Supplementary Material:

SI Materials and Methods;

Figure S1. Effects of different concentrations of oxacillin on the SOS induction, *ccrC1* expression and *mecA* expression;

Figure S2. Identification of SCC*mec* excision;

Table S1. Isolates and plasmids used in this study;

Table S2. Primers for cloning, sequencing and qPCR;

Table S3. The minimum inhibitory concentrations (MIC) of the antibiotics for *S. haemolyticus* NW19

SI Materials and Methods

Construction of Reporter Strains for Gene Overexpression and Reduction. To construct the promoterless reporter plasmid pCL::*gfp*, a fragment containing the *gfp* coding region with no promoter was amplified from pGLO with the primers gfpF and gfpR, and another fragment containing most of the pCL15Z elements was amplified from pCL15Z using the primers pCLF and pCLR. Two fragments were digested and ligated to obtain pCL::*gfp*. To construct the reporter plasmid pCL::*ccr-gfp* for the detection of *ccrC1* expression, a fragment containing 224 bp of the promoter upstream of the *ccrC1* initiation codon was amplified by PCR (ccrOPF and ccrOPR) from the NW19 genome and cloned into pCL::*gfp* upstream of the *gfp* coding region. The plasmid pCL::*ccr-gfp* was transformed into NW19 to obtain 19*ccrgfp*.

The plasmid pCL::*ccr-gfp* was used to construct gene overexpression and reduction reporters. The *recA* and *lexA* reading frames were amplified using the primers recAF/R and lexAF/R (Table S2) and cloned into pCL::*ccr-gfp* to yield pCL*recA*::*ccr-gfp* and pCL*lexA*::*ccr-gfp*, respectively. The plasmids were transformed into NW19 to obtain strains 19recUp and 19lexUp, respectively, to achieve exogenous expression of RecA and LexA. To construct the antisense sequences of *recA* and *lexA*, two regions of *recA* (471 bp) and *lexA* (515 bp) were amplified using irecAF/R and ilexAF/R and cloned into pCL::*ccr-gfp*, yielding pCL*recA*i::*ccr-gfp* and pCL*lexA*i::*ccr-gfp*, respectively. The plasmids were transformed into NW19 to give strains 19recDw and 19lexDw, respectively.

Plasmid pCL::*ccr-gfp* was used to further construct pCL::mut*ccr-gfp* by site-directed mutagenesis of the putative SOS box in the *ccrC1* promoter. The primers ccrZmutF and ccrZmutR were used. In pCL::mut*ccr-gfp*, the wild type SOS box TGAAACGAAATTATAAATA was mutated to TGAAA<u>AA</u>AAA<u>AA</u>ATAAATA, the wild type -35 and -10 elements were retained, and the key LexA recognition sites were removed. Plasmid pCL::*ccr-gfp* was transformed into strain NW19 to give strain 19ccrgfp. All resulting plasmids were transferred via electroporation first to *S. aureus* RN4220 and subsequently to strain NW19.

Gene Deletion and Complementation In the Mu50 Strain. The lambda recombination cassette in

pKOR1 was replaced by multiple cloning sites (KpnI-EcoRI-NotI-BglII-SalI). The bleomycin resistance gene operon was amplified from the Mu50 genome and cloned downstream of the ColE1 plasmid replication origin (ori) by overlap PCR. The resulting vector pKZ2 was used for gene deletion in chloramphenicol-resistant MRS, and 1000 bp of the DNA regions located upstream (recA50upF and recA50upR) and downstream (recA50dwF and recA50dwR) of the *recA* gene were obtained by PCR of Mu50 genomic DNA. Two fragments were digested with KpnI/EcoRI (upstream) and EcoRI/SalI (downstream) and cloned into pKZ2, respectively, to yield plasmid pKZΔrecA50. The complementing plasmid pCLrecA50 was transformed into Mu50ΔrecA to give strain Mu50ΔrecA::precA. All plasmids from *E. coli* were transferred via electroporation first to *S. aureus* RN4220 and subsequently to strain Mu50.

