Supplemental Material to the manuscript:

CidR and CcpA synergistically regulate *Staphylococcus aureus cidABC* expression.

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The Supplemental Material contains three tables (Table S1, Table S2 and Table S3) and two figures (Fig. S1 and Fig. S2).

Table S1. Strains and plasmids used in this study.

Bacterial strain or plasmid	Relevant properties	Source
Strains		
E.coli:		
DH5α	cloning host	Invitrogen
BL21	Expression strain, *F-*, *ompT, hsdS* ($r_B^-m_B^-$), gal, dcm (DE3)	Invitrogen
Rosetta 2	F^- *ompT hsdS*$_B$ (r_B^- m_B^-) gal dcm pRARE2 (Cam^R)	Invitrogen
S. aureus:		
RN4220	restriction-deficient mutant of strain 8325-4	(Novick, 1991)
UAMS-1	clinical osteomyelitis isolate	(Gillaspy *et al.*, 1995)
JE2	plasmid-cured derivative of USA300 LAC	(Fey *et al.*, 2013)
KB1090	UAMS-1 *cidR::tet*; Tet^R	(Yang *et al.*, 2005)
KB6001	JE2; Δ*geh*-P*cidABC::lacZ*	This study
JE2 *ccpA*::*tet*	JE2; Δ*ccpA::tetL;* Tet^R	(Halsey *et al.*, 2017)
UAMS-1-*ccpA*	UAMS-1; Δ*ccpA::tetL;* Tet^R	This study
UAMS-1-*ackA*	UAMS-1; Δ*ackA::ermB*; Erm^R;	(Sadykov *et al.*, 2013)

UAMS-1-*ackA/ccpA*	UAMS-1; Δ*ackA::ermB*; Erm^R; Δ*ccpA::tetL*; Tet^R	This study
UAMS-1-*ackA/cidR*	UAMS-1; Δ*ackA::ermB*; Erm^R; *cidR::tet*; Tet^R	This study
UAMS-1-*pta*	UAMS-1; Δ*pta*	(Sadykov *et al.*, 2013)
UAMS-1-*pta/ccpA*	UAMS-1; Δ*pta*; Δ*ccpA::tetL*; Tet^R	This study
UAMS-1-*pta/cidR*	UAMS-1; Δ*pta*; *cidR::tet*; Tet^R	This study
Plasmids		
pIHW7	Promoterless *lacZ* reporter plasmid, derivative of pCN51; Amp^R Erm^R	(Windham *et al.*, 2016)
pIHW9	*cidABC* reporter plasmid; Amp^R Erm^R, 658 bp	This study
pIHW10-lac	*cidABC* reporter plasmid; Amp^R Erm^R, truncation -177 upstream of transcription start site	This study
pIHW11-lac	*cidABC* reporter plasmid; Amp^R Erm^R, truncation -138 upstream of transcription start site	This study
pIHW17-lac	*cidABC* reporter plasmid; Amp^R Erm^R, truncation -57 upstream of transcription start site	This study
pIHW22-lac	*cidABC* reporter plasmid; Amp^R Erm^R, truncation -93 upstream of transcription start site	This study
pIHW25	*geh* allellic exchange plasmid with P*cidABC::lacZ* reporter (pCL52.2 derivative); Tet^R Spc^R	This study
pSW1	derivative of pIHW10-lac lacking *cre*-site; Amp^R Erm^R	This study
pCL52.2	Temperature-sensitive shuttle vector; Tet^R Spc^R	(Sau *et al.*, 1997)
pET24b	IPTG-inducible *E. coli* expression plasmid; Kan^R	Invitrogen
pET24b-*ccpA*	pET24b with *ccpA* gene	This study
pET24b-*cidR*	pET24b with *cidR* gene	This study

Table S2. Primers used in this study.

Primer designation	Nucleotide sequence (5′ - 3′)
IW10	GGGATCCAGCAAATTATCAATGATGAAGTAGATATAGGC
IW11	GGCTAGCGCCATCCCTTTCTAAATATGTCTAAATTGTTAC
IW15	GGATCCCATATTAATAAAGCACTCATTATTTGTGATTCC
IW16	GGATCCCTTGGATCATTGAAATAATGAGTGTTTTTTTTG
IW22	GGATCCCAAACCATAAAAAAAGAGTATTTTTATATTG
IW27	GAATTCTACATCCCTTGCTTATAGACACGATTAGTAATC
IW28	GAGCTCGAAAAACAACTGCACTTTCAATATAACATGACA
IW29	CTGCAGGTGCTACTAACATGGCACGGAAGATATAAGTAG
IW30	AAGCTTCAACCAACAAAAGGTGCCATTGTCTACATTCAT
IW31	GGATCCGAAATTTAGAGAGCGTTTCCATAGAAAATAGTA
IW44	CTGCAGTGTCACTTTGCTTGATATATGAGAATTATTTAA
IW110	AGTGAAATTTAGAGAGCGTTTCCATAGAAAATAGTAATACAAACC ATAAAAAAAGAGTAT
IW113	ATACTCTTTTTTTATGGTTTGTATTACTATTTTCTATGGAAACGCTC TCTAAATTTCACT
IW116	GGCTAGCGAGGAAATTATGACAGTTACTATATATGATGTAGC
IW117	GGCTCGAGTTTTGTAGTTCCTCGGTATTCAATTCTGTGTGG
IW128cc	GGGCTAGCGTGGATATCAAACATATGAAATATTTTATT
IW129cc	GGCTCGAGGCCTAAACGATCTTTCAAAAATTCTATCCA
IW176	CATAGAAAAAAAAAATACAAACCATAAAAAAAGAGTATTT
IW177	AAATACTCTTTTTTTATGGTTTGTATTTTTTTTTTCTAT
cre*-cidABC-f	GCAGTGTATCCAGGCTAGAAAATAGTAATACAAACTATAAAAAAA GAGT
cre*-cidABC-r	AATTTCACTTCATTTTCACAAAAAAACACTCA
pCN51-S2-f	CTGTGGATAACCGTATTACCGCC
B-gal-S-r	GATGACCATGATTACGGATTCACTGG
SAV1737-f	GCAACAAAGGACCATTTAACGATAATAC
acuC-f	GGTGGACTTGAAATATTCGCTACAG

