

Supplementary Table S1 Primers used in this study.

Name	Sequence (5'-3')	Purpose
<i>RarcR</i> -UP-F- EcoRI	ATCGCAGTGCAGCGGAATTCTTGTGGTGCAA	<i>arcR</i> deletion
<i>RarcR</i> -UP-R	TGTCACAGGGTATG	
	TGTTTGCTTACTAACCAATCTGCTAATGCCTTT AGTTCATG	
<i>RarcR</i> -DN-F	CATGAACTAAAGGCATTAGCAGATTGGTTAGT AAGCAAACA	
<i>RarcR</i> -DN-R- HindIII	AAACTACCGCATTAAGCTTAGCATCAGCAG CATTTACTACCG	<i>icaA</i> deletion
<i>RicaA</i> -UP-F- EcoRI	ATCGCAGTGCAGCGGAATTCAGTAGCGAATA	
<i>RicaA</i> -UP-R	CACTTCATC	
	TCCAGTATACTGTCTGGATACGACAACGTATCT TCAATCGT	
<i>RicaA</i> -DN-F	ACGATTGAAGATACGTTGTCGTATCCGACAGT ATACTGGA	Expression of <i>arcR</i> in <i>S. aureus</i>
<i>RicaA</i> -DN-R- HindIII	AAACTACCGCATTAAGCTTAAGCCATAAGG ATAGGCTATAG	
pCN51- <i>arcR</i> -F	GGTCAATGTCTGAACCTGCAG	
	AGTTGTCTGCTGACACTTTGC	
pCN51- <i>arcR</i> -R	TCCTCTAGAGTCGACCTGCAGTACGTTAGAC CTCATGTTCAAC	β -Galactosidase activity assay of <i>hla</i> promoter
pOS1- <i>Phla</i> F	CAAAGCCTTAAAGACGATCCGGGGAATTCCC GACGAAATTCCAAACATA	
pOS1- <i>Phla</i> R	TCACGACGTTGTAAAACGACGGGATCCGGCA TTAGCGACAGGATT	
pOS1- <i>Phlb</i> F	CAAAGCCTTAAAGACGATCCGGGGAATTCTG CAACTTAATTATAGCCAG	
pOS1- <i>Phlb</i> R	TCACGACGTTGTAAAACGACGGGATCCTGCT AATGCAAGTGTTGCAAC	β -Galactosidase activity assay of <i>hlb</i> promoter
pOS1- <i>Psb</i> iF	CAAAGCCTTAAAGACGATCCGGGGAATTCTC TACTCTACCAAACCTCTCTTCG	
pOS1- <i>Psb</i> iR	TCACGACGTTGTAAAACGACGGGATCCAGCT AACGTAATTGTTGCTG	
pOS1- <i>Pvra</i> XF	CAAAGCCTTAAAGACGATCCGGGGAATTCAC TCATCTATATGCTCAATACCTC	
pOS1- <i>Pvra</i> XR	TCACGACGTTGTAAAACGACGGGATCCTTCA TGGTGATACTGTTCGAT	β -Galactosidase activity assay of <i>vraX</i> promoter
pOS1- <i>Pspl</i> AF	CAAAGCCTTAAAGACGATCCGGGGAATTCAC ATGAATTGTTACCATTC	
pOS1- <i>Pspl</i> AR	TCACGACGTTGTAAAACGACGGGATCCAGCA GTAAACCTTTAACC	
pET28a- <i>arcR</i> -F	TTGAAGGAGTTTAACTTATGCATCATCATCAT CATCACACAGAAAACCTTTATTTTGG	

