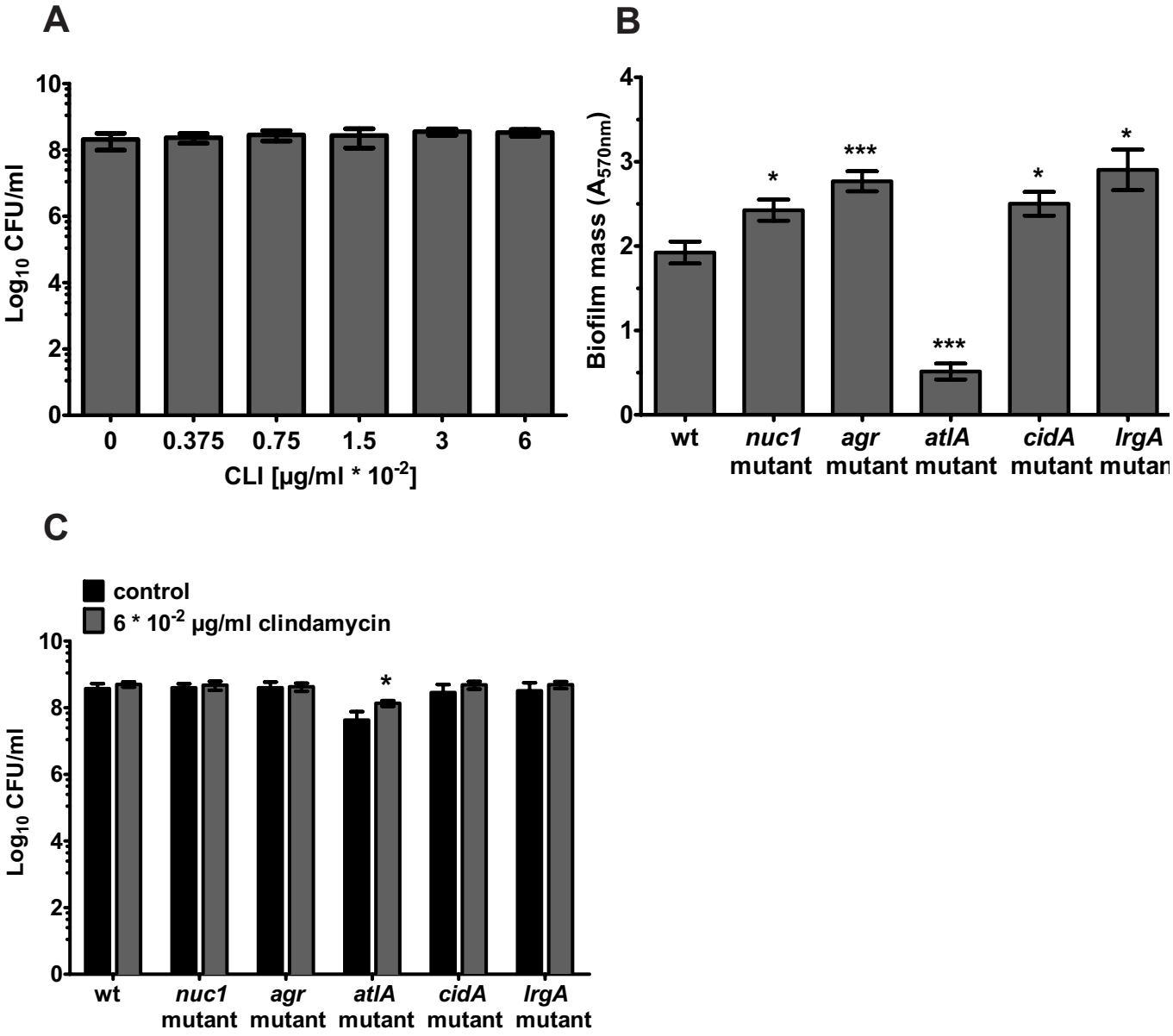

FIG S1: Viable bacteria counts and biofilm formation of LAC wt and isogenic mutants. A) CFU counts (Log_{10} CFU/ml) of the LAC wt strain in the absence and presence of different concentrations of clindamycin after 18 h of biofilm growth. No significant difference is visible between the samples incubated with or without subinhibitory concentrations of clindamycin. Results presented are average data from at least three independent replicates ± SEM (P = 0.2982, not significant; Kruskal-Wallis test followed by post hoc Dunn`s multiple comparisons). B) Biofilm mass quantification with crystal violet of *S. aureus* LAC wt and all mutants used in this study after 18 h of static incubation in 96-well plates. Absorbance was measured at 570 nm (A_{570nm}). All results presented are average data from at least three replicates ± SEM. An asterisk on top of the respective mutant data set indicates a significant difference to the LAC wt (*P<0.05, ***P<0.001, Mann-Whitney U test). C) CFU counts (Log_{10} CFU/ml) of the LAC wt and all mutants used in this study in the absence and presence of clindamycin (0.06 µg/ml) after 18 h of biofilm growth. Results presented are average data from at least three independent replicates ± SEM. An asterisk on top of the bar indicates a significant difference to the control without clindamycin (*P<0.05, Mann-Whitney U test).

