Appendix for

Structural and functional insights into a novel signaling network regulating biofilm formation

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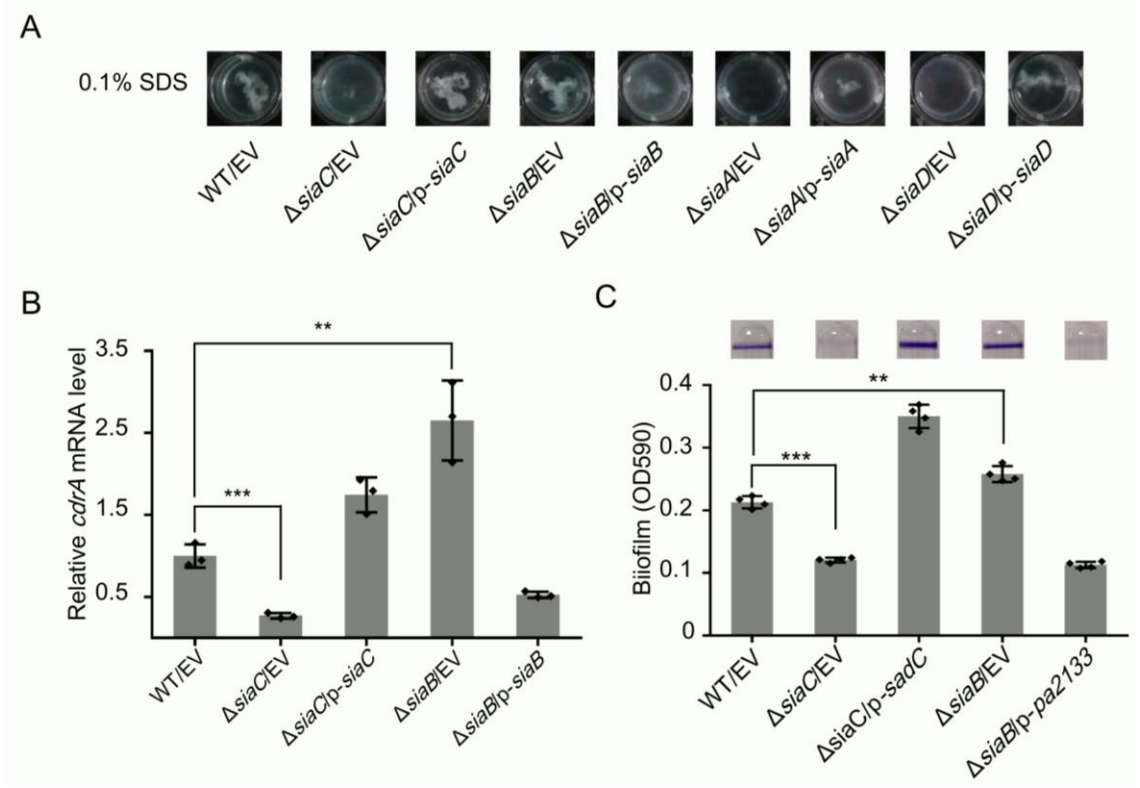
Appendix Figures

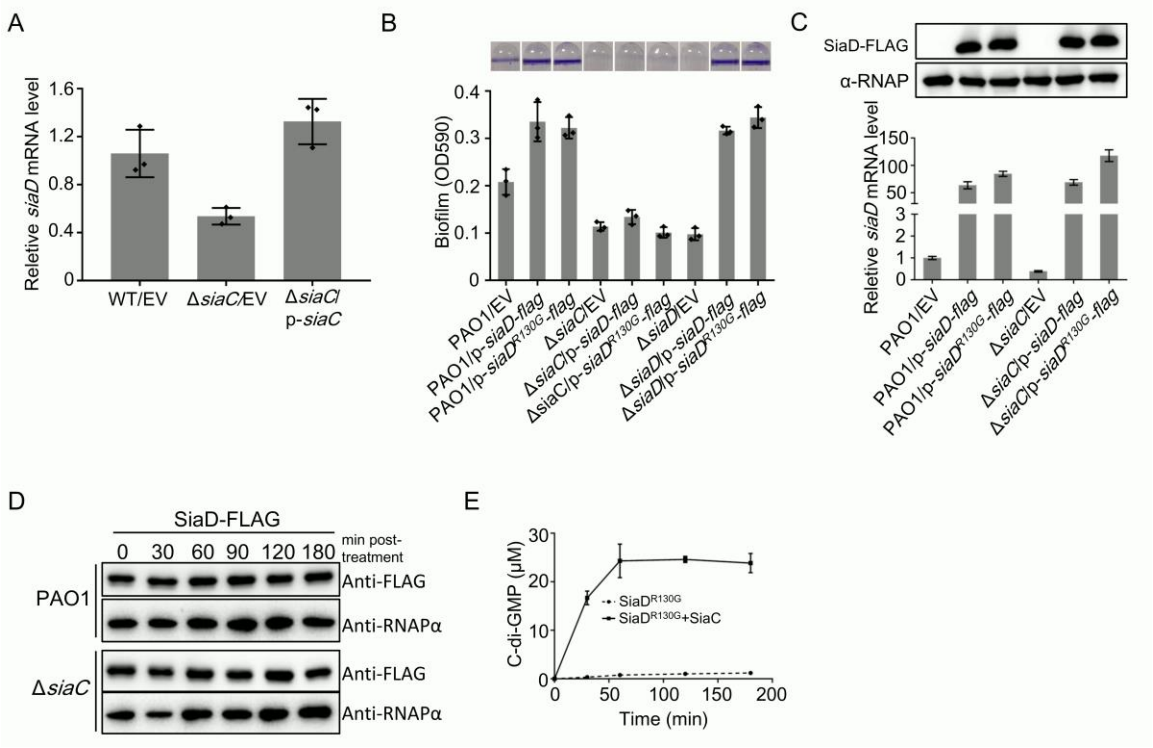


Appendix Figure S1. SiaB and SiaC regulates aggregates via c-di-GMP.

A SDS-induced aggregates formation by PAO1/EV, Δ*siaC/EV*, Δ*siaB/EV* and the corresponding complemented strains. Strains were grown in M9 medium with 10 mM succinate or 0.1% SDS as the sole carbon source for 12 h at 37 ºC with shaking at 200 rpm.

B The mRNA levels of *cdrA* in PAO1, Δ*siaC*, Δ*siaB* and the corresponding complemented strains were detected by qRT-PCR and normalized to the level of PAO1. The 30s ribosomal protein-encoding gene *rpsL* was used as an internal control.

C Biofilm formation by the indicated strains was displayed with crystal violet staining (up) and quantified with optical density measurement (down). Error bars represent the means and SDs of three biological replicates. ***P*<0.01 and ****P*<0.001 compared to WT based on one-way ANOVA test. EV represents the empty vector pUCP20 in this and subsequent assays.



Appendix Figure S2. SiaC is required for the DGC activity of SiaD.