pET28a- <i>arcR</i> -R	TCCTCTAGAGTCGACCTGCAGTACGTTAGAC CTCATGTTCAAC	
Q-16S rRNA-F	ACAAAGTGACAGGTGGTGCA	qRT-PCR
Q-16S rRNA-R	GTTTGTCAACCGGCAGTCAAC	
Q- <i>hla</i> -F	ACAACACTATTGCTAGGTTCC	qRT-PCR
Q- <i>hla</i> -R	TCGTTCTAATAACTAGCAG	
Q- <i>hlb</i> -F	AGTTGCAACACTTGCATTAGC	qRT-PCR
Q- <i>hlb</i> -R	AAGACTATCGAAGCAAGCG	
Q- <i>icaA</i> -F	TACGTTGTCTAATGTTCTTGC	qRT-PCR
Q- <i>icaA</i> -R	AGTATCTGCATCCAAGCAC	
Q- <i>icaB</i> -F	TGCAGATGACGATTCACC	qRT-PCR
Q- <i>icaB</i> -R	TAGCATCATGTGATTTTAGCC	
Q- <i>icaC</i> -F	TCTTGTCACAGTTACTGACAACC	qRT-PCR
Q- <i>icaC</i> -R	TACCATTGACCTAATAGGAC	
Q- <i>icaD</i> -F	AGAGAAACAGCACTTATCGC	qRT-PCR
Q- <i>icaD</i> -R	AGCAACACGTATTGTATTG	
Q- <i>sbi</i> -F	ACAACACTACGTAACAGATCAAC	qRT-PCR
Q- <i>sbi</i> -R	TGCAACACGTCGGTCTGGGGTTC	
Q- <i>vraX</i> -F	ATCGACAGTATCACCATGAAGG	qRT-PCR
Q- <i>vraX</i> -R	CATATGATCTATATCGTCTTG	
Q- <i>splA</i> -F	TCAGCCTCATTCAATTGCC	qRT-PCR
Q- <i>splA</i> -R	ACTCGAATGATGTGCTGATACTC	
Q- <i>yycF</i> -F	ACCTGGTCGTGATGGTATGG	qRT-PCR
Q- <i>yycF</i> -R	CCAGTGTCTTGTGCTGGTTG	
Q- <i>yycG</i> -F	ACCAGACGGTCGAAATGTCC	qRT-PCR
Q- <i>yycG</i> -R	ACCAAGCATCTTGAGTGCC	
E- <i>icaA</i> -F	AGCTATATCATCAAGTGTTG	EMSA
E- <i>icaA</i> -R	ACCTACCTTTCGTTAGTTAG	
E- <i>hla</i> -F	TCAACTTTGACTAACCCTCG	EMSA
E- <i>hla</i> -R	AACCTAGCAATAGTGTGTTG	
E- <i>hlb</i> -F	AGTTGTTGTAATCAATGAC	EMSA
E- <i>hlb</i> -R	TGCTAATGCAAGTGTGCAAC	
E- <i>tetA</i> -F	AGCGCGTGTTGTTATGTCCG	EMSA
E- <i>tetA</i> -R	ACCTCCTTATTAGACAATGTG	

Supplementary Table S2 The quantitative hemolytic activity

Samples	OD543		
NCTC8325	0.786	0.963	0.910
Δ <i>arcR</i>	1.505	1.488	1.400
Δ <i>arcR/arcR</i>	0.700	0.665	0.788
Negative	0.087	0.035	0.071
Positive	1.750	1.663	1.575

Supplementary Table S3 Genes downregulated by ArcR. (P < 0.05 and fold change log₂ < -2)