Purification of *S. aureus* LexA from *E.coli*. BL21(DE3) cells (Invitrogen) carrying pCold-lexA were diluted 1:100 in the LB broth supplemented with ampicillin (100 μg/mL) and grown at 37°C until OD600=0.5. Cells were induced with 0.1 mM IPTG at 16°C for 24h. Induced cells were harvested and resuspended in a ice-cold binding buffer (50 mM Tris, 0.5 mM EDTA-2Na, 50 mM NaCl, 10% (v/v) Glycerol, 20 mM Imidazole). Resuspended cells were sonicated and centrifuged at 20,000 × g and 4°C for 1h. The supernatant was filtered through a 0.22-μm filter before it was applied to the column (HisTrapTM HP, GE). The purification experiments were performed according to the manufacturer's instructions. The elution buffer (50 mM Tris, 0.5 mM EDTA-2Na, 50 mM NaCl, 10% (v/v) Glycerol, 200 mM Imidazole) was pre-cooled before being used. The eluate was dialyzed in the stock buffer (10 mM Tris, 50 mM KCl, 10 mM DTT, 30% (v/v) Glycerol; pH 7.5) at 4°C for 12h. Dialyzed LexA protein was stored in a -80°C freezer.

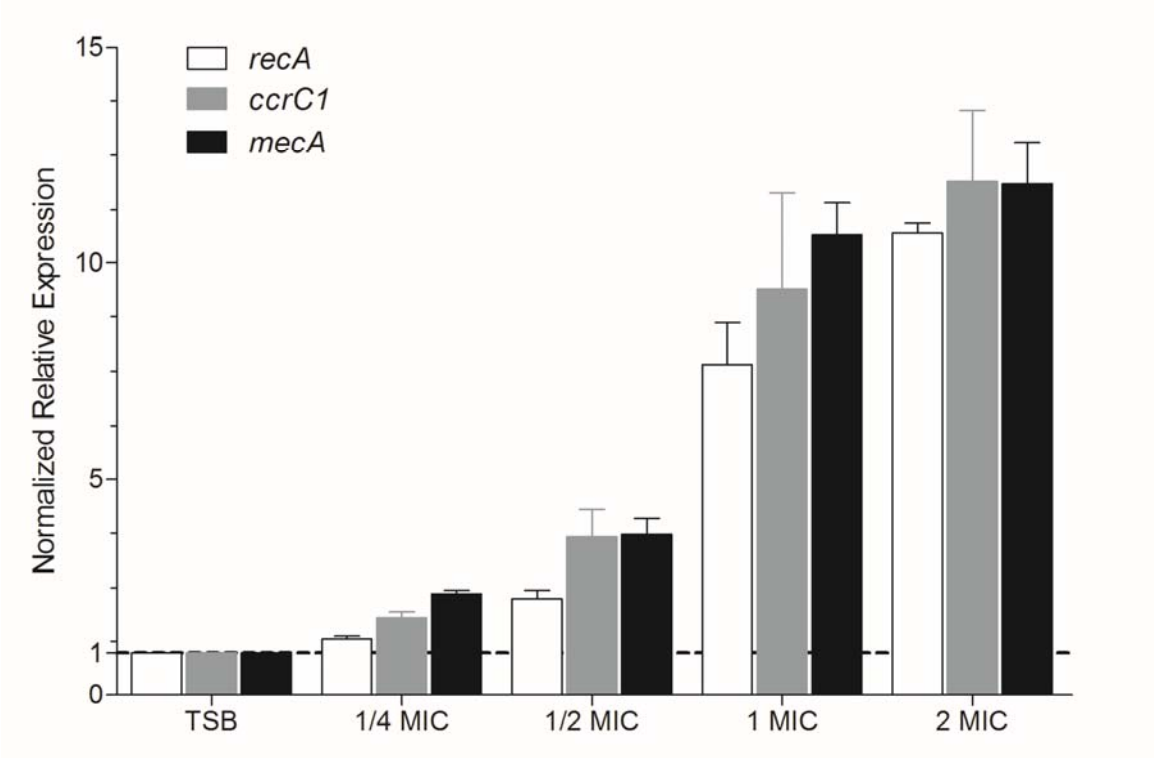
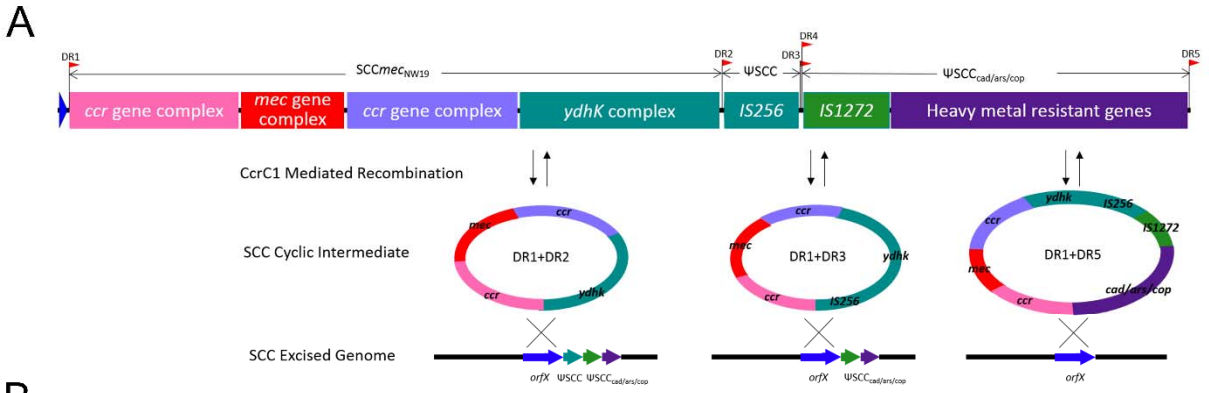