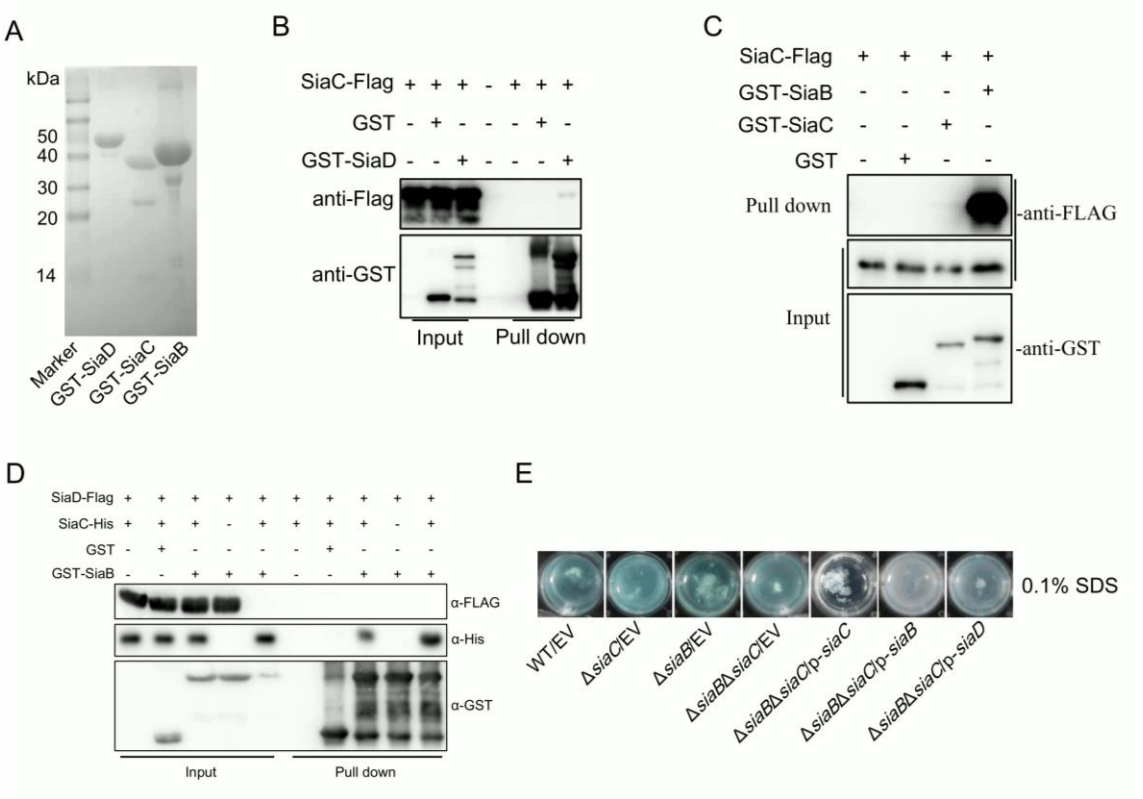
A Expression of *siaD* was decreased after *siaC* deletion. The mRNA levels of *siaD* in PAO1, Δ*siaC* and the corresponding complemented strains were detected by qRT-PCR and normalized to the level of PAO1.

B The C-terminus FLAG tag did not influence the function of SiaD. Biofilm formation by the indicated strains was displayed with crystal violet staining (up) and quantified with optical density measurement (down).

C SiaD was sufficiently expressed on the overexpressing plasmid. Expression levels of SiaD-Flag were tested by western blotting (up) and qRT (down). For western blotting assays, RNA polymerase alpha unit (α-RNAP) was used as a control for whole cells. For qRT-PCR assays, the 30s ribosomal protein-encoding gene *rpsL* was used as an internal control.

D Deletion of SiaC did not influence SiaD stability. Wild type PAO1 and Δ*siaC* mutant carrying pUCP20-*siaD-flag* were cultured for 3 h. The 50 μg ml^{-1} streptomycin was added to the medium. At the indicated time points, the protein levels of SiaD-FLAG were determined by western blotting assay. RNA polymerase alpha unit was used as an internal control.

E Production of c-di-GMP at the indicated time-point by SiaD^{R130G} with SiaC was determined by HPLC.



Appendix Figure S3. SiaC interacts with SiaB or SiaD.

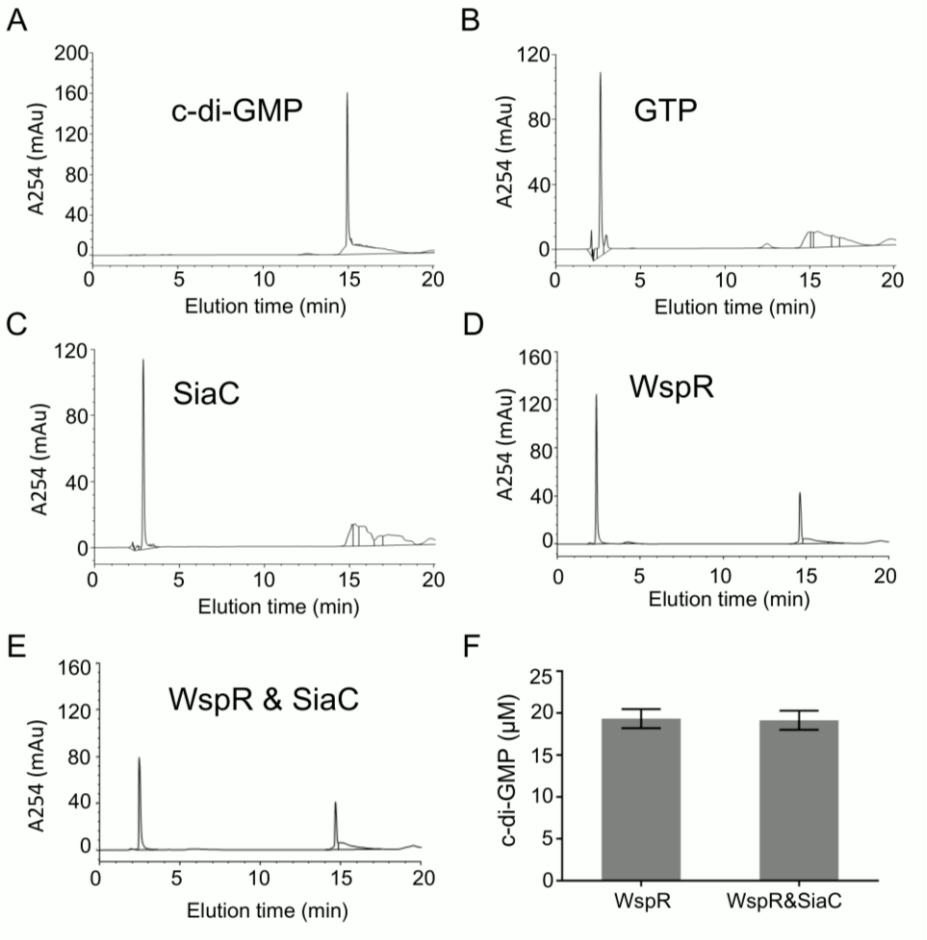
A Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis of purified GST-SiaD, GST-SiaC and GST-SiaB.

B GST pull down revealed direct interaction between SiaC and SiaD. Cell lysates of *P. aeruginosa* containing SiaC-Flag were incubated with GST and GST-SiaD individually, and protein complexes were captured by glutathione beads.

C GST-pull down assays showed that SiaC failed to interact with itself. Cell lysates of *P. aeruginosa* containing SiaC-Flag were incubated with GST and GST-SiaC individually, and protein complexes were captured by glutathione beads. GST-SiaB was used as a positive control.

D GST pull down assay showed no formation of SiaB-SiaC-SiaD complex. GST-SiaB was firstly incubated with purified SiaC-His. After SiaC-His binding, cell lysates of DH5α containing SiaD-Flag was added, and protein complex were captured by glutathione beads. GST protein was used as negative control.

E SDS-induced aggregates formation by the indicated strains. Strains were grown in M9 medium with 10 mM succinate or 0.1% SDS as the sole carbon source for 12 h at 37 ^{o}C with shaking at 200 rpm.