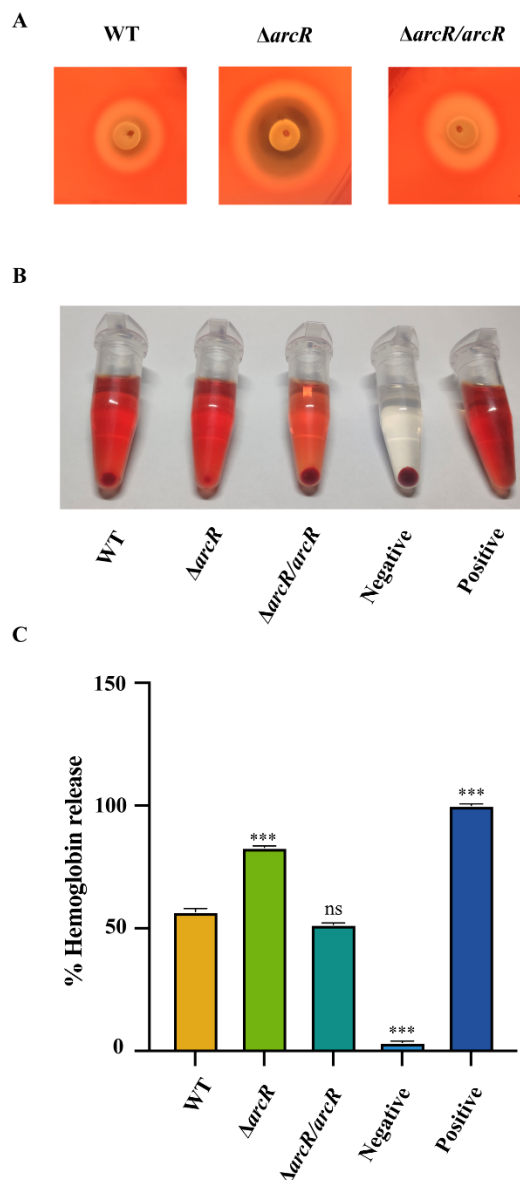
Gene_id	Log2Fold Change	Gene_name	Gene_description
NCTC8325_02680	-7.12	<i>arcA</i>	Arginine deiminase
NCTC8325_00169	-5.54	<i>pflB</i>	formate acetyltransferase
NCTC8325_00183	-5.27	<i>hmp</i>	Flavohemoprotein (Hemoglobin-like protein)
NCTC8325_00170	-5.26	<i>pflA</i>	Pyruvate formate-lyase activating enzyme
NCTC8325_01308	-5.41	<i>aldI</i>	Alanine dehydrogenase
NCTC8325_01307	-5.29	<i>ilvA_1</i>	Threonine dehydratase%2C catabolic
NCTC8325_00181	-5.31	NCTC8325_00181	Putative cytosolic protein
NCTC8325_00182	-4.86	NCTC8325_00182	membrane protein
NCTC8325_01306	-4.89	<i>steT</i>	amino acid permease
NCTC8325_00202	-4.63	<i>scdA</i>	DnrN
NCTC8325_02679	-4.98	<i>arcB</i>	ornithine carbamoyltransferase
NCTC8325_02660	-4.52	NCTC8325_02660	Ribonucleotide reductase of class III
NCTC8325_02661	-4.13	<i>nrdD</i>	Ribonucleotide reductase of class III
NCTC8325_02526	-3.99	NCTC8325_02526	Uncharacterised protein
NCTC8325_00099	-3.77	<i>adhE</i>	acetaldehyde dehydrogenase
NCTC8325_02753	-4.76	NCTC8325_02753	Permease
NCTC8325_00949	-4.07	NCTC8325_00949	Cytochrome d ubiquinol oxidase subunit II
NCTC8325_00557	-3.35	<i>adhI</i>	Alcohol dehydrogenase
NCTC8325_01027	-3.56	<i>argF</i>	ornithine carbamoyltransferase
NCTC8325_01305	-3.18	<i>norB_4</i>	Multidrug resistance protein B
NCTC8325_01311	-3.38	NCTC8325_01311	pepSY-associated TM helix family protein
NCTC8325_00948	-3.21	<i>cydA</i>	Cytochrome d ubiquinol oxidase subunit I
NCTC8325_02594	-2.76	<i>clpL</i>	putative ATP-dependent protease ATP-binding subunit ClpL
NCTC8325_00180	-2.63	<i>gsiB</i>	ABC superfamily ATP binding cassette transporter
NCTC8325_02678	-2.64	<i>arcD_2</i>	arginine/ornithine antiporter PF13520:Amino acid permease
NCTC8325_00055	-2.60	NCTC8325_00055	myosin-crossreactive antigen

NCTC8325_02752	-2.60	NCTC8325_02752	membrane protein
NCTC8325_02751	-2.22	<i>immR_2</i>	Transcriptional regulator
NCTC8325_01028	-2.34	<i>arcC1</i>	carbamate kinase

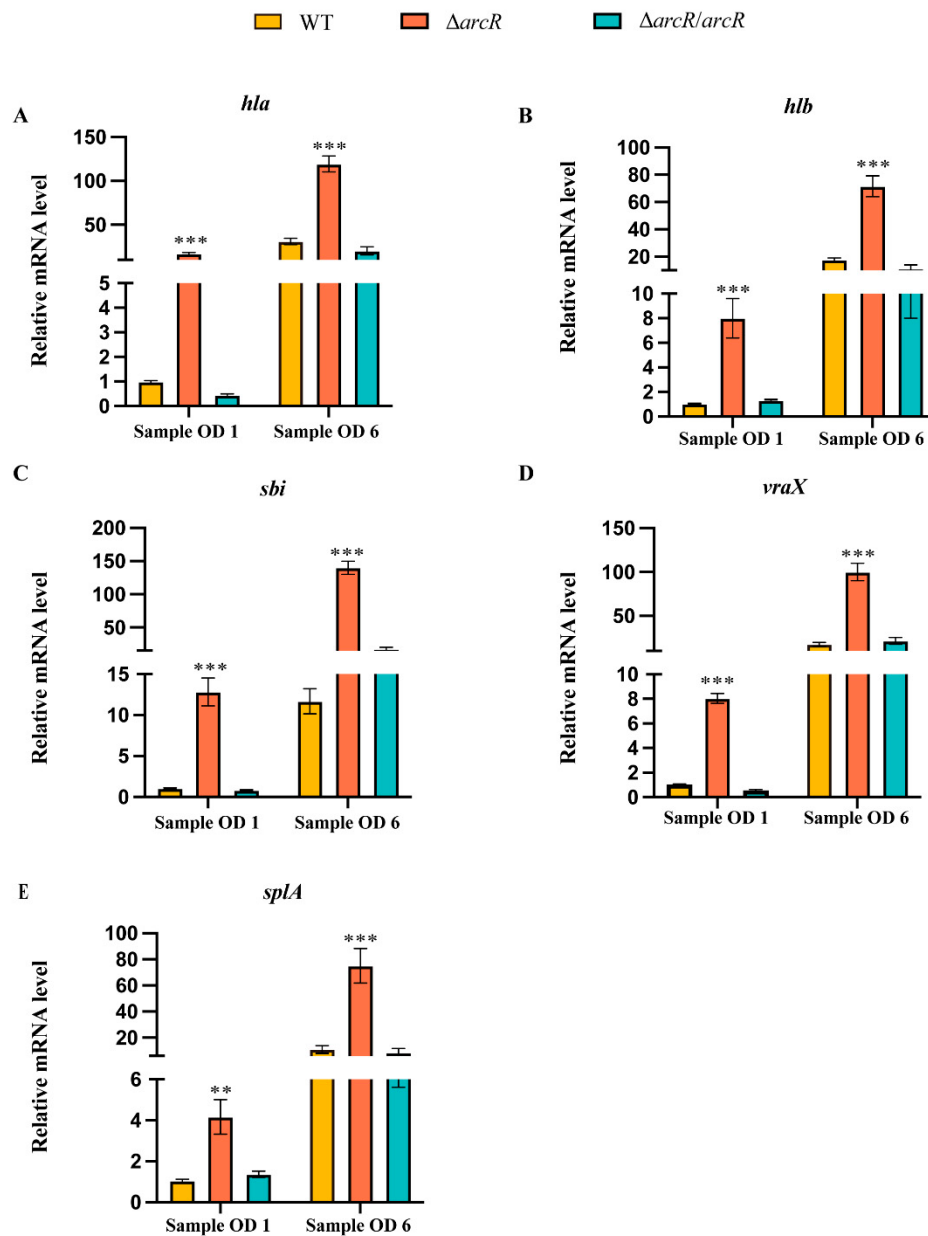
Supplementary Table S4 Genes upregulated by ArcR. (P < 0.05 and fold change log₂ >2)

