Supplementary Information

Targeting catabolite control protein A from *Staphylococcus aureus* by auranofin

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Strains, plasmids and primers.

Strains, plasmids and primers are listed in Table S1. The *Escherichia coli* (*E. coli*) XL1-Blue and BL21(DE_3) strains was employed for CcpA expression and purification. *Staphylococcus aureus* (*S. aureus*) strains were cultured in TSB medium. Custom polyclonal antisera to *S. aureus* CcpA were prepared by immunizing rabbit with purified CcpA protein.

Purification of CcpA proteins

CcpA proteins from *S. aureus* were purified by reference method[1].

***S. aureus* mutant construction**

The *S. aureus* mutant was obtained as previously described[1]. Primers used to generate flanking DNA fragments for allelic replacement are listed in Table S1.

Fluorescence Thermal shift analysis (FTSA)

Briefly, 20 μL reactions buffer (3O-C12HSL, 100 μM; auranofin, 50 μM); purified CcpA, 10 μM; SYPRO dye, 1:125 *v/v*) was transferred into a 8-tube strip. Then the melting curves of CcpA was recorded after incubated for 30 min at 4°C using Real-Time PCR. The temperature were elevated from 25 to 99°C at a rate of 1°C/min and the transition midpoint (T_m) values of the protein were quantified.

Cellular thermal shift assay (CETSA)

S. aureus was co-incubated with auranofin at 37 ºC for 150 min. Afterwards, collected the bacteria and then washed with PBS. After equally divided into 10 aliquots in a volume of 50 μL, bacteria was heated individually for 3 min at different temperatures and then cooled using ice. After repeated this steps 3 times, three intense freeze-thaw cycles were conducted using liquid nitrogen to lyse the bacterial cells. The resulting solution were centrifuged at 15000×g at 4 ºC for 10 min to remove the denatured proteins. At last, the soluble CcpA in the supernatants were quantified through western blotting.

β-Gal assays

The *S. aureus* RN4220 or mutant strains containing the *lacZ* reporter plasmid

(pALC-*pckA::lacZ* or pALC-*hla::lacZ*) were used for this assay. In brief, overnight culture of the bacteria was diluted into fresh glucose-replete LB medium (containing 50 mM Hepes, 10 mM glucose). The culture was grown at 37°C until at OD_{600} reached to 1.0. The bacteria were then treated with auranofin and cultured for 3 h. Subsequently, the bacteria were collected and cell density was recorded. After cell lysis, the β-Gal activity of the cell lysate was determined using the 4-methylumbelliferyl-β-d-galactoside as substrates. Fluorescence at 460 nm was monitored for 30 min to determine the produced 7-hydroxy-4-methylcoumarin with an excitation wavelength of 365nm. At last, the fluorescence was normalized to bacterial density to obtain the relative β-Gal activity.

Transcriptomics analysis

Bacteria at logarithmic phase was diluted 100 times with TSB, then auranofin was added. After co-incubated in a shaker at 37 ℃ for 8 h, collected the bacteria and then wash 3 times with sterile PBS at 4 ℃. Afterwards, collected bacterial precipitation. The resulted bacteria were frozen in liquid nitrogen for 15 minutes and stored at -80℃. At last, all samples was employed for transcriptomic analysis.

Inhibition of hemolytic toxin secretion

The effect of auranofin on the release of hemolytic toxin from *S. aureus* was investigated by rabbit red blood cells. The *S. aureus* was cultured in TSB medium until OD_{600} reached 1. Then the bacterial suspension were diluted with fresh TSB medium at a ratio of 1:100. Take out 3 mL diluted bacterial suspension and 1 mL auranofin into 10 mL shaker tube, incubated at 37℃ with 220 rpm. After 20 h, the bacterial solution was centrifuged at 5000 rpm for 2 min to retain the supernatant. Fresh rabbit blood cell were centrifuged for 2 min at 2000 rpm and washed with sterile PBS 3 times. Subsequently, 1 mL sterile PBS, 50 μL rabbit blood cell and 100 μL bacterial supernatant were added into 1.5 mL centrifuge tube and incubated at 37 ℃ for 30 min. The supernatant was subsequently centrifuged to remove the supernatant and the absorbance value of OD_{543} was measured as a measure of the amount of α-hemolysin.