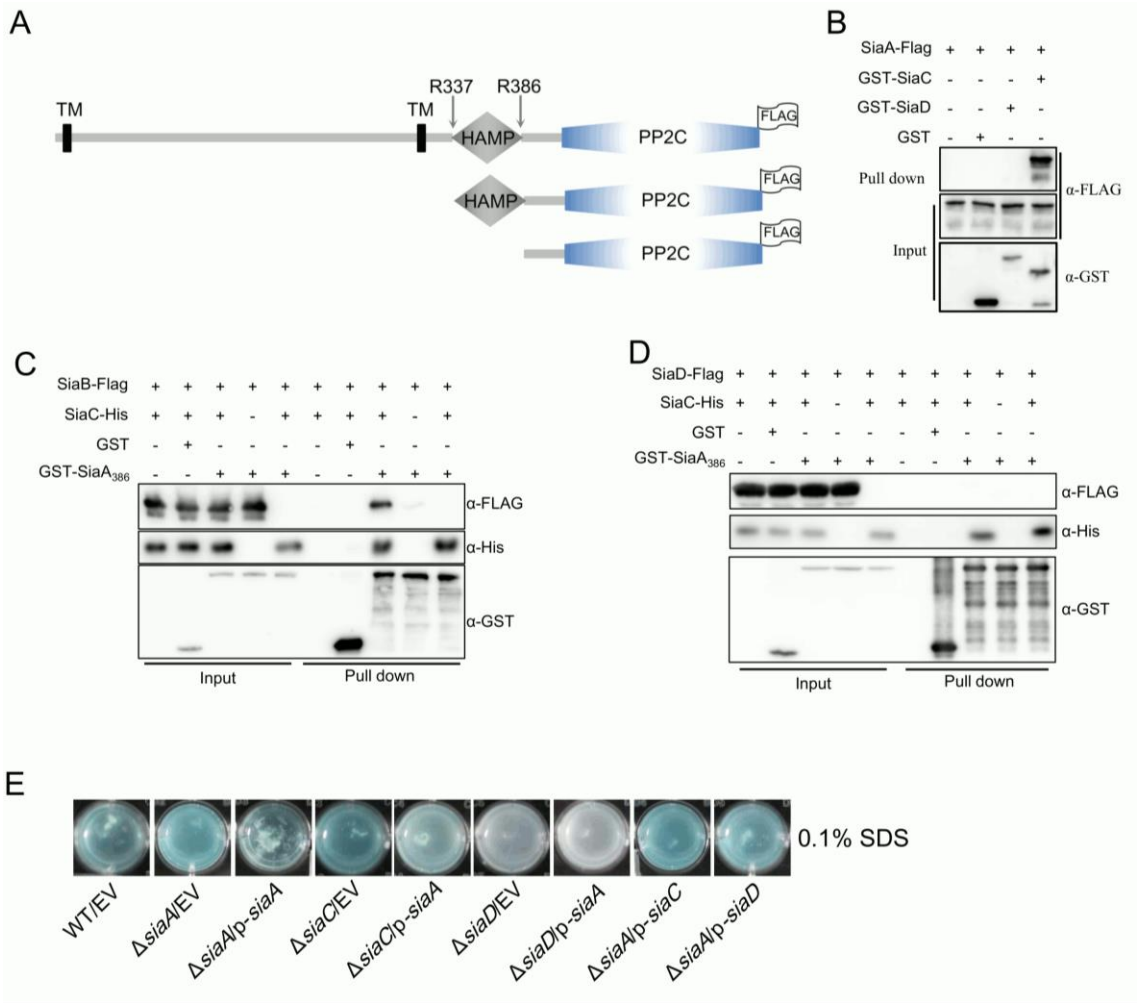
Appendix Figure S4. SiaC is unable to promote the DGC activity of WspR.

A The retained times of c-di-GMP (50 μM) standard

B The retained time of GTP (50 μM) standard.

C-E The c-di-GMP levels produced by SiaC (C), WspR (D), or WspR with SiaC (E) after 3 h or 10 min reaction were determined.

F Quantification analysis showed that SiaC did not promote WspR to produce more c-di-GMP production. Data are presented as the mean ± s.d. of three independent experiments.



Appendix Figure S5. SiaA regulates aggregates formation via direct interaction with SiaC.

A Predicted domain of SiaA.

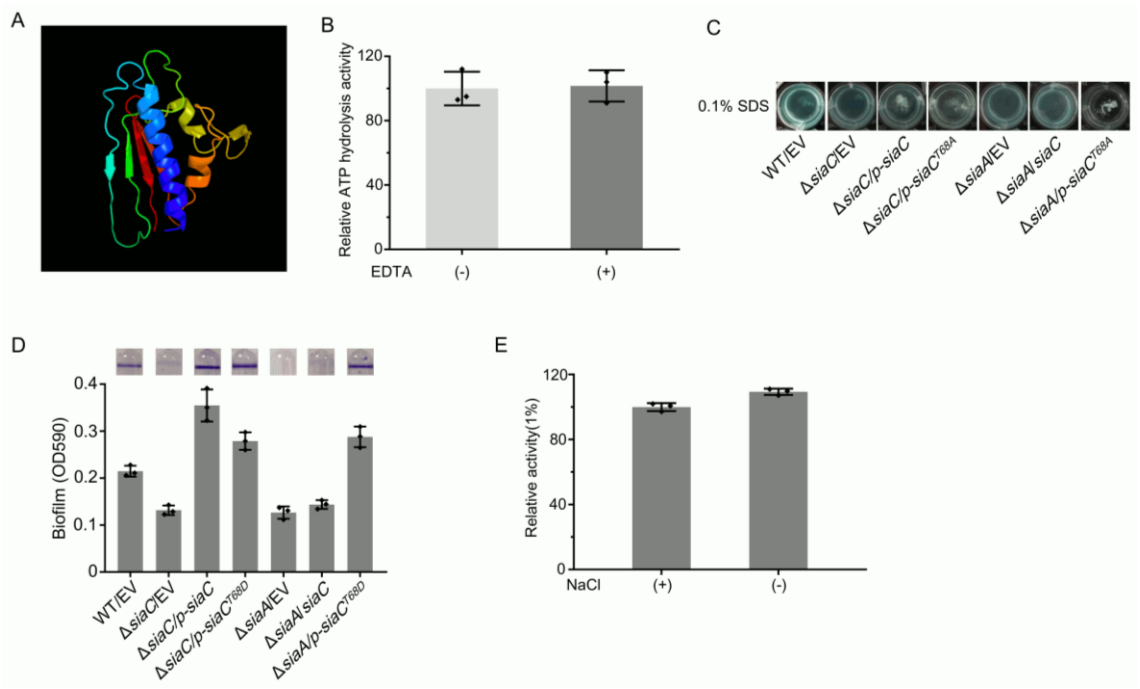
B GST pull-down assays showed no interaction between SiaA and SiaD. Cell lysates of *P. aeruginosa* expressing SiaA-Flag were incubated with GST and GST-SiaD individually, and protein complexes were captured by glutathione beads. GST-SiaC was used as a positive control.

C GST pull down assay revealed formation of SiaA-SiaC-SiaB complex *in vitro*. GST-$SiaA_{386}$ was firstly incubated with purified SiaC-His. After SiaC-His binding, cell lysates of DH5α containing SiaB-Flag was added, and protein complex were captured by glutathione beads. GST protein was used as negative control.

D GST pull down assay showed no formation of SiaA-SiaC-SiaD complex. GST-$SiaA_{386}$ was firstly incubated with purified SiaC-His. After SiaC-His binding, cell

lysates of DH5α containing SiaD-Flag was added, and protein complex were captured by glutathione beads. GST protein was used as negative control.

E SDS-induced aggregates formation by the indicated strains. Strains were grown in M9 medium with 10 mM succinate or 0.1% SDS as the sole carbon source for 12 h at 37 ^{o}C with shaking at 200 rpm.



Appendix Figure S6. SiaB is a kinse that specifically phosphorylates SiaC.