FIG S2

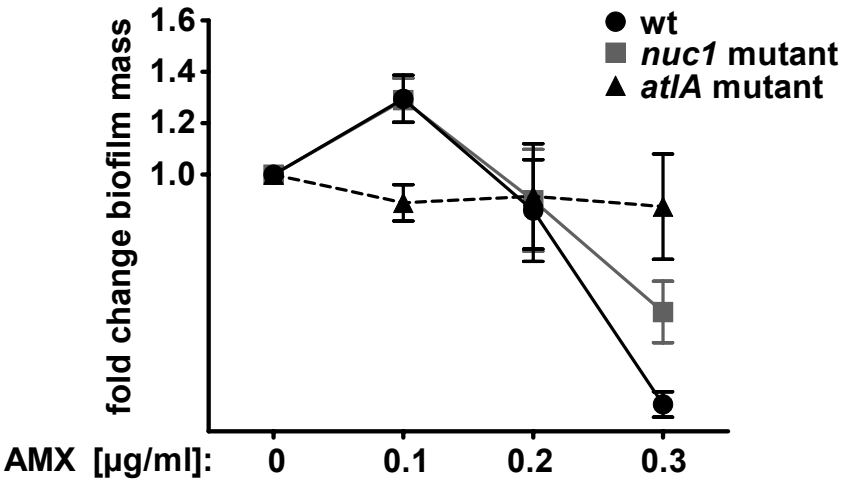

FIG S2: Modulation of biofilm formation in *S. aureus* LAC in response to subinhibitory concentrations of amoxicillin (AMX). LAC wt and the *nuc1* mutant showed an increase of biofilm formation in response to low-dose amoxicillin. No induction of biofilm formation was observed in the *atlA* mutant background. The biofilm mass was quantified with crystal violet after 18 h of static incubation. Biofilm mass without amoxicillin was set to 1 and fold changes are presented. Results presented are average data from two replicates ± SD.