gene_id	log2Fold Change	gene_name	gene_description
NCTC8325_01872	13.60	NCTC8325_01872	Phage head protein
NCTC8325_01873	13.06	NCTC8325_01873	phi 11 orf33 && PF14265
NCTC8325_01870	11.82	NCTC8325_01870	phi 11 orf36 && PF05135
NCTC8325_01869	11.54	NCTC8325_01869	phage-like protein
NCTC8325_01875	11.49	NCTC8325_01875	phage putative head morphogenesis protein
NCTC8325_01868	11.38	NCTC8325_01868	phi 11 orf37 && PF04883
NCTC8325_01871	11.18	NCTC8325_01871	phi 11 orf35 && -
NCTC8325_01874	8.63	NCTC8325_01874	Uncharacterised protein && -
NCTC8325_00457	6.77	NCTC8325_00457	16S ribosomal RNA
NCTC8325_00571	5.97	NCTC8325_00571	Protein of uncharacterised function (DUF2922)
NCTC8325_02348	4.5	NCTC8325_02348	Uncharacterised protein
NCTC8325_01867	4.43	NCTC8325_01867	phi 11 orf38
NCTC8325_01188	4.12	NCTC8325_01188	Uncharacterised protein
NCTC8325_01707	4	NCTC8325_01707	comK family protein
NCTC8325_01408	3.66	NCTC8325_01408	phage protein
NCTC8325_01982	3.46	<i>chp</i>	Chemotaxis-inhibiting protei
NCTC8325_02713	3.45	<i>icaB</i>	polysaccharide deacetylase
NCTC8325_01979	3.44	<i>hly_1</i>	beta-hemolysin
NCTC8325_01411	3.34	NCTC8325_01411	DNA-binding protein
NCTC8325_02649	3.15	NCTC8325_02649	Uncharacterised protein
NCTC8325_01876	3.03	NCTC8325_01876	phage-like protein
NCTC8325_00172	2.96	<i>scn_1</i>	complement inhibitor SCIN family protein
NCTC8325_02711	2.87	<i>icaA</i>	Polysaccharide intercellular adhesin (PIA) biosynthesis N-glycosyltransferase IcaA
NCTC8325_02722	2.74	<i>hisD</i>	Histidinol dehydrogenase
NCTC8325_01415	2.66	NCTC8325_01415	putative phage transcriptional regulator
NCTC8325_01749	2.63	NCTC8325_01749	probable beta-lactamase
NCTC8325_00453	2.62	NCTC8325_00453	16S ribosomal RNA
NCTC8325_02724	2.55	NCTC8325_02724	ATP phosphoribosyltransferase%2C regulatory subunit
NCTC8325_00247	2.53	NCTC8325_00247	lipoprotein%2C putative

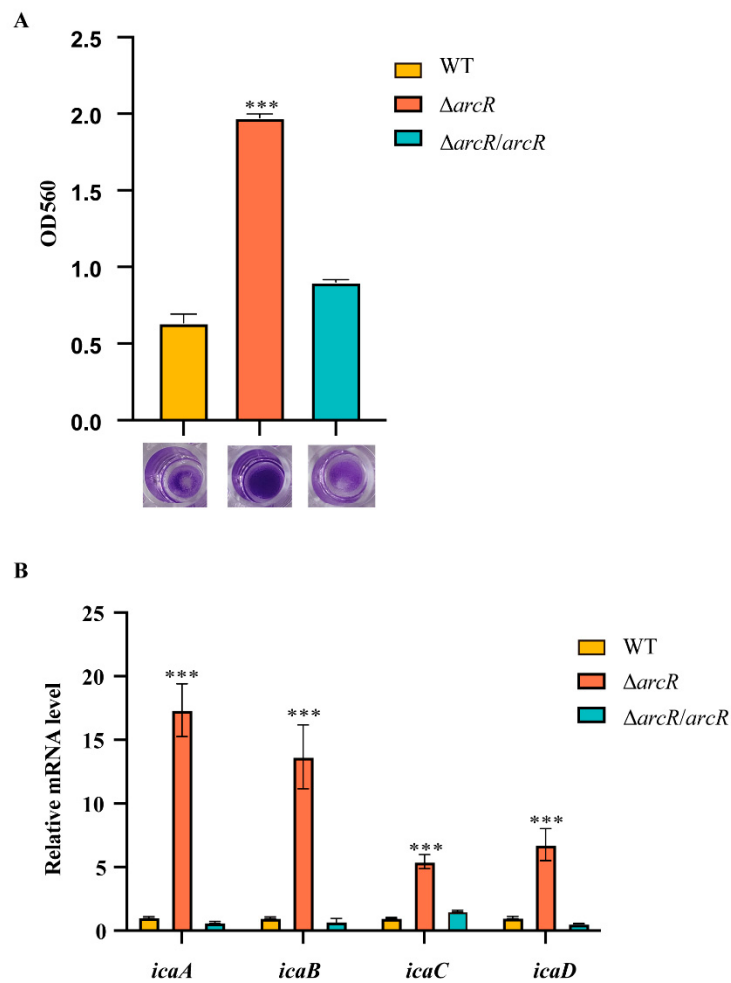
NCTC8325_01828	2.52	NCTC8325_01828	23S ribosomal RNA
NCTC8325_02208	2.51	NCTC8325_02208	16S ribosomal RNA
NCTC8325_00393	2.43	NCTC8325_00393	tRNA-Ser
NCTC8325_02602	2.43	NCTC8325_02602	exported protein
NCTC8325_01021	2.42	<i>hla</i>	alpha-hemolysin
NCTC8325_02714	2.35	<i>icaC</i>	intercellular adhesion protein icaC
NCTC8325_02556	2.31	<i>yvnA</i>	MarR family transcriptional regulator
NCTC8325_01474	2.29	<i>comGD</i>	Late competence protein ComGD%2C access of DNA to ComEA
NCTC8325_02541	2.27	<i>sarT</i>	Transcriptional regulator SarT
NCTC8325_00750	2.26	NCTC8325_00750	membrane protein
NCTC8325_00404	2.25	NCTC8325_00404	16S ribosomal RNA
NCTC8325_02720	2.22	<i>hisB</i>	imidazoleglycerol-phosphate dehydratase
NCTC8325_00174	2.21	<i>thlA</i>	3-ketoacyl-CoA thiolase
NCTC8325_01706	2.2	<i>sigS</i>	RNA polymerase sigma factor sigS
NCTC8325_02119	2.2	NCTC8325_02119	Uncharacterised protein
NCTC8325_00970	2.19	NCTC8325_00970	Uncharacterised protein
NCTC8325_01150	2.19	NCTC8325_01150	Uncharacterised protein
NCTC8325_00392	2.18	<i>gltD</i>	Glutamate synthase [NADPH] small chain
NCTC8325_01748	2.17	<i>splA</i>	serine protease SplA
NCTC8325_02462	2.08	<i>sbi</i>	IgG-binding protein SBI
NCTC8325_01016	2.07	NCTC8325_01016	membrane protein
NCTC8325_02723	2.05	<i>hisG</i>	ATP phosphoribosyltransferase subunit
NCTC8325_01866	2.01	NCTC8325_01866	phage major tail protein%2C TP901-1 family%3B phi 11 orf39
NCTC8325_00391	1.98	<i>gltB_1</i>	glutamate synthase [NADPH] large subunit
NCTC8325_00175	1.97	NCTC8325_00175	Enoyl-CoA hydratase / Enoyl-CoA hydratase [valine degradation] / 3-hydroxyacyl-CoA dehydrogenase
NCTC8325_00521	1.95	<i>vraX</i>	protein vraX
NCTC8325_00165	1.94	<i>uhpT</i>	sugar phosphate transporter
NCTC8325_02712	1.93	<i>icaD</i>	Polysaccharide intercellular adhesin (PIA) biosynthesis protein IcaD