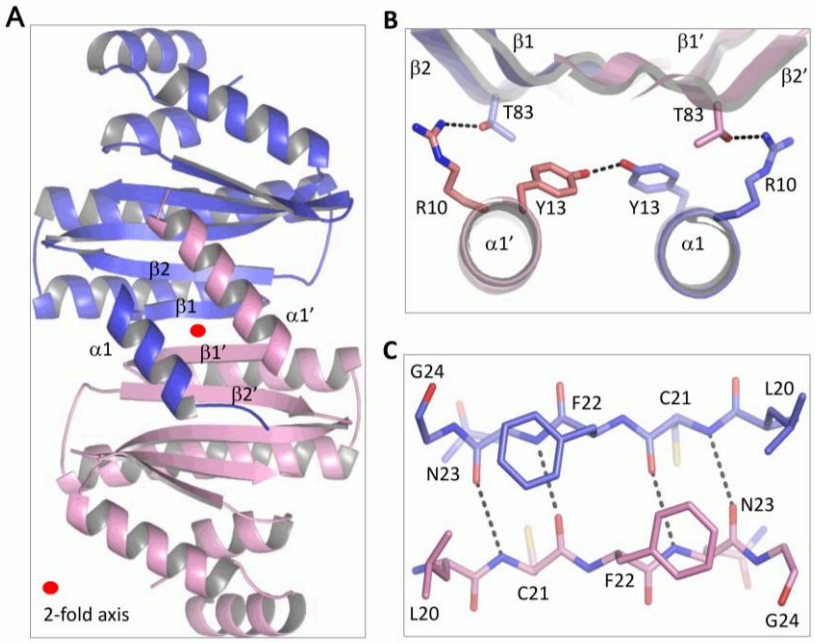
A Structure modeling of SiaB using online server Phyre 2.0.

B SiaB retains ATP hydrolysis activity in the presence of EDTA. (-) and (+) represent reaction buffer (10 mM Tris-HCl, pH 8.0, 10 mM NaCl) with or without 1 mM EDTA.

C SDS-induced aggregates formation by the indicated strains. Strains were grown in M9 medium with 10 mM succinate or 0.1% SDS as the sole carbon source for 12 h at 37 ^{o}C with shaking at 200 rpm.

D Biofilm formation by the indicated strains was displayed with crystal violet staining (up) and quantified with optical density measurement (down).

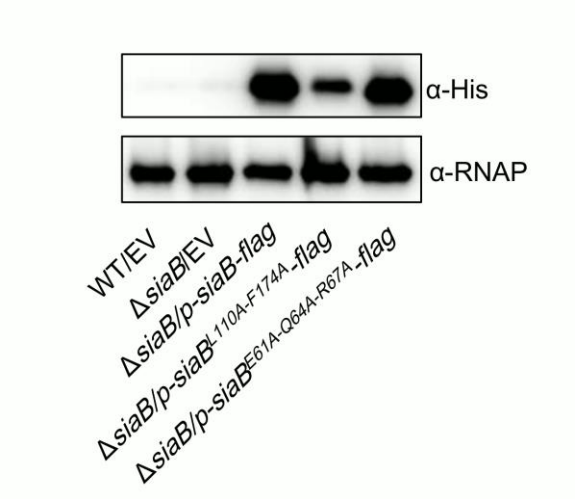
E Na^{+} ion is not strictly required for the ATP hydrolysis activity of SiaB. SiaB retains ATP hydrolysis activity in ammonium phosphate buffer without Na^{+} (50 mM ammonium phosphate, pH 7.5).



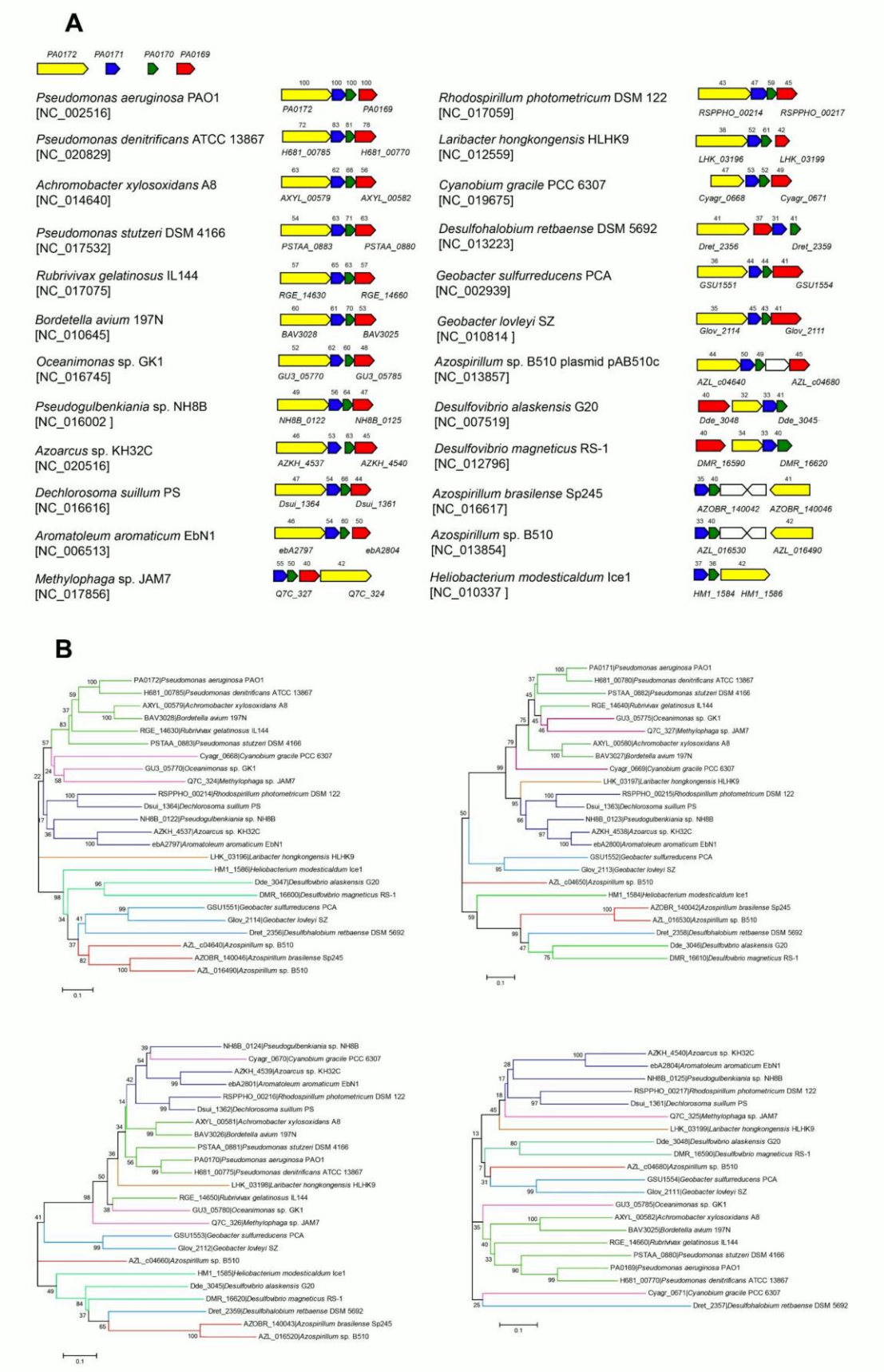
Appendix Figure S7. Dimerization of SiaB.

A The overall conformation of SiaB dimer. The two SiaB molecules are shown as cartoons in blue and pink, respectively.

B, C Detailed interactions between the two SiaB molecules.



Appendix Figure S8. Expression of SiaB and its mutants during biofilm formation. The biofilm formed by the indicated strains were scraped and subject to western blotting assay. The expression levels of SiaB-Flag or its mutants from these strains were determined. RNA polymerase alpha unit was used as an internal control.