FIG S3

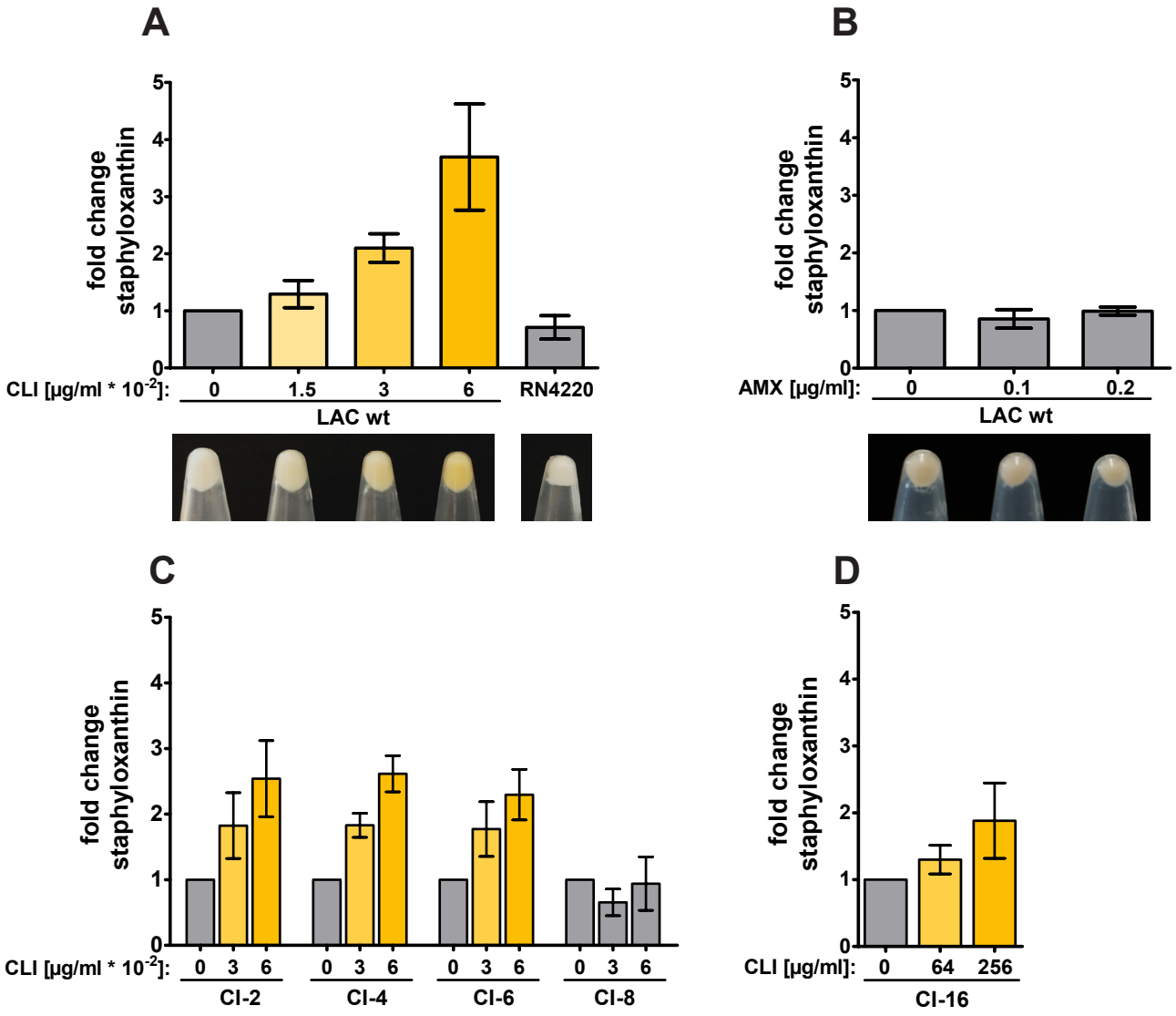

FIG S3: Alteration of staphyloxanthin production by subinhibitory antibiotic concentrations. Quantification of staphyloxantin production in the presence and absence of subinhibitory concentrations of clindamycin (CLI; A, C and D) and amoxicillin (AMX; B). Extracted staphyloxanthin from LAC wt (A and B), RN4220 (A), clindamycin-sensitive clinical isolates (C) and a clindamycin-resistant clinical isolate (D) was measured by spectrophotometry. Strains grown without antibiotics were set to 1 and fold changes are presented. Representative pellets of bacterial cultures used for staphyloxanthin extraction are shown below the bar graph (A and B). Results presented are average data from at least three (A, means ± SEM) or two replicates (B, C, D, means ± SD).

FIG S4

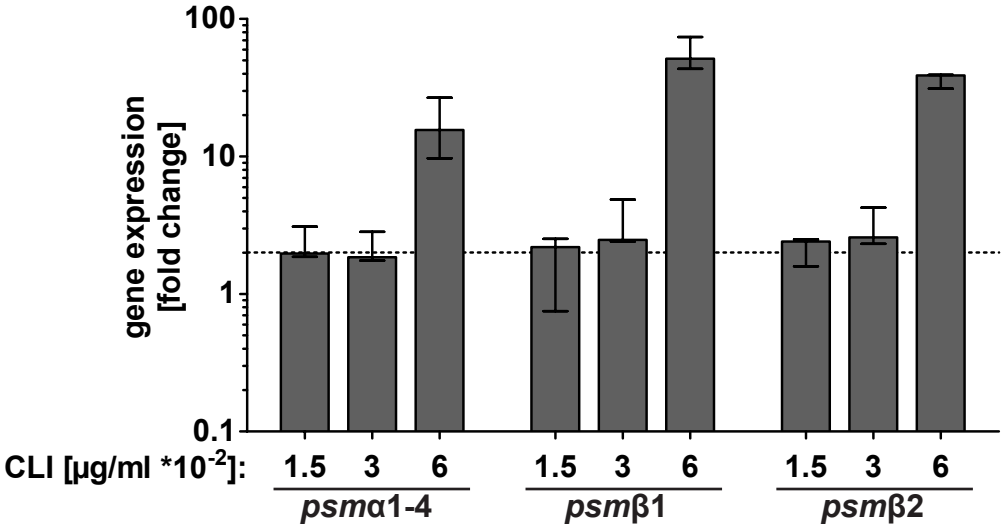

FIG S4: Overexpression of *psm* genes in *S. aureus atlA* mutant biofilms in response to clindamycin. Relative transcript levels of *psm* genes derived from LAC *atlA* mutant biofilm mass after 18 h with and without clindamycin determined by qRT-PCR. Fold change ratios were calculated by normalizing complementary DNA levels of the gene of interest against the housekeeping gene *gyrB*. Data are presented as fold change compared to biofilms grown without clindamycin (set to 1) and error bars indicate interquartile range of at least three independent experiments. The dotted line indicates a 2-fold upregulation.