Figure S1. Effects of different concentrations of oxacillin on the SOS induction, *ccrC1* expression and *mecA* expression. Gene expression levels were normalized and are presented relative to the TSB cultured control. The expression of *recA* increases once SOS response is activated. The protein PBP2a encoded by *mecA* confers staphylococcus β-lactam resistance.



B

DR1	GTTGTCTTAATCGAGCAAGTATACAGAGCATTTAAAATCATGCGAGGCGAGGCTTATCATAAATAAAACTAAAAATTAGATTGTGTATAATTTAAAAATTTAATGAGATGTGGA
DR2	TTATTAAATGAAATTATATAATTAAAAATCGCATCATTAACCGATACGCAGAAGCATATCATAAATAAAACTAAAAATTAAATATAACGATTAATATTTTAATTTCAATGAGGTAAG
DR3	TATTAAGCCATTATAACGATTTGTTACTTTAGTAATATCATTGATAAGGGGAAGCATATCATAAATGATGAGGTTTTTTTAGCCGCTTCATAAAGGGATTTTGAACGTATCAGAA
DR5	TTCTACAAAAAGACGTTAATTAAAAACCGCATCATTAACCGATACGCAGAAGCGTATCACAAATAAAACTAAAAAATAGATTGTGTATAGTATAAAAGGAAGGGATTTATATTA
DR1+DR2	GTTGTCTTAATCGAGCAAGTATACAGAGCATTTAAAATCATGCGAGGCGAAGCATATCATAAATAAAACTAAAAATTAAATATAACGATTAATATTTTAATTTCAATGAGGTAAG
DR1+DR3	GTTGTCTTAATCGAGCAAGTATACAGAGCATTTAAAATCATGCGAGGCGAAGCATATCATAAATGATGAGGTTTTTTTAGCCGCTTCATAAAGGGATTTTGAACGTATCAGAA
DR1+DR5	GTTGTCTTAATCGAGCAAGTATACAGAGCATTTAAAATCATGCGAGGCGAAGCGTATCACAAATAAAACTAAAAAATAGATTGTGTATAGTATAAAAGGAAGGGATTTATATTA

Figure S2. Identification of SCC*mec* excision. (*A*) Schematic diagram of the recombination of the SCC composite island in *S. haemolyticus* NW19. SCC excision occurs between different DR sites yielding various excised products. Gene complexes are shown as boxes, and the colors of the boxes represent different gene clusters. Red arrowheads (DR1, DR2, DR3, DR4 and DR5) indicate the locations of integration site sequences for the SCC. SCC*mec*$_{NW19}$ consists of the *ccrC1* allele 8 gene complex, the *mec* gene complex, the *ccrC1* allele 2 gene complex and the *ydhK* gene cluster, which might be associated with heavy metal resistance, metallo β-lactamase resistance and anion transposition. ΨSCC harbors *IS256*, and ΨSCCcad/ars/cop harbors resistance genes for cadmium, arsenic and copper. The three circles designate the SCC cyclic intermediates. The *orfX* gene is marked as a blue arrow. The sequences of the recombination sites are shown below. (*B*) Identification of recombination sites in the genome before and after the excision of SCC elements. The core sequences in direct repeats in the recombination site are underlined. The DNA sequences containing DR1, DR2, DR3 and DR5 are marked with black, red, purple and blue, respectively. The DNA sequences of DR1 + DR2, DR1 + DR3 and DR1 + DR5 are black if they are derived solely from DR1, red if they are from DR2, purple if they are from DR3 and blue if from DR5 after SCC excision by CcrC1-mediated recombination.