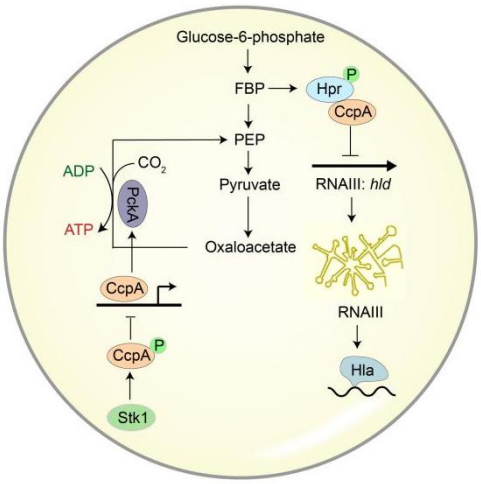
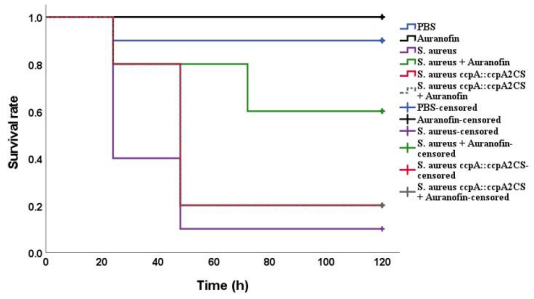


Figure S1 The pathway map for the downstream gene-gene (*pckA* and *hla*) interaction network of CcpA.

A)



B)

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	31.158	5	.000

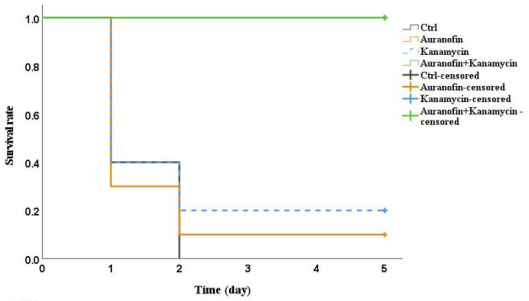
C)

Pairwise Comparisons

		PBS		Auranofin		*S. aureus*		*S. aureus* + Auranofin		*S. aureus ccpA::ccpA*^{2CS}		*S. aureus ccpA::ccpA*^{2CS} + Auranofin	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	PBS			1.000	.317	12.109	.001	2.135	.144	7.957	.005	7.957	.005
	Auranofin	1.000	.317			16.293	.000	4.749	.029	12.358	.000	12.358	.000
	S. aureus	12.109	.001	16.293	.000			7.203	.007	2.212	.137	2.212	.137
	S. aureus + Auranofin	2.135	.144	4.749	.029	7.203	.007			3.179	.075	3.179	.075
	S. aureus ccpA::ccpA ^{2CS}	7.957	.005	12.358	.000	2.212	.137	3.179	.075			.000	1.000
	S. aureus ccpA::ccpA ^{2CS} + Auranofin	7.957	.005	12.358	.000	2.212	.137	3.179	.075	.000	1.000		

Figure S2. (A) After treated with auranofin, the survival rate of *G. mellonella* which infected by *S. aureus* or *ccpA::ccpA*^{2CS} mutant; **(B)** The results of overall log-rank test; **(C)** Pairwise comparison analysis among groups. (In the log-rank test graph, only three decimal places are retained; P<0.05 indicates a significant difference)

A)



B)

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	23.445	3	.000

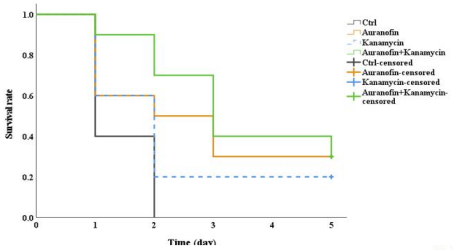
C)

Pairwise Comp:

		Ctrl		Auranotin		Kanamycin		Auranotin + Kanamycin	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	Ctrl			.004	.953	.591	.442	19.793	.000
	Auranofin	.004	.953			.366	.545	16.671	.000
	Kanamycin	.591	.442	.366	.545			13.233	.000
	Auranofin+Kanamycin	19.793	.000	16.671	.000	13.233	.000		

Figure S3. (A) The survival rate of *S. aureus*-infected *G. mellonella* after treated with kanamycin in the presence of auranofin during 5 days; **(B)** The results of overall log-rank test; **(C)** Pairwise comparison analysis among groups. (In the log-rank test graph, only three decimal places are retained; P<0.05 indicates a significant difference)

A)



B)

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	9.148	3	.027

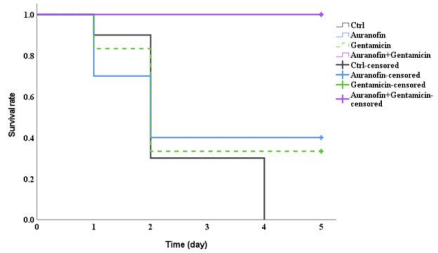
C)