RT-cidR-f	TGAAGTAGATATAGGCGTGACCAC
RT-cidR-r	GTTTCATATTTTGCGAGTCGATGC
RT-cidA-f	GCACAAAGTCCAATTAATAATCAAATTATTACTACAAC
RT-cidA-r	GTAAATAAAATAAAAATAGACCAACAATACTGCCG
sigA-rt-F	AACTGAATCCAAGTCATCTTAGTC
sigA-rt-R	TCATCACCTTGTTCAATACGTTTG
ptsI-F	CGGTAGTGATGAATCTGACG
ptsI-R	CTTACAATGAATACTTCGGG
ptsH-F	GAAGATTCTAACTATACGAAGGAG
ptsH-R	ATCAGATGCGGCAATACC
glkA-F	CCAGAAGATATGTACGAAATG
glkA-R	CCGTTCCAACTGGAATAAC

Table S3. Transposon mutants with decreased P_{cidABC}::*lacZ* activity.

Locus	**Description**	**Gene Name**
SAUSA300_1327	cell surface protein	*ebh*
SAUSA300_0659	sugar efflux protein, MFS family, sugar:cation symporter	
SAUSA300_0352	ABC transporter, ATP-binding protein	
SAUSA300_2645	glucose-inhibited division protein A	*gidA*
SAUSA300_0983	phosphocarrier protein HPr	*ptsH*
SAUSA300_0984	phosphoenolpyruvate-protein phosphotransferase	*ptsI*
SAUSA300_0388	inosine-5'-monophosphate dehydrogenase	*guaB*
SAUSA300_1507	glucokinase	*glkA*
SAUSA300_2059	ATP synthase F1, gamma subunit	*atpG*
SAUSA300_0495	hypothetical protein	
SAUSA300_1902	Conserved hypothetical protein, Lactonase	
SAUSA300_0552	Conserved hypothetical protein, LmbE family protein	
SAUSA300_1624	upstream of MutT/nudix family protein	

SAUSA300_0192	Conserved hypothetical protein	*murQ?*
Intergenic	metallopeptidase	
SAUSA300_2588	preprotein translocase	*SecY*
Intergenic	upstream of rrsC, 16s ribosomal RNA	
SAUSA300_1393	phiSLT ORF2067-like protein, phage tail tape measure protein	
SAUSA300_1015	cytochrome oxidase assembly protein	*ctaA*
SAUSA300_0764	ribonuclease R	*rnr*
SAUSA300_1259	ImpB/MucB/SamB family protein; DNA damage repair	
SAUSA300_0123	siderophore biosynthesis protein, IucC family	
SAUSA300_1286	aspartate kinase	

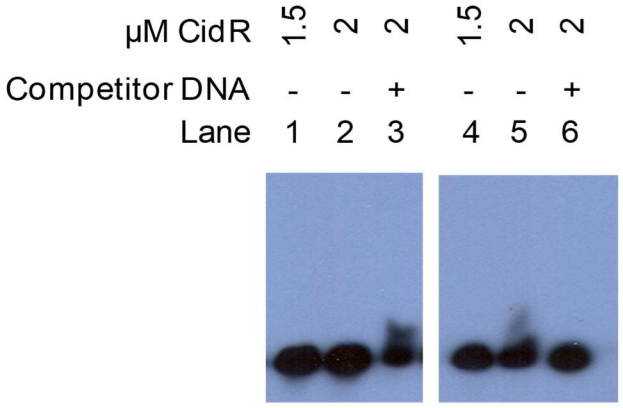

Fig. S1. CidR does not bind to the regions upstream and downstream of the CidR binding site.

EMSAs were performed using 1.5 and 2 μM concentrations of purified CidR protein and biotin-labeled DNA fragments located downstream (lanes 1-3) and upstream (lanes 4-6) of the putative CidR-binding site.

TAGAGAGCGTTTCCA
WTGNAANCGNWNNCW

Fig. S2. Alignment of the *S. aureus* UAMS-1 *cre*-box with *cre*-site consensus of *B. subtilis*.

N – A, T, C or G; W - T or A

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