FIG S5

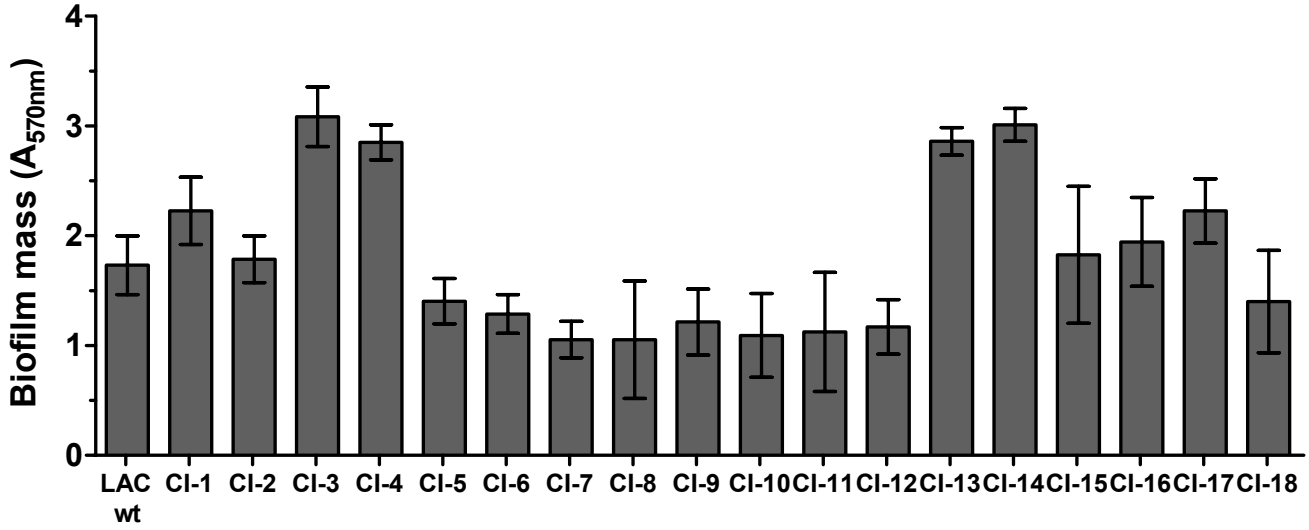

FIG S5: Biofilm formation of *S. aureus* LAC wt and clinical isolates used in this study. Biofilm mass quantification with crystal violet of *S. aureus* clinical isolates after 18 h of static incubation in 96-well plates. Absorbance was measured at 570 nm ($A570_{nm}$). All results presented are average data from at least three replicates ± SEM.

Table S1 – Strains used in this study

Strains	Relevant characteristics	Reference/ Source
LAC wt	CA-MRSA, pulsed-field type USA300 (ST8), Erm^s	(1)
nuc1 mutant	LAC *nuc1* deficient, Erm^s	(2)
RN4220	Restriction-deficient derivative of NCTC8325-4, *agr*$^-$, *tcaR*$^-$, *rsbU*$^-$	(3)
RN6911	NCTC8325-4 *agr*::*tet*(M), Tc^r	(4)
agr mutant	LAC *agr*::*tet*(M), Tc^r	This study
RN4220 pTnT	RN4220 carrying pTnT, Amp^r, Cm^r	(5)
NE1438	JE2 *lrgA* transposon mutant, Erm^r	(6)
NE1692	JE2 *cidA* transposon mutant, Erm^r	(6)
NE460	JE2 *atlA* transposon mutant, Erm^r	(6)
lrgA mutant	LAC *lrgA* transposon mutant, Erm^s	This study
cidA mutant	LAC *cidA* transposon mutant, Erm^s	This study
atlA mutant	LAC *atlA* transposon mutant, Erm^s	This study

Erm^s = erythromycin sensitive, Tc^r = tetracycline resistant, Erm^r = erythromycin resistant, Amp^r = ampicillin resistant, Cm^r = chloramphenicol resistant

References

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Table S2 – Primers used in this study