A
PA0172 PA0171 PA0170 PA0169
Pseudomonas aeruginosa PAO1 [NC_002516]
PA0172 PA0169
Pseudomonas denitrificans ATCC 13867 [NC_020829]
H681_00785 H681_00770
Achromobacter xylosoxidans A8 [NC_014640]
AXYL_00579 AXYL_00582
Pseudomonas stutzeri DSM 4166 [NC_017532]
PSTAA_0883 PSTAA_0880
Rubrivivax gelatinosus IL144 [NC_017075]
RGE_14630 RGE_14660
Bordetella avium 197N [NC_010645]
BAV3028 BAV3025
Oceanimonas sp. GK1 [NC_016745]
GU3_05770 GU3_05785
Pseudogulbenkiania sp. NH8B [NC_016002]
NH8B_0122 NH8B_0125
Azoarcus sp. KH32C [NC_020516]
AZKH_4537 AZKH_4540
Dechlorosoma suillum PS [NC_016616]
Dsui_1364 Dsui_1361
Aromatoleum aromaticum EbN1 [NC_006513]
ebA2797 ebA2804
Methylophaga sp. JAM7 [NC_017856]
Q7C_327 Q7C_324
Rhodospirillum photometricum DSM 122 [NC_017059]
RSPPHO_00214 RSPPHO_00217
Laribacter hongkongensis HLHK9 [NC_012559]
LHK_03196 LHK_03199
Cyanobium gracile PCC 6307 [NC_019675]
Cyagr_0668 Cyagr_0671
Desulfohalobium retbaense DSM 5692 [NC_013223]
Dret_2356 Dret_2359
Geobacter sulfurreducens PCA [NC_002939]
GSU1551 GSU1554
Geobacter lovleyi SZ [NC_010814]
Glov_2114 Glov_2111
Azospirillum sp. B510 plasmid pAB510c [NC_013857]
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Desulfovibrio alaskensis G20 [NC_007519]
Dde_3048 Dde_3045
Desulfovibrio magneticus RS-1 [NC_012796]
DMR_16590 DMR_16620
Azospirillum brasilense Sp245 [NC_016617]
AZOBR_140042 AZOBR_140046
Azospirillum sp. B510 [NC_013854]
AZL_016530 AZL_016490
Heliobacterium modesticaldum Ice1 [NC_010337]
HM1_1584 HM1_1586
B
PA0172|Pseudomonas aeruginosa PAO1
H681_00785|Pseudomonas denitrificans ATCC 13867
AXYL_00579|Achromobacter xylosoxidans A8
BAV3028|Bordetella avium 197N
RGE_14630|Rubrivivax gelatinosus IL144
PSTAA_0883|Pseudomonas stutzeri DSM 4166
Cyagr_0668|Cyanobium gracile PCC 6307
GU3_05770|Oceanimonas sp. GK1
Q7C_324|Methylophaga sp. JAM7
RSPPHO_00214|Rhodospirillum photometricum DSM 122
Dsui_1364|Dechlorosoma suillum PS
NH8B_0122|Pseudogulbenkiania sp. NH8B
AZKH_4537|Azoarcus sp. KH32C
ebA2797|Aromatoleum aromaticum EbN1
LHK_03196|Laribacter hongkongensis HLHK9
HM1_1586|Heliobacterium modesticaldum Ice1
Dde_3047|Desulfovibrio alaskensis G20
DMR_16600|Desulfovibrio magneticus RS-1
GSU1551|Geobacter sulfurreducens PCA
Glov_2114|Geobacter lovleyi SZ
Dret_2356|Desulfohalobium retbaense DSM 5692
AZL_c04640|Azospirillum sp. B510
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0.1
PA0171|Pseudomonas aeruginosa PAO1
H681_00780|Pseudomonas denitrificans ATCC 13867
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RSPPHO_00215|Rhodospirillum photometricum DSM 122
Dsui_1363|Dechlorosoma suillum PS
NH8B_0123|Pseudogulbenkiania sp. NH8B
AZKH_4538|Azoarcus sp. KH32C
ebA2800|Aromatoleum aromaticum EbN1
GSU1552|Geobacter sulfurreducens PCA
Glov_2113|Geobacter lovleyi SZ
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DMR_16610|Desulfovibrio magneticus RS-1
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AZKH_4539|Azoarcus sp. KH32C
ebA2801|Aromatoleum aromaticum EbN1
RSPPHO_00216|Rhodospirillum photometricum DSM 122
Dsui_1362|Dechlorosoma suillum PS
AXYL_00581|Achromobacter xylosoxidans A8
BAV3026|Bordetella avium 197N
PSTAA_0881|Pseudomonas stutzeri DSM 4166
PA0170|Pseudomonas aeruginosa PAO1
H681_00775|Pseudomonas denitrificans ATCC 13867
LHK_03198|Laribacter hongkongensis HLHK9
RGE_14650|Rubrivivax gelatinosus IL144
GU3_05780|Oceanimonas sp. GK1
Q7C_326|Methylophaga sp. JAM7
GSU1553|Geobacter sulfurreducens PCA
Glov_2112|Geobacter lovleyi SZ
AZL_c04660|Azospirillum sp. B510
HM1_1585|Heliobacterium modesticaldum Ice1
Dde_3045|Desulfovibrio alaskensis G20
DMR_16620|Desulfovibrio magneticus RS-1
Dret_2359|Desulfohalobium retbaense DSM 5692
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AZL_016520|Azospirillum sp. B510
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AZKH_4540|Azoarcus sp. KH32C
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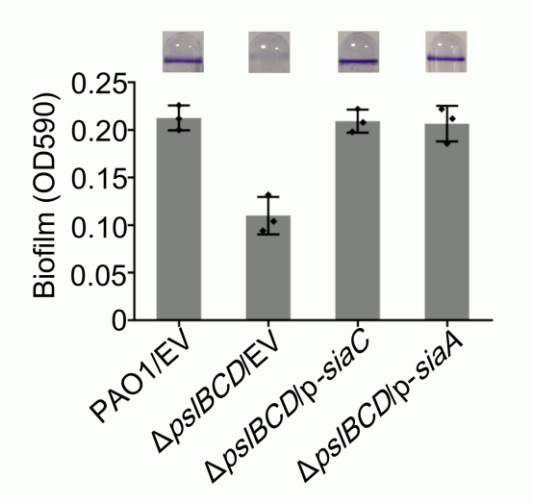
Q7C_325|Methylophaga sp. JAM7
LHK_03199|Laribacter hongkongensis HLHK9
Dde_3048|Desulfovibrio alaskensis G20
DMR_16590|Desulfovibrio magneticus RS-1
AZL_c04680|Azospirillum sp. B510
GSU1554|Geobacter sulfurreducens PCA
Glov_2111|Geobacter lovleyi SZ
GU3_05785|Oceanimonas sp. GK1
AXYL_00582|Achromobacter xylosoxidans A8
BAV3025|Bordetella avium 197N
RGE_14660|Rubrivivax gelatinosus IL144
PSTAA_0880|Pseudomonas stutzeri DSM 4166
PA0169|Pseudomonas aeruginosa PAO1
H681_00770|Pseudomonas denitrificans ATCC 13867
Cyagr_0671|Cyanobium gracile PCC 6307
Dret_2357|Desulfohalobium retbaense DSM 5692
0.1

Appendix Figure S9. Syteny and phylogeny of *siaABCD*.

A Genetic organization and co-linear alignment of *siaABCD*-like homologues. Sequence identities of protein homologs respectively compared to *siaABCD* prototypes are indicated above corresponding genes.

B The phylogeny relationships of SiaABCD protein homologs were conducted in MEGA 7 by using the NJ method (bootstrap: 1000 replicates). The percentage of consenus trees are shown close to the clades.



Appendix Figure S10. The activity of SiaD is independent on exopolysaccharide Psl. Biofilm formation by the indicated strains was displayed with crystal violet staining (up) and quantified with optical density measurement (down).

Appendix Tables

Appendix Table S1. Data collection and refinement statistics

Structure (PDB ID)	SiaB/SiaC complex 6KKO	apo-SiaC 6KKP
Data collection[a]		
Space group	$P2_1$	I222
Cell parameter		
a (Å)	75.6	35.6
b (Å)	42.0	72.1
c (Å)	96.6	96.1
β (°)	93.45	90.0
Wavelength (Å)	0.9793	0.9793
Resolution (Å)	39.6-2.1	30-2.5
Last shell (Å)	2.24-2.1	2.59-2.5
Completeness (%)[a]	98.7 (97.5)	98.5 (95.2)
Redundancy [a]	3.3(3.3)	6.4 (3.8)
I/σ(I) [a]	9.4(2.0)	15.4 (4.5)
Rmerge (%)[a]	8.4/57.4	13.0/26.3
Refinement		
Resolution (Å)	37.7-2.1	30.0-2.5
R_{work} (%) / R_{free} (%)	23.2/27.8	20.9/25.1
No. of atoms		
Protein	4633	966
Ligand	54	0
water	135	11
R. m. s. deviations		
Bond length (Å)	0.005	0.007
Bond angle ( )	0.757	1.164
Ramachandran plot (%)		

Most favored	99.1	94.9
Additional allowed	0.9	5.1

[a]: Values in parentheses are for the last resolution shell.

Appendix Table S2. Strains and plasmids used in this study.

Strains or plasmids	**Relevant characteristics or function**	**Reference or origin**
Plasmids		
mini-CTX-*lacZ*	Integration plasmid; TcR[a]	(Becher & Schweizer, 2000)
mini-CTX-*lacZ-siaD*-flag		This study
pEX18Tc	*oriT*+ *sacB*+ gene replacement vector with multiple-cloning site from pUC18; TcR	(Hoang, Karkhoff-Schweizer et al., 1998)
pEX-*siaC*	*siaC* deletion plasmid; TcR	This study
pEX-*siaB*	*siaB* deletion plasmid; TcR	This study
pEX-*siaB-C*	*siaC-siaB* double deletion plasmid; Tc^r	This study
pEX-*siaA*	*siaA* deletion plasmid, Tc^r	This study
pEX-*siaD*	*siaD* deletion plasmid, Tc^r	This study
pUCP20	*Escherichia-Pseudomonas* shuttle vector	(West, Schweizer et al., 1994)
pUCP20-*siaC*	pUCP20 derived plasmid expressing *siaC*; Amp^r	This study
pUCP20-*siaC*^{T68A}	pUCP20 derived plasmid expressing *siaC*^{T68A}; Amp^r	This study
pUCP20-*siaC*^{K72A}	pUCP20 derived plasmid expressing *siaC*^{K72A}; Amp^r	This study
pUCP20-*siaC*^{E33A}	pUCP20 derived plasmid expressing *siaC*^{E33A}; Amp^r	This study
pUCP20-*siaC*^{Y65A}	pUCP20 derived plasmid expressing *siaC*^{Y65A}; Amp^r	This study
pUCP20-*siaC*^{N67A}	pUCP20 derived plasmid expressing *siaC*^{N67A}; Amp^r	This study
pUCP20-*siaC*^{R103A}	pUCP20 derived plasmid expressing *siaC*^{R103A}; Amp^r	This study
pUCP20-*siaC*^{E110A}	pUCP20 derived plasmid expressing *siaC*^{E110A}; Amp^r	This study
pUCP20-*siaB*	pUCP20 derived plasmid expressing *siaB*; Amp^r	This study
pUCP20-*siaB*^{E32A}	pUCP20 derived plasmid expressing *siaB*^{E32A}; Amp^r	This study
pUCP20-*siaB*^{E61A}	pUCP20 derived plasmid expressing *siaB*^{E61A}; Amp^r	This study
pUCP20-*siaB*^{Q64A}	pUCP20 derived plasmid expressing *siaB*^{Q64A}; Amp^r	This study
pUCP20-*siaB*$^{E61A-Q64A}$	pUCP20 derived plasmid expressing *siaB*$^{E61A-Q64A}$; Amp^r	This study
pUCP20-*siaB*$^{E61A-Q64A-R67A}$	pUCP20 derived plasmid expressing *siaB*$^{E61A-Q64A-R67A}$; Amp^r	This study
pUCP20-*siaB*^{N65A}	pUCP20 derived plasmid expressing *siaB*^{N65A}; Amp^r	This study
pUCP20-*siaB*^{Y69A}	pUCP20 derived plasmid expressing *siaB*^{Y69A}; Amp^r	This study
pUCP20-*siaB*^{N100A}	pUCP20 derived plasmid expressing *siaB*^{N100A}; Amp^r	This study
pUCP20-*siaB*^{L110A}	pUCP20 derived plasmid expressing *siaB*^{L110AA}; Amp^r	This study
pUCP20-*siaB*^{F174A}	pUCP20 derived plasmid expressing *siaB*^{F174A}; Amp^r	This study
pUCP20-*siaB*$^{L110A-F174A}$	pUCP20 derived plasmid expressing *siaB*$^{L110A-F174A}$; Amp^r	This study
pUCP20-*siaA*	pUCP20 derived plasmid expressing *siaA*; Amp^r	This study
pUCP20-*siaD*	pUCP20 derived plasmid expressing *siaD*; Amp^r	This study

pUCP20-*siaD*^{G140A}	pUCP20 derived plasmid expressing *siaD*^{G140A}; Amp^r	This study
pUCP20-*siaC-siaD*	pUCP20 derived plasmid expressing *siaC* and *siaD*; Amp^r	This study
pUCP20-*siaC-siaD*^{G140A}	pUCP20 derived plasmid expressing *siaC* and *siaD*^{G140A}; Amp^r	This study
pUCP20-*sadC*	pUCP20 derived plasmid expressing *sadC*; Amp^r	This study
pUCP20-*PA2133*	pUCP20 derived plasmid expressing *PA2133*; Amp^r	This study
pUCP20-*siaD-flag*	pUCP20 derived plasmid expressing SiaD-FLAG, Amp^r	This study
pMMB67EH	*Escherichi-Pseudomonas* Shuttle vector between; Amp^r	S. Jin lab
pMMB67EH-Flag	pMMB67EH vector with FLAG tag coding sequence	This study
pMMB67EH-*siaD*-Flag	pMMB67EH-Flag derived plasmid expressing *siaD-flag*	This study
pMMB67EH-*siaC*-Flag	pMMB67EH-Flag derived plasmid expressing *siaC-flag*	This study
pMMB67EH-*siaC*^{T68A}-Flag	pMMB67EH-Flag derived plasmid expressing *siaC*^{T68A}*-flag*	This study
pMMB67EH-*siaB*-Flag	pMMB67EH-Flag derived plasmid expressing *siaB-flag*	This study
pMMB67EH-*siaA*-Flag	pMMB67EH-Flag derived plasmid expressing *siaA-flag*	This study
pMMB67EH-*siaA337*-Flag	pMMB67EH-Flag derived plasmid expressing *siaA337-flag*	This study
pMMB67EH-*siaA386*-Flag	pMMB67EH-Flag derived plasmid expressing *siaA386-flag*	This study
pBT	p15A origin of replication, *lac-UV5* promoter, λ cI open reading frame; Cm^r	Agilent
pTRG	ColE1 origin of replication, *lpp* promoter, *lac-UV5* promoter, α-RNAP open reading frame; Tc^r	Agilent
pBT-LGF2	Interaction control plasmid encoding the dimerization domain (40 amino acid residues) of the Gal4 transcriptional activator protein; Cm^r	Agilent
pTRG-Gal11	Interaction control plasmid encoding a domain (90 amino acid residues) of the mutant form of the Gal11 protein; Tc^r	Agilent
pTRG-*siaD*	pTRG vector inserted with *siaD* coding sequence	This study
pTRG-*siaC*	pTRG vector inserted with *siaC* coding sequence	This study
pTRG-*siaA*$_{337}$	pTRG vector inserted with *siaA*$_{337}$ coding sequence	This study
pTRG-*siaA*$_{386}$	pTRG vector inserted with *siaA*$_{386}$ coding sequence	This study
pBT-*siaD*	pBT vector inserted with *siaD* coding sequence	This study
pBT-*siaC*	pBT vector inserted with *siaC* coding sequence	This study
pBT-*siaB*	pBT vector inserted with *siaB* coding sequence	This study
pGEX-6p-1	Expression vector with N-terminal GST tag, Apr	Novagen
pGEX-*siaD*	Plasmid for GST-SiaD expression	This study
pGEX-*siaC*	Plasmid for GST-SiaC expression	This study
pGEX-*siaB*	Plasmid for GST-SiaB expression	This study
pET-*siaC*	Plasmid for SiaC-His expression	This study

pET-*siaC*^{T68A}	Plasmid for SiaC^{T68A}-His expression	This study
pET-Sumo	Plasmid for Sumo expression	(Yang, Wu et al., 2018)
pSumo-*SiaA*$_{386}$	Plasmid for Sumo-SiaA$_{386}$ expression	This study
pSumo-*siaD*	Plasmid for Sumo-SiaD expression	This study
pSumo-*siaC*	Plasmid for Sumo-SiaC expression	This study
pSumo-*siaC*^{T68A}	Plasmid for Sumo-SiaC^{T68A} expression	This study
pSumo-*siaC*^{K72A}	Plasmid for Sumo-SiaC^{K72A} expression	This study
pSumo-*siaC*^{E33A}	Plasmid for Sumo-SiaC^{E33A} expression	This study
pSumo-*siaB*	Plasmid for Sumo-SiaB expression	This study
pSumo-*siaB*$^{E61A\text{-}Q64A\text{-}R67A}$	Plasmid for Sumo-SiaB$^{E61A\text{-}Q64A\text{-}R67A}$ expression	This study
pSumo-*siaB*$^{L110A\text{-}F174A}$	Plasmid for Sumo-SiaB$^{L110A\text{-}F174A}$ expression	This study
Strains		
P. aeruginosa		
PAO1	Wild type	This lab
Δ*siaC*	*siaC* deletion mutant of PAO1	This study
Δ*siaB*	*siaB* deletion mutant of PAO1	This study
Δ*siaA*	*siaA* deletion mutant of PAO1	This study
Δ*siaD*	*siaD* deletion mutant of PAO1	This study
Δ*siaC*Δ*siaB*	*siaC-siaB* double deletion mutant of PAO1	This study
Δ*siaB*Δ*siaC*	*SiaB-siaC* double deletion mutant of PAO1	
Δ*sadC*	*sadC* deletion mutant of PAO1	This lab
E. coli		
DH5α	F– *φ80lacZ ΔM15* Δ(*lacZYA-argF)U169 recA1 endA1 hsdR17(rk–, mk+)phoA supE44 thi-1 gyrA96 relA1 tonA*	Invitrogen
BL21(DE3)	F- *ompT hsdS$_B$(r$_B^-$m$_B^-$) gal dcm* (DE3)	Invitrogen

Table S3. Primes used in this study.

Primer	Sequence (5'→3')[a]	Application
mini-*siaD*-F1	TTActcgagAACCGCCTCTGAGCGGGAT	For constructing mini-CTX-*siaD*-Flag
mini-*siaD*-R1	GCGCTCCAGCCGCACGGCTATCCCTATCAG	For constructing mini-CTX-*siaD*-Flag
mini-*siaD*-F2	CTGATAGGGATAGCCGTGCGGCTGGAGCGC	For constructing mini-CTX-*siaD*-Flag
mini-*siaD*-R2	CCCaagcttGCGCGCTGGAGCCGGGCG	For constructing mini-CTX-*siaD*-Flag
pUC-*siaC*-F	AGCgaattcCGTGTTTTAGGAAGAACACCATC	For constructing pUCP-*siaC*
pUC-*siaC*-R	CCCaagcttCTACTCGTCGTGGGCCTG	For constructing pUCP-*siaC*
siaC-E33A –F	GGCGATTCCTACCCGGCAAACTCCTATGAACT	For siaC E33A mutation
siaC-E33A –R	AGTTCATAGGAGTTTGCCGGGTAGGAATCGCC	For siaC E33A mutation
siaC-Y65A –F	CGCCTGCTGGCCCTGAACACCAGTTCGAT	For siaC Y65A mutation
siaC-Y65A –R	ATCGAACTGGTGTTCAGGGCCAGCAGGCG	For siaC Y65A mutation
siaC-N67A –F	CTGTACCTGGCCACCAGTTCGATCAAGGCCAT	For siaC N67A mutation
siaC-N67A –R	ATGGCCTTGATCGAACTGGTGGCCAGGTACAG	For siaC N67A mutation
siaC-K72A –F	AACACCAGTTCGATCGCGGCCATGATGGACATC	For siaC K72A mutation
siaC-K72A –R	GATGTCCATCATGGCCGCGATCGAACTGGTGTT	For siaC K72A mutation
siaC-R103A –F	GACCGGCGCAACGAAGCGGTCGCCGAGCTGGC	For siaC R103A mutation
siaC-R103A –R	GCCAGCTCGGCGACCGCTTCGTTGCGCCGGTC	For siaC R103A mutation
siaC-E110A –F	GCCGAGCTGGCCGAGGCGTTCCGCGAGGACTGC	For siaC E110A mutation
siaC-E110A –R	GCAGTCCTCGCGGAACGCCTCGGCCAGCTCGGC	For siaC E110A mutation
pUC-*siaB*-F	AGCgaattcCCTTCCGATTCGACTAGGGG	For constructing pUCP-*siaB*
pUC-*siaB*-R	CCCaagcttTCAGATCACGGCGCGCAG	For constructing pUCP-*siaB*
siaB-E61A-F	GTGTACATCGCCATGACCCAGAACATCCGC	For siaB-E61A mutation
siaB-E61A-F	GCGGATGTTCTGGGTCATGGCGATGTACAC	For siaB-E61A mutation
siaB-Q64A-F	GAGATGACCGCCAACATCCGCCACTACGCC	For siaB-Q64A mutation
siaB-Q64A-F	GGCGTAGTGGCGGATGTTGGCGGTCATCTC	For siaB-Q64A mutation
siaB-R67A-F	CAGAACATCGCCCACTACGCCAATCTCAAGGGCTA	For siaB-R67A mutation
siaB-R67A-F	TAGCCCTTGAGATTGGCGTAGTGGGCGATGTTCTG	For siaB-R67A mutation
siaB-N65A-F	TCGAGATGACCCAGGCCATCCGCCACTACGC	For siaB N65A mutation
siaB-N65A-R	GCGTAGTGGCGGATGGCCTGGGTCATCTCGA	For siaB N65A mutation
siaB-N100A-F	GTGGTGTCCGCCGGCGCCCTGGTGGAACGCGAC	For siaB N100A mutation
siaB-N100A-R	GTCGCGTTCCACCAGGGCGCCGGCGGACACCAC	For siaB N100A mutation
siaB-Y69A-F	CAGAACATCCGCCACGCCGCCAATCTCAAGGGC	For siaB Y69A mutation
siaB-Y69A-R	GCCCTTGAGATTGGCGGCGTGGCGGATGTTCTG	For siaB Y69A mutation
siaB-L110A-F	GACGACGGCCAGAGCGCGGTGCGCAGCATCCAG	For siaB L110A mutation
siaB-L110A-R	CTGGATGCTGCGCACCGCGCTCTGGCCGTCGTC	For siaB L110A mutation

siaB-F174A-R	AGCgaattcTCAGATCACGGCGCGCAGGCTGGCGAA GGCGCGCCCGTCGGG	For siaB F174A mutation
pUC-*siaA*-F	AGCgaattcCAACCTGCTCGCCGGCCT	For constructing pUCP-*siaA*
pUC-*siaA*-R	AGCaagcttGTCCGTTGAAGCAGAGCAGGATG	For constructing pUCP-*siaA*
pUC-*siaD*-F	AGCgaattcGGACCTGCGCCTGCTGTACC	For constructing pUCP-*siaD*
pUC-*siaD*-R	CCCaagcttTCAGCGCGCTGGAGCCGGG	For constructing pUCP-*siaD*
pUC-*siaD-flag*-R	CCCaagcttTCACTTGTCATCGTCGTCCTTGTAG TCGCGCGCTGGAGCCGGG	For constructing pUCP-*siaD-flag*
siaD-G-F	GCCGCTGGGGCGCCGAGGAATTCCTC	For constructing pUCP-*siaD*^{R130G}
siaD-G-R	GAGGAATTCCTCGGCGCCCCAGCGGC	For constructing pUCP-*siaD*^{R130G}
pUC-*sadC*-F	AGCgaattc CAGTCGACGATCGAGTCGGAC	For constructing pUCP-*sadC*
pUC-*sadC*-R	CCCaagcttTCAGGCACTGGTGACCTCCC	For constructing pUCP-*sadC*
pUC-*PA2133*-F	AGCgaattcGTGAACGGTTCCCCACAGG	For constructing pUCP-*PA2133*
pUC-*PA2133*-R	AGCaagcttTCACCCCTGGCGGCTCGC	For constructing pUCP-*PA2133*
pmm-up	TGCctgcacaagcttGACTACAAGGACGACGATGA CAAGGATTACAAAGATGACGACGA	For constructing pMMB67EH-Flag
pmm-down	GCAgatatcTCATTTATCATCATCGTCCTTATAG TCCTTATCGTCGTCATCTTTGTAAT	For constructing pMMB67EH-Flag
pmm-*siaC*-F	AGCgaattcATGAGTGACCTGCACATACCCG	For constructing pMMB67EH-*siaC*-Flag
pmm-*siaC*-R	CCCaagcttCTCGTCGTGGGCCTGGAT	For constructing pMMB67EH-*siaC*-Flag
pmm-*siaB*-F	AGCgaattcATGGAAACGCTAGACCTGCTG	For constructing pMMB67EH-*siaB*-Flag
pmm-*siaB*-R	CCCaagcttGATCACGGCGCGCAGGCT	For constructing pMMB67EH-*siaB*-Flag
pmm-*siaA*-F	AGCgaattcATGGCGGCGAACTGGGGG	For constructing pMMB67EH-*siaA*-Flag
pmm-*siaA*-R	CCCaagcttGTCGAATCGGAAGGACAGGAT	For constructing pMMB67EH-*siaA*-Flag
pmm-*siaD*-F	AGCgagctcATGCGGCTGGAGCGCATCG	For constructing pMMB67EH-*siaD*-Flag
pmm-*siaD*-R	CCCaagcttGCGCGCTGGAGCCGGGCG	For constructing pMMB67EH-*siaD*-Flag
pmm-*siaA337*-F	AGCgaattcCGCCTGCTGTTGCGCCCC	For constructing pMMB67EH-*siaA*^{337}-Flag
pmm- *siaA386*-F	AGCgaattcCGGCACACCGCCGAGCTG	For constructing pMMB67EH-*siaA*^{386}-Flag
ptrg-*siaC*-F	AGCggatccAGTGACCTGCACATACCCGG	For constructing pTRG-*siaC*
ptrg-*siaC*-R	AGCgaattcCTACTCGTCGTGGGCCTGG	For constructing pTRG-*siaC*
ptrg-*siaA337*-F	AGCggatccCGCCTGCTGTTGCGCCCC	For constructing pTRG-*siaA*^{337}
ptrg-*siaA337*-R	AGCgaattcCTAGTCGAATCGGAAGGACAGG	For constructing pTRG-*siaA*^{337}
ptrg-*siaA386*-F	AGCggatccCGGCACACCGCCGAGCTG	For constructing pTRG-*siaA*^{386}
ptrg-*siaA386*-R	AGCgaattcCTAGTCGAATCGGAAGGACAGG	For constructing pTRG-*siaA*^{386}
siaD-bam-F	AGCggatccCGGCTGGAGCGCATCGCC	For constructing pBT-*siaD* and pTRG-*siaD*
siaD-sal1-R	CCGgtcgacTCAGCGCGCTGGAGCCGG	For constructing pBT-*siaD* and pTRG-*siaD*
pbt-*siaB*-F	AGCgaattccATGGAAACGCTAGACCTGCTG	For constructing pBT-*siaB*

pbt-*siaB*-R	AGCggatccTCAGATCACGGCGCGCAG	For constructing pBT-*siaB*
pbt-*siaC*-F	AGCgaattccATGAGTGACCTGCACATACCCG	For constructing pBT-*siaC*
pbt-*siaC*-R	AGCggatccCTACTCGTCGTGGGCCTGG	For constructing pBT-*siaC*
$siaA_{386}$-ecor-F	AGCgaattcCACACCGCCGAGCTGGAA	For constructing pSumo-$siaA_{386}$ and pGEX-$siaA_{386}$
$siaA_{386}$-hind-R	CCCaagcttCTAGTCGAATCGGAAGGACAGG	For constructing pSumo-$siaA_{386}$
$siaA_{386}$-xho-R	CCCctcgagCTAGTCGAATCGGAAGGACAGG	For constructing pGEX-$siaA_{386}$
siaC-bam-F	TTAggatccGGCGGAGGAATGAGTGACCTGCAC	For constructing pET-siaC, pSumo-siaC and pGEX-siaC
siaC-eco-R	CCCgaattcCTACTCGTCGTGGGCC	For constructing pET-siaC, pSumo-siaC and pGEX-siaC
siaB-bam-F	ATAggatccATGGAAACGCTAGACCTGCTGG	For constructing pSumo-siaB and pGEX-siaB
siaB-eco-R	CTAgaattcTCAGATCACGGCGCGCA	For constructing pSumo-siaB and pGEX-siaB
siaD-bam-F	ATAggatccGTGCGGCTGGAGCGCAT	For constructing pSumo-siaD and pGEX-siaD
siaD-sal-R	CCCgtcgacGCGCGCTGGAGCC	For constructing pSumo-siaD and pGEX-siaD
siaB-H1F	AGCgaattcTGGCTGCTGAAGGAAGACG	For construction of pEX-*siaB*-H
siaB-H1R	TGCtctagaCAGCAGGTCTAGCGTTTCCAT	For construction of pEX-*siaB*-H
siaB-H2F	TGCtctagaTACAAGGAGCAGCTACGCCG	For construction of pEX-*siaB*-H
siaB-H2R	CCCaagcttAGGGACAGGACATGGACTGC	For construction of pEX-*siaB*-H
siaC-H1F	AGCgaattcGGCCGCCAGCAACTCTTC	For construction of pEX-*siaC*-H
siaC-H1R	TGCtctagaCCGGGTATGTGCAGGTCACT	For construction of pEX-*siaC*-H
siaC-H2F	TGCtctagaGAGGACTGCAGCTTCCCCTT	For construction of pEX-*siaC*-H
siaC-H2R	CCCaagcttACCCAGCACTACGCCGACT	For construction of pEX-*siaC*-H
siaD-H1F	AGCgaattcCATCCTGCTCTGCTTCAACG	For construction of pEX-*siaD*-H
siaD-H1R	TGCtctagaGGCGATGCGCTCCAGCCGCA	For construction of pEX-*siaD*-H
siaD-H2F	TGCtctagaCGCGACAAATGCGTGTTCG	For construction of pEX-*siaD*-H
siaD-H2D	CCCaagcttTCACGCCGATGGAAACCTG	For construction of pEX-*siaD*-H
siaA-H1F	AGCgaattcGACCCGCTCAACGGCATC	For construction of pEX-*siaA*-H
siaA-H1R	TGCtctagaGGCACAGGCCAGCAACAG	For construction of pEX-*siaA*-H
siaA-H2F	TGCtctagaACTGACGGTTTCCTCGACCAG	For construction of pEX-*siaA*-H
siaA-H2R	CCCaagcttCGATGCGGTGTTCGGTAAC	For construction of pEX-*siaA*-H
siaB-C-H1F-bam	AGCggatccCCTACATTCCCCAACTGCG	For construction of pEX-*siaC-siaB*-H
siaB-C-H1R-xba	TGCtctagaCAGCAGGTCTAGCGTTTCCAT	For construction of pEX-*siaC-siaB*-H
siaB-C-H2F-xba	TGCtctagaGACGAGTAGCCGACGATGAG	For construction of pEX-*siaC-siaB*-H
siaB-C-H2R-hind	CCCaagcttAGGGACAGGACATGGACTGC	For construction of pEX-*siaC*-siaB-H

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