Table S1. Isolates and plasmids used in this study

Strain and Plasmid	Characteristics	Sources
Strain		
NW19	MRSH, type V(5C2&5) SCC*mec*, *ccrC1* allele 2 & *ccrC1* allele 8	(1)
19ccrgfp	NW19 with pCL::*ccr-gfp* reporter	This study
19Mutccrgfp	NW19 with pCL::mut*ccr-gfp* reporter	This study
19recUp	NW19 with pCL*recA::ccr-gfp*	This study
19lexUp	NW19 with pCL*lexA::ccr-gfp*	This study
19recDw	NW19 with pCL*recA*i*::ccr-gfp*	This study
19lexDw	NW19 with pCL*lexA*i*::ccr-gfp*	This study
Mu50	MRSA, type II SCC*mec*, *ccrA2B2*	Lab stocks
ΔrecA	Mu50 with recA gene deleted	This study
ΔrecA::precA	Mu50ΔrecA with pCLrecA50	This study
Plasmid		
pCL15Z	*S. aureus* expression vector, Amp^R, Cm^R, $Bleo^R$	(1)
pCL::*gfp*	pCL15Z carrying *gfp* coding sequence with no promoter	This study
pCL::*ccr-gfp*	pCL15Z with *ccrC1::gfp* fusion	This study
pCL*recA*::*ccr-gfp*	pCL::*ccr-gfp* expressing RecA with IPTG induced promoter	This study
pCL*lexA*::*ccr-gfp*	pCL::*ccr-gfp* expressing LexA with IPTG induced promoter	This study
pCL*recA*i::*ccr-gfp*	pCL::*ccr-gfp* decreasing the expression of RecA with IPTG induced promoter	This study
pCL*lexA*i::*ccr-gfp*	pCL::*ccr-gfp* decreasing the expression of LexA with IPTG induced promoter	This study
pCL::mut*ccr-gfp*	pCL::*ccr-gfp* with mutations in SOS box of *ccrC1* promoter	This study
pCold II	*E.coli* expression vector, Amp^R, cspA promoter	Lab stocks
pCold II-lexA	NW19 LexA expression plasmid in *E. coli*	This study
pCold II-lexA50	Mu50 LexA expression plasmid in *E. coli*	This study
pKOR1	*S. aureus* shuttle vector	(2)
pKZ2	*S. aureus* shuttle vector modified from pKOR1	This study
pKZΔrecA50	Plasmid used for *recA* gene deletion in strain Mu50	This study
pCLrecA50	Complemental plasmid for *recA* gene with its own promoter in Mu50	This study

1. Liu P, Xue H, Wu Z, Ma J, Zhao X (2016) Effect of *bla* regulators on the susceptible phenotype and phenotypic conversion for oxacillin-susceptible *mecA*-positive staphylococcal isolates. *J Antimicrob Chemother* 71(8):2105-2112.
2. Bae T, Schneewind O (2006) Allelic replacement in *Staphylococcus aureus* with inducible counter-selection. *Plasmid* 55(1):58-63.