Primer	Sequence (5´- 3´)	Application	Reference/ Source
Upstream	CTCGATTCTATTAACAAGGG	PCR	(1)
Buster	GCTTTTTCTAAATGTTTTTTAAGTAAATCAAGTAC	PCR	(1)
cidA	ATATTGGCACAGAAATTCAA	PCR	This study
lrgA	ATCAAAACCAGCACACTTT	PCR/sequencing	This study
altA	ATCGTACACACACACGACTA	PCR	This study
agrlocus-F	AACTCAGTAAGAACCCATTTCG	PCR/sequencing	This study
agrlocus-R	AGTTTGCCAACATTACAAGAGG	PCR/sequencing	This study
cidASeq1	AACCTGAAGATAATGCAACGA	sequencing	This study
cidASeq2	CCGGCAGTATTGTTGGTCTA	sequencing	This study
atlASeq1	TGTTGTTGGTTTTGATGGTG	sequencing	This study
atlASeq2	TTTGCACGTTCAATGAATAA	sequencing	This study
gyrB.MB-F2	CGCAGGCGATTTTACCATTA	qRT PCR	(2)
gyrB.MB-R2	GCTTTCGCTAGATCAAAGTCG	qRT PCR	(2)
nuc1-F	TGAAGCAAGTGCATTTACGA	qRT PCR	(3)
nuc1-R	CCAAGCCTTGACGAACTAAA	qRT PCR	(3)
atlA-F	AACAAGTTGCTGGTAGTGTG	qRT PCR	This study
atlA-R	CCATTCACAGAGCCATATAA	qRT PCR	This study
cidA-F	CGCAGTCATTATCATAGGAA	qRT PCR	This study
cidA-R	TAAGCGTCTACACCTTTACG	qRT PCR	This study
lrgA-F	TATGCCTGCATCAGTAATCG	qRT PCR	This study
lrgA-R	CGGCTGGTACGAAGAGTAAG	PCR/qRT PCR/sequencing	This study
agrA-F	CAGACTCATTGCCCATTTA	qRT PCR	This study
agrA-R	TTGACGACAAAGCTATTATGAC	qRT PCR	This study

Primer	Sequence (5´- 3´)	Application	Reference/ Source
sigB-F	ATGGAAATGGGACAAAGTTA	qRT PCR	This study
sigB-R	TCTTGTTGCCCCATAATATC	qRT PCR	This study
fnbA-F	ATAGCGGAGATCAAAGACAA	qRT PCR	This study
fnbA-R	CTTACTTTCTGATGCCGTTC	qRT PCR	This study
fnbB-F	CTCAACCAAGTAACGTCTCA	qRT PCR	This study
fnbB-R	CATCTGTACCTGTCGCTTTA	qRT PCR	This study
psmβ1-F	TTAACGCAATTAAAGATACCG	qRT PCR	This study
psmβ1-R	ACCTAATAAACCTACGCCATT	qRT PCR	This study
psmβ2-F	CTGGACTAGCAGAAGCAATC	qRT PCR	This study
psmβ2-R	GTAAACCCACACCGTTAGC	qRT PCR	This study
psmα1-4-F	AACGATCAACAACTCATCACTA	qRT PCR	This study
psmα1-4-R	TGTCGATAATTGCTTTGATG	qRT PCR	This study
spa-1113f	TAAAGACGATCCTTCGGTGAGC	*spa* typing	(4)
spa-1514r	CAGCAGTAGTGCCGTTTGCTT	*spa* typing	(4)

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Table S3 - Clinical isolates

Strains	Site of isolation	Clinical presentation	*spa* type
MSSA clinical isolate CI-1	tissue	necrotizing fasciitis	t4235
MSSA clinical isolate CI-2	tissue	vascular prosthesis infection	t005
MSSA clinical isolate CI-3	wound	panaritium	t10973
MSSA clinical isolate CI-4	bone	Brodie abscess	t3613
MSSA clinical isolate CI-5	aortic valve	endocarditis	t091
MRSA clinical isolate CI-6	blood	bacteremia	t002
MRSA clinical isolate CI-7	blood	bacteremia	t002
MRSA clinical isolate CI-8	arthrocentesis	knee prosthesis infection	t1183
MRSA clinical isolate CI-9	pacemaker wires	pacemaker endocarditis	t1036
MRSA clinical isolate CI-10	breast implants	breast implant infection	*
MRSA clinical isolate CI-11	tissue	no information available	t021
MRSA clinical isolate CI-12	puncture fluid	hip prosthesis infection	t044
MRSA clinical isolate CI-13 **	axilla	no information available	t032
MRSA clinical isolate CI-14 **	nose	no information available	t032
MRSA clinical isolate CI-15 **	urine	no information available	t586
MRSA clinical isolate CI-16 **	abscess	skin and soft tissue infection	t008
MRSA clinical isolate CI-17 **	wound	skin and soft tissue infection	t008
MRSA clinical isolate CI-18 **	blood	central nervous system (CNS) shunt infection	t586

* potential new *spa* type
** clindamycin resistant >256 mg/L