Supplementary Figure S1 ArcR regulates the hemolytic activity of *S. aureus* USA300. (A) Hemolytic activities of the WT, $\Delta arcR$ mutant, and complemented *arcR* mutant were evaluated on SBA plates. (B) Hemolytic activities of the WT, $\Delta arcR$ mutant, and complemented *arcR* mutant were determined by incubating samples with 3% sheep red blood cells, PBS and ddH₂O were used as negative control and positive control, respectively. (C) Hemolytic activities of the WT, $\Delta arcR$ mutant, and complemented *arcR* mutant were determined by measuring the absorption of supernatants at 543 nm. The error bars indicate the standard errors of the means of three biological replicates. *** $P < 0.001$, analyzed by one-way ANOVA.

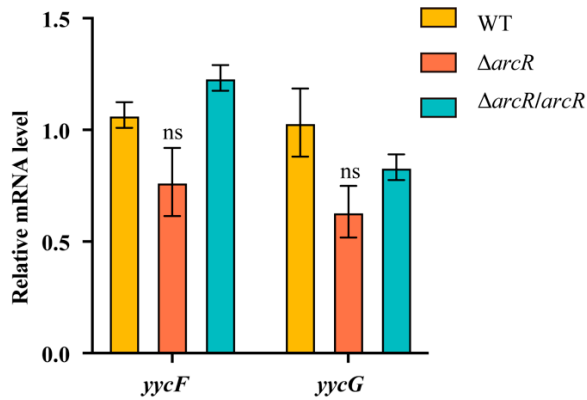


Supplementary Figure S2 ArcR negatively regulates the transcription of hemolytic genes in *S. aureus* USA300. The transcript levels of *hla* (A), *hlb* (B), *sbi* (C), *vraX* (D) and *splA* (E) in the WT of *S. aureus* USA300, $\Delta arcR$ mutant, and complemented *arcR* mutant at different growth phases. **, $P < 0.01$, *** $P < 0.001$, analyzed by one-way ANOVA.



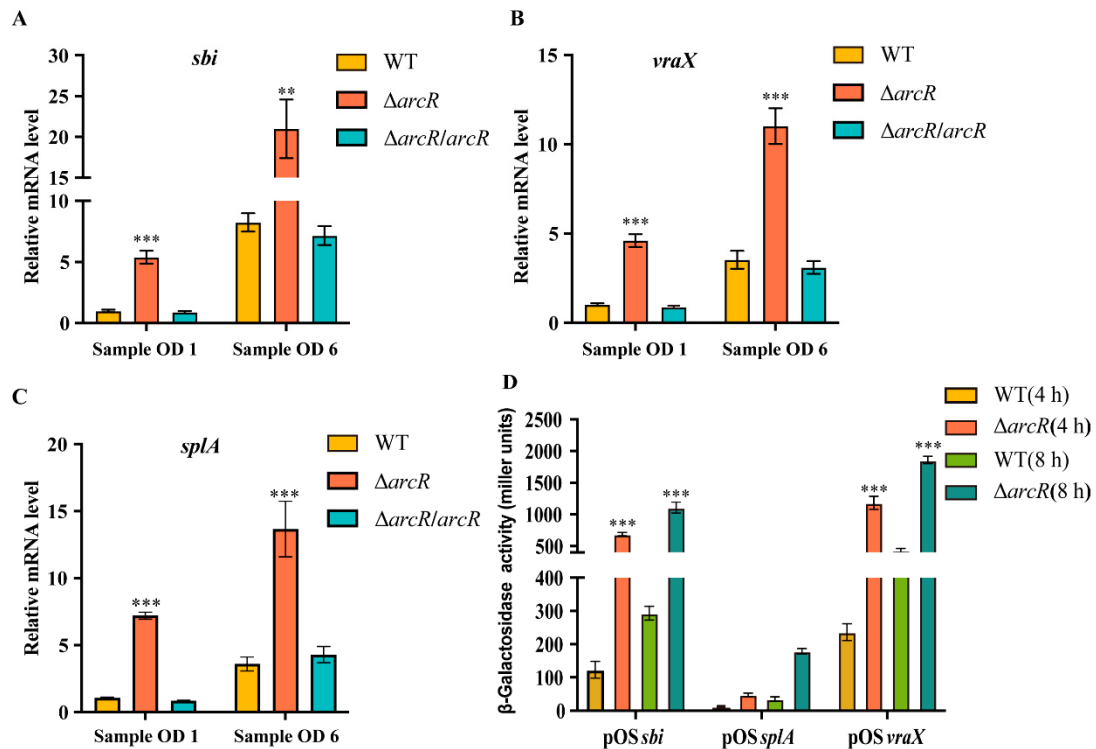
Supplementary Figure S3 Roles of ArcR in biofilm formation of *S. aureus* USA300.

(A) Biofilm formation of the WT of *S. aureus* USA300, $\Delta arcR$ mutant, and complemented *arcR* mutant was detected by 96-well microtiter plate assays. *** $P < 0.001$, compared to each other by one-way ANOVA. (B) The transcript levels of *icaA*, *icaB*, *icaC* and *icaD* in the WT of *S. aureus* USA300, $\Delta arcR$ mutant, and complemented *arcR* mutant detected by qRT-PCR. *** $P < 0.001$, analyzed by one-way ANOVA.



Supplementary Figure S4 The transcriptional levels of *yycFG* in *S. aureus* NCTC8325.

The transcript levels of *yycF* and *yycG* (B) in the WT, $\Delta arcR$ mutant, and *arcR* complementary strains. The error bars indicate the standard errors of the means of three biological replicates. ns: no significant difference. ns, indicated no statistical difference, analyzed by one-way ANOVA.



Supplementary Figure S5 ArcR negatively regulates the transcriptional of virulence genes in *S. aureus* NCTC8325.

The transcript levels of *sbi* (A), *vraX* (B) and *splA*(C)in the WT NCTC8325, $\Delta arcR$ mutant, and *arcR* complementary strains at different growth phases. (D) The β -galactosidase activities of *sbi*, *vraX* and *splA* promoter in the WT and $\Delta arcR$ mutant strains. Cells were collected at 4 and 8 h, and the β -galactosidase activity was detected with ONPG. The error bars indicate the standard errors of the means of three biological replicates. **P < 0.01, ***P < 0.001, analyzed by one-way ANOVA.