Pairwise Comparisons

		Ctrl		Auranofin		Kanamycin		Auranofin + Kanamycin	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	Ctrl			4.540	.033	1.859	.173	11.147	.001
	Auranofin	4.540	.033			.359	.549	.237	.626
	Kanamycin	1.859	.173	.359	.549			1.747	.186
	Auranofin + Kanamycin	11.147	.001	.237	.626	1.747	.186		

Figure S4. (A) The survival rate of *S. aureus ccpA::ccpA*^{2CS}-infected *G. mellonella* after treated with kanamycin in the presence of auranofin during 5 days; **(B)** The results of overall log-rank test; **(C)** Pairwise comparison analysis among groups. (In the log-rank test graph, only three decimal places are retained; P<0.05 indicates a significant difference)

A)



B)

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	16.099	3	.001

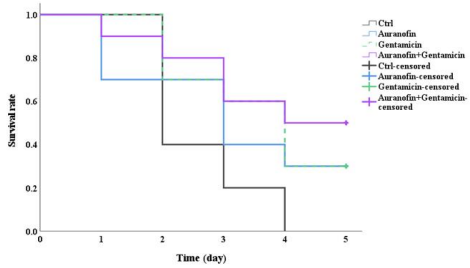
C)

Pairwise Comparisons

		Ctrl		Auranofin		Gentamicin		Auranofin+Gentamicin	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	Ctrl			1.153	.283	1.194	.275	20.060	.000
	Auranofin	1.153	.283			.000	.988	8.156	.004
	Gentamicin	1.194	.275	.000	.988			9.687	.002
	Auranofin+Gentamicin	20.060	.000	8.156	.004	9.687	.002		

Figure S5. (A) The survival rate of *S. aureus*-infected *G. mellonella* after treated with gentamicin in the presence of auranofin during 5 days; **(B)** The results of overall log-rank test; **(C)** Pairwise comparison analysis among groups. (In the log-rank test graph, only three decimal places are retained; P<0.05 indicates a significant difference)

A)



B)

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	6.310	3	.097

C)

Pairwise Comparisons

		Ctrl		Auranofin		Gentamicin		Auranofin+Gentamicin	
		Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.	Chi-Square	Sig.
Log Rank (Mantel-Cox)	Ctrl			1.676	.195	4.416	.036	5.659	.017
	Auranofin	1.676	.195			.163	.687	.850	.357
	Gentamicin	4.416	.036	.163	.687			.404	.525
	Auranofin+Gentamicin	5.659	.017	.850	.357	.404	.525		

Figure S6. (A) The survival rate of *S. aureus ccpA::ccpA*^{2CS}-infected *G. mellonella* after treated with gentamicin in the presence of auranofin during 5 days; **(B)** The results of overall log-rank test; **(C)** Pairwise comparison analysis among groups. (In the log-rank test graph, only three decimal places are retained; P<0.05 indicates a significant difference)

Table S1 Strains, plasmids and primers

Strain, plasmids or primers	**Application**	
***E.coli* strains**		
BL21(DE_3)	Protein expression	
***Staphylococcus aureus* strains**		
RN4220	Modification plus strain	
Newman	Wild-type strain. Used in CETSA, biofilm and erythrocyte lysis assay	
Newman *ccpA::ccpA*^{2CS}	Wild-type *ccpA* gene was replaced by *ccpA*^{2CS}. Used in CETSA, biofilm and erythrocyte lysis assay	
Plasmids		
pET47b		
pET47b-*ccpA*	Wild-type CcpA protein expression	
pET47b-*ccpA*^{2CS}	CcpA^{2CS} mutant protein expression	
pKOR1	*S. aureus* mutants construction	
Primers for *S. aureus* mutants construction		
pKOR1-*ccpA::ccpA*^{2CS}	ATTGGTACCAAATATGCAAAACTTC GAGTTATTAAAAGAAG	ATTGGATCCGGATTAAATGAT GAA GAG AAT GGT CAA T

Table S2 The minimum inhibitory concentration (MIC) of auranofin and some clinical antibiotics against *S. aureus* or *S. aureus ccpA::ccpA*^{2CS} mutant.

Complex	*S. aureus* (μg/mL)	*S. aureus ccpA::ccpA*^{2CS} (μg/mL)
Auranofin	0.195	0.39
Kanamycin	6.25	6.25
Gentamycin	0.78	1.56
Amikacin	6.25	12.5

Reference

[1] Liao X., *et al*. Identification of catabolite control protein A from *Staphylococcus aureus* as a target of silver ions. *Chem. Sci*. **8**, 8061-8066 (2017).