Table S2. Primers for cloning, sequencing and qPCR

Primers	Sequences (5'>3')
LexAexpF	CATGCCATGGATGAGAGAATTAACTAAACGTCAAAGTG
LexAexpR	CCGCTCGAGCAGTTCTCTGTATAAACCAATAACTTTACCG
Lex50expF	CGAGCTCAGAGAATTAACAAAACGACAAAGCG
Lex50expR	GGGGTACCTTACATTTCGCGGTACAAACCAATTACTTTCC
recAF	AAGGAAAAAAGCGGCCGCGATTAACTCGTTAATAGGAGGT TTCG
recAR	CGGGATCCCTATTCTTCAACGAATAATGTTTGAGGTG
irecAF	CGCGGATCCGATATTAATACGATGATTAACTCGTTAATAGGA GG
irecAR	AAAACTGCAGGTCAACTACTACAATATCAACCGCACCA
lexAF	AAGGAAAAAAGCGGCCGCGAGAGAATTAACTAAACGTCAA AGTG
lexAR	CGGGATCCCTACAGTTCTCTGTATAAACCAATAACTTTACCG
ilexAF	CGCGGATCCTAAAATTAGGGAGTGCCTATATATGAGAGAATT
ilexAR	CCCAAGCTTCTTCATCATCTTCAGTCATCGCAAC
ccrCOPF	TGAACAAATTGAAGCATTTG
ccrCOPR	CATATCATACCGCTCCTTTT
mut1F	AAAAACGAAATTATAAATATATATTATCT
mut1R	TATTTATAATTTCGTTTTTTTTTTATCA
mut2F	TGAAAAAAAATTATAAATATATATTATCT
mut2R	TATTTATAATTTTTTTTCATTTTTATCA
mut3F	TGAAACGAAAAAATAAATATATATTATCT
mut3R	TATTTATTTTTTCGTTTCATTTTTATCA
recA50upF	GGGGTACCTGTATTAGAACATAAAGTTATTGGAGAT
recA50upR	GGAATTCATATTTTTATCGATAATACAATTAATTTG
recA50dwF	GGAATTCTATCTATAGTTAAACTTAGCAAATATCCTT
recA50dwR	ACGCGTCGACATGTTTCGTCCTTCTCGTCC
rec50-F	AACTGCAGGTATTATCGATAAAAATATAAGCACGT
rec50-R	CGGGATCCCTTATAGTCTCGATTTGTAGTGTATCC
pCLF	CCGCTCGAGCCAAGCTAATTCGGTGGAAACG
pCLR	GGAATTCAACGGACAAAACCACTCAAAATAAA
gfpF	ATCTAATAAAAGGAGCGGTATGATATGGCTAGCAAAGGAGA AGAACTTTTC
gfpR	AACTGCAGTTATTTGTACAGTTCATCCATGCCATGTG
ccrOPF	GAAGATCTTGAACAAATTGAAGCATTTGTAGAAG
ccrOPR	GGCCGACGTCGACATCATACCGCTCCTTTTATTAGATTATT
ccrZmutF	AGTGGTAAAAGTGATAAAAATGAAAAAAAAAAATAAATATA TATTATCTATATGTTGTT
ccrZmutR	AACAACATATAGATAATATATATTTATTTTTTTTTTTCATTTTT ATCACTTTTACCACT
ccrAOPF	CCATAAATATATATTATTACTGTGAAAGGAGGAGCCGATATG AAACAAGTCATAGGCTA
ccrAOPR	TAGCCTATGACTTGTTTCATATCGGCTCCTCCTTTCACAGTA ATAATATATATTTATGG
Primers for detection of excision	
DR1F	AAAGCACCTGAAAATATGAGC

DR1R	TTTCATTCTTTCTTGATTCCATTA
DR2F	GCTGGTATTTTAGCATTTATCGG
DR2R	TGTGCCAATTTCGCTTCATTC
DR3F	TTCGTTGATTTTCCATTAGTTG
DR3R	TGTAGATTTTGGCTTATTCACTTC
DR5F	GCTTGGGACAGTTGTTGCTTT
DR5R	ATAGGAGCAATTCTCATAGCC
Primers for RT-PCR	
ccrC1RTF	GTGGAGATTTCTACTTACGTG
ccrC1RTR	TATAATACGTCCGTCGACA
recARTF	GATAAATGCAGCAACGCCAC
recARTR	CGCCGTGTTTCAAGTGTT
lexARTF	TACGAAACCGAGAGCCAT
lexARTR	CCGGCAGTAACTTTACCA
ccrARTF	ATGGGCTTCAAGAAAAAGCA
ccrARTR	GCCTTTACCGTTGGTGACTC
rec50RTF	AAATAAAGTGGCACCACCATT
rec50RTR	TTCTTCAACATCACCATCAGA
lex50RTF	TAACAAAACGACAAAGCGAA
lex50RTR	CGTGAAAGATGACCATGAAC
B1F†	CACACTGGAACTGAGACACG
B1R†	CTGCTGGCACGTAGTTAG
DR12EGRT	TACAACGCAGCAACTACGCA
DR12EGRT	ATTCTTTAGATTTTCATTTTTAGCCC
tpiRTF‡	AGAAACTTCTCCAGTGGCATTAGCTGA
tpiRTR‡	ATTTCATTAGCTTTGCCATTTTCGCGTTC
EG50RTF	GCAGTAACTACGCACTATCATTCAG
EG50RTR	GATATATCTTATATATTGAATGAACGTGGA
tpi50RTF§	TAAAGAAGGAAAAGCACAAGG
tpi50RTR§	TTCAGAATGACCGATAACAAC

†, reference gene, *Staphylococcus aureus* 16S RNA (from 320bp-532bp); ‡, one copy housekeeping gene as reference for excision detection in NW19; §, reference gene for excision detection in Mu50.

Table S3. The minimum inhibitory concentrations (MIC) of the antibiotics for *S. haemolyticus* NW19.

Antibiotic	**MIC (mg/L)**
Oxacillin	0.25
Teicoplanin	16
Vancomycin	2
Gentamicin	512
Kanamycin	512
Tetracycline	64
Sulfamethoxazole	256
Ciprofloxacin	32
Trimethoprim	1