Supplementary Information for

hipBA toxin-antitoxin systems mediate persistence in *Caulobacter crescentus*

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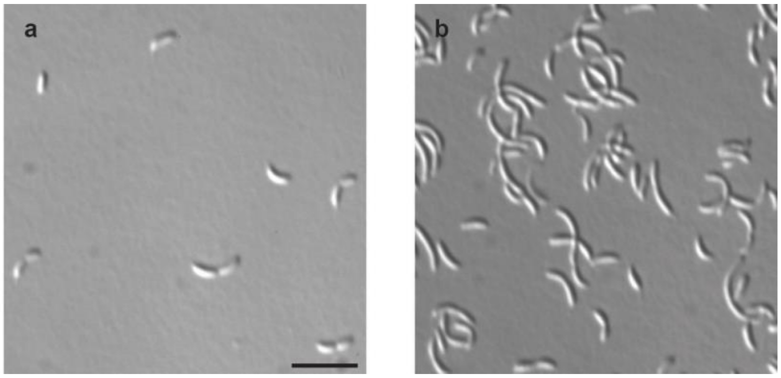
This PDF includes Supplementary Figures S1-S10, Supplementary Tables S2-S4, and references cited in these elements.



Supplementary Figure S1. $HipA_3$ induction causes cell elongation. a, NA1000 cells harboring p*FLAG-hipA$_3$* in exponential growth phase were diluted to OD_{660} = 0.02 and split into two cultures. At time 0, $HipA_3$ expression was induced in one culture using IPTG and theophylline. Differential interference contrast micrographs were obtained after 9 h of growth at 30°C. **a**, Uninduced culture. **b**, $HipA_3$-expressing culture. Scale bar, 5 µm.

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E.coli ---MPKLVTWMN--NQRVGEL-TKLANGAHTFKYAPEWLASRYARPLSLSLPLQRGNITS 54
HipA1  MAMTRVLTVW--WGDGVVGSL-ALDETGDIRFAYDAAWLADEACPAVSVSLPKQPGAFSR 57
HipA2  MSGPAGLIVRMDGFNLPAGYL-ASDEARAISFAYDDRYIAAG-GPPLSLSMPLEQVSFGD 58
HipA3  MTTVAEVRLW---GS-RIGAVSLEDGAETAVFAYEPSFIASGI-QPAPLMMPLKAGVFSF 55

E.coli D--AVFNFF------DNLLPDSPI--VRDRIVKRY--HAKSRQPFDLLSEIGRDSVGAVT 102
HipA1  R--KTRSFF------AGLLPDDL---QRDNVARAL--GVSRQNDFGLLERLGGDVAGALT 104
HipA2  V--TARAFF------DNLLPEND---QMQRVMDRE--GLARDDIVGLLSHLGADCSGAIS 105
HipA3  PDLPPRSFHGLPGMLADALPDKYGHVLIDAWLATQGRSPESFNAVERLCYTGRRGMGALE 115
                                                                †
E.coli LIPEDETVTHPI----MA------WEKLTEARLEEVLTAYKADIPLGMIREENDFRISVA 152
HipA1  LWPEGEAPPKRQGGRTAE--------PLNDKRLLAILDELPR---RPFLAGEDGVRLSLA 153
HipA2  CLPIGADPIKVPGVLSED------YELLAPGAIAEIARSLAE---RQRLPDTITDPSPVA 156
HipA3  FSPMAGPRRRVSSKIDIDALVTLASEVLTHRH--DLRASFADADKADALRDILSVGTSAG 173

                                         #
E.coli GAQEKTALLRIGNDW--CIPK----GITPTTHIIKLPIGEIRQPNATLDLSQSVDNEYYC 206
HipA1  GAQEKLPVVLVDGAV--ALPA----LGQPSTHILKPANARWPA---------MTENEALA 198
HipA2  GVQRKIALTHTPQGFAKPRPG----RKVPTTHILKVPETRLRR---------DARLEAAA 203
HipA3  GARAKAVIAWNPATN-EVRSGQVEAGAGFGYWLLKFDGVSGNRDKELADPKGYGAVEHAY 232

E.coli LLLAKELGLNVPDAEIIKAGNVRALAVERFDRRWNAERTVLLRLPQEDMCQT----FGLP 262
HipA1  MRLAAAVGLPTAGVEPRRIGERTFLLVTRYDRVVSSD-GAVRRLHQEDFCQA----LGVS 253
HipA2  ARLASALGLDVSIPEAIVIDGVDALLITRFDRVV-RD-GVVYRLHQEDFAQA----MGLP 257
HipA3  GQMAAAAGIDVAESRLLEEGGRRHFMSKRFDRLDGGGK-----LHMQSLAAIAHLDFNDP 287

                                                         *    ‡ #
E.coli SSVKYESDGGPGIARIMA----FLMGSSEALKDRYDFMKFQVFQWLIGATDGHAKNFSVF 318
HipA1  PEHKYAAEGGPIFPDCF-NLVRNVCQPS--APAVLALLDAAIFNVIVGNADAHGKNYSLL 310
HipA2  ATLKYQRNGAPGRQFDAQAIARVLDQTEAPALSRTAFLSATIFNLLIGNTDNHAKNHGLL 317
HipA3  VANSYEQA-----LFTMR-----RMGLS--MAQLEEQFRRMVFNVLARNQDDHVKNIAFL 335

                    ‡
E.coli IQAGGSYRLTPFYDIISAFPVLGGTGIHISDLKLAMGLNASKGKKTAIDKIYPRHFLATA 378
HipA1  HQAG-AIVMAPLYDLMCTAAYPEVH------AKLAMKIAGR----AQLEAFKADTWRDFG 359
HipA2  YRQGRAPILAPLYDLLPSRMNLDFN------DQLSFNIGAA----DHPDAITFDDMMAFF 367
HipA3  MDRAGRWRLSPAFDITWSYNPDGEW---T--SRHQMSINGKR------DGFDFADLEACA 384

E.coli KVLRFPEVQMHEILSDFA---RMIPAALDNVKTSLPTDFPENVVT----AVESNVLRLHG 431
HipA1  RDIG------------------MGPAFVER-RANAQARRVLDQVGPVADAI--AACGFDR 398
HipA2  EVFG------------------MRRAAA--------ARFIENVIKPMIEALERATLGLAG 401
HipA3  KTASISRGHVGRIFDEVREAVMRWPTFADAA--GV-DERWRDQIG--------ATVRLEL 433

E.coli R-------------------------LSREYGSK-------- 440
HipA1  EALRGLRDLIAERARAVLK---LGQGER--------------- 423
HipA2  ERLKDMDDLIGRETEQLVEVLGLDVAVRERDYYPKVRHALAPS 444
HipA3  RR----------------------------------------- 435
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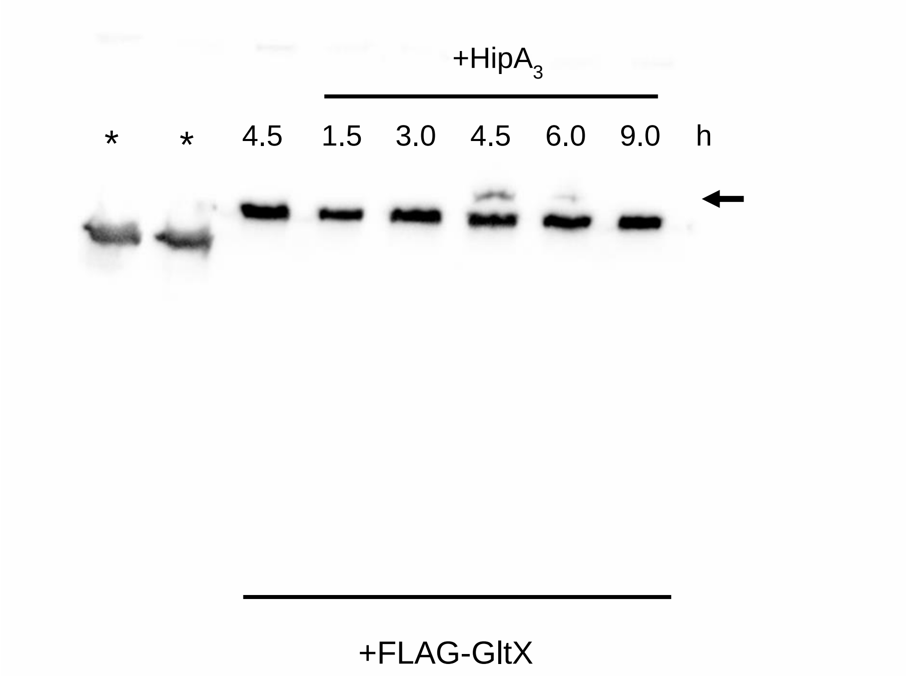
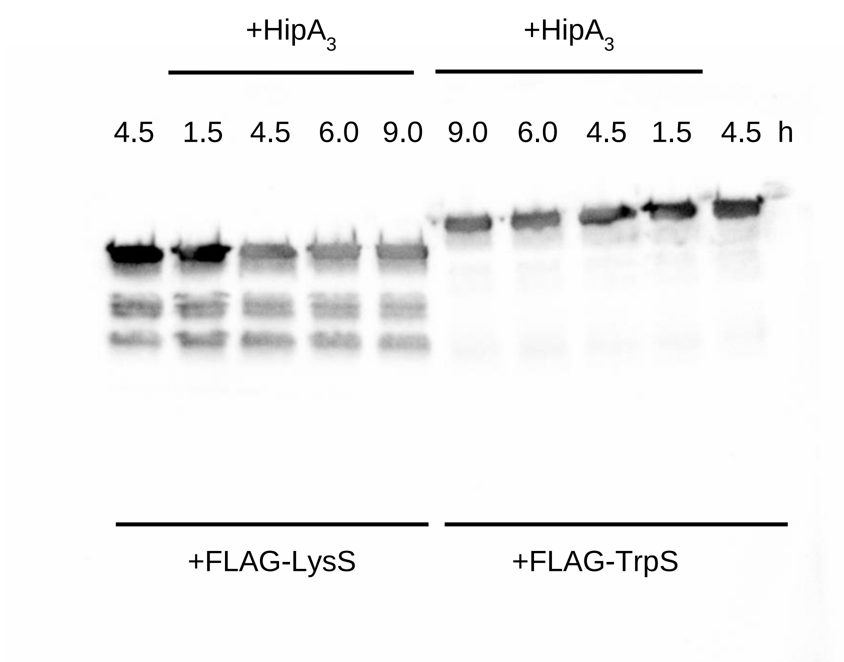
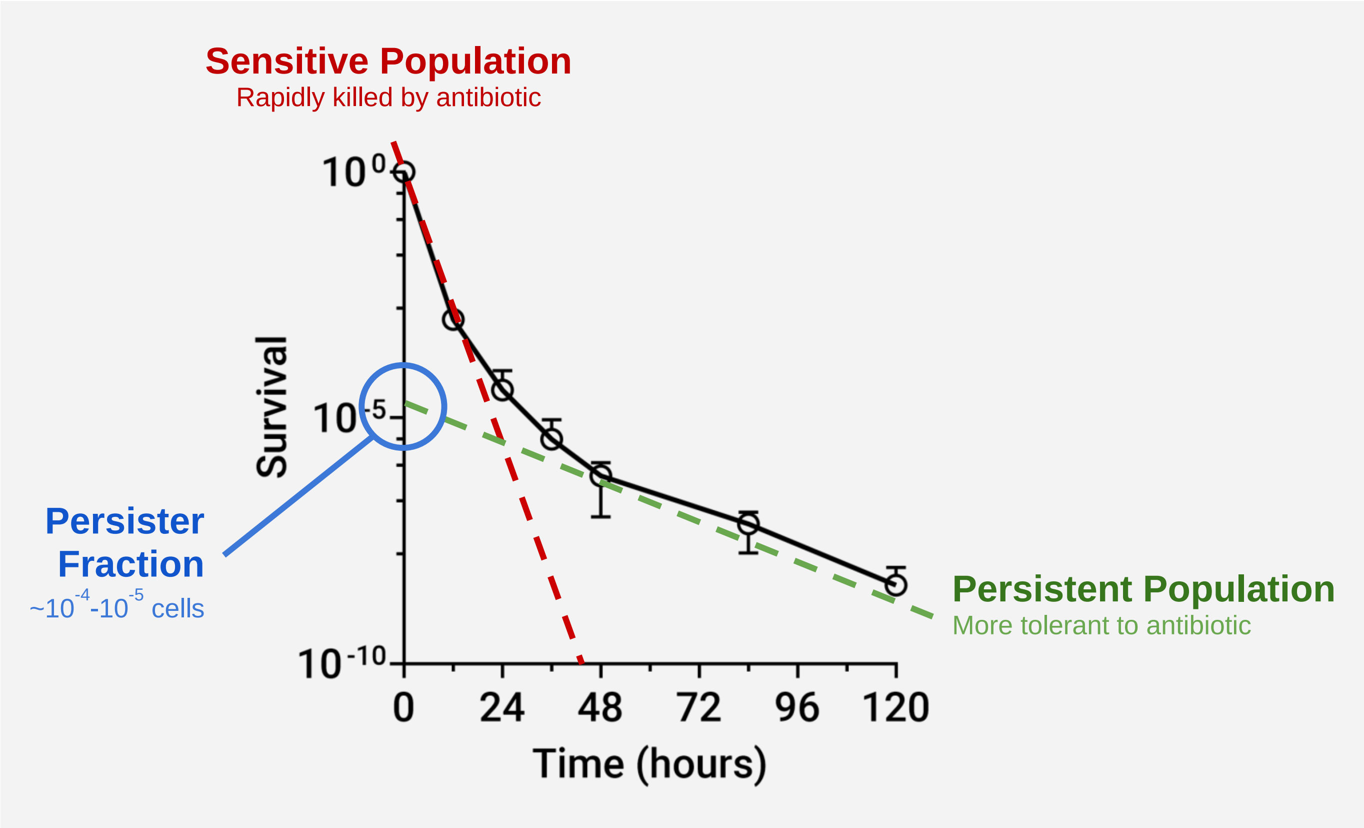
Supplementary Figure S2. Protein alignment of *E. coli* HipA and NA1000 HipA toxins. Alignment was obtained using ClustalOmega. Functionally critical residues are indicated: *****, catalytic aspartate residue targeted for mutation; **‡**, Mg^{++} binding residues; **#**, ATP-binding residues; **†**, autophosphorylated serine residue[1].

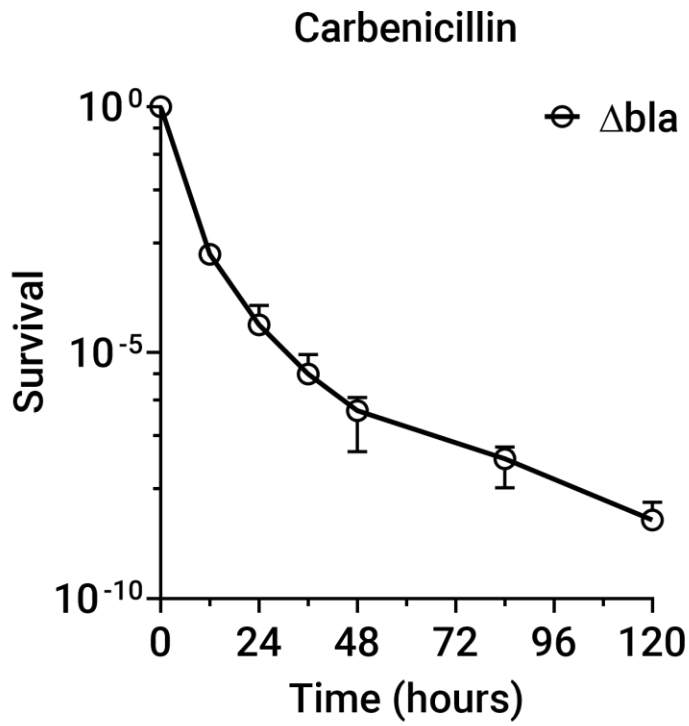




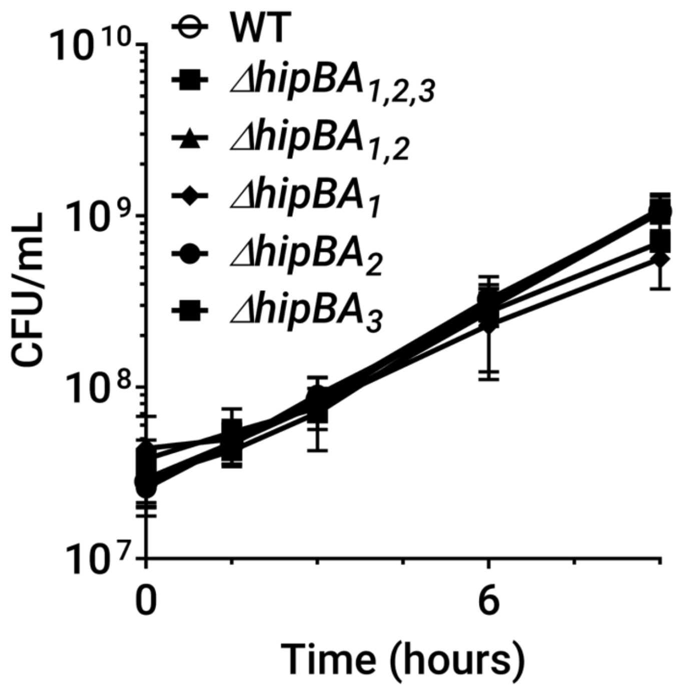
Supplementary Figure S3. HipA3 does not phosphorylate LysS or TrpS, and only weakly phosphorylates GltX. Full images are shown. *, protein samples not related to this experiment are shown in order to provide complete gel images. Arrow indicates phosphorylated FLAG-GltX species with reduced mobility in *Caulobacter* coexpressing HipA$_3$. Molecular size standards are omitted because they do not migrate at the correct sizes in Phos-tag gels, according to manufacturer's information (APExBIO).



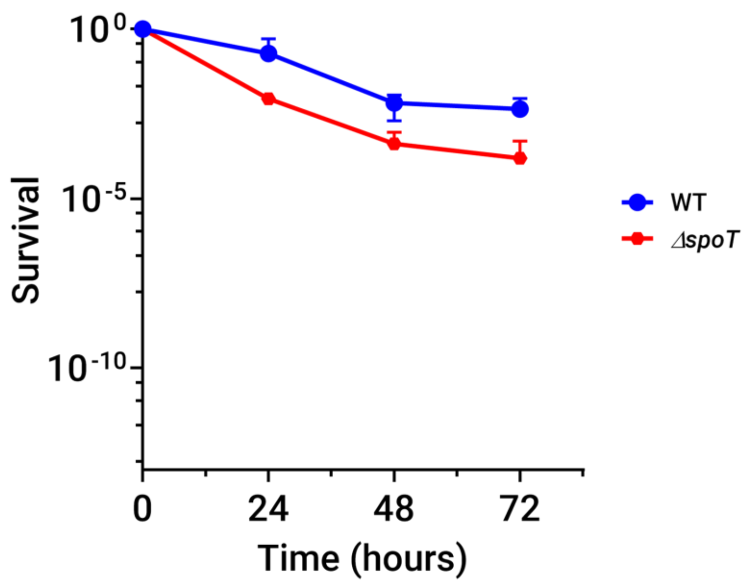
Supplementary Figure S4. Biphasic killing curves describe two sub-populations (sensitive and persistent) and their different rates of killing by antibiotics. The first phase primarily describes the survival of the sensitive fraction, while the second phase describes the killing of the remaining persistent population. The persister fraction can be approximated using the y-intercept of the second phase of the curve. This explanatory diagram was created using actual experimental data that re-appears in Supplementary Fig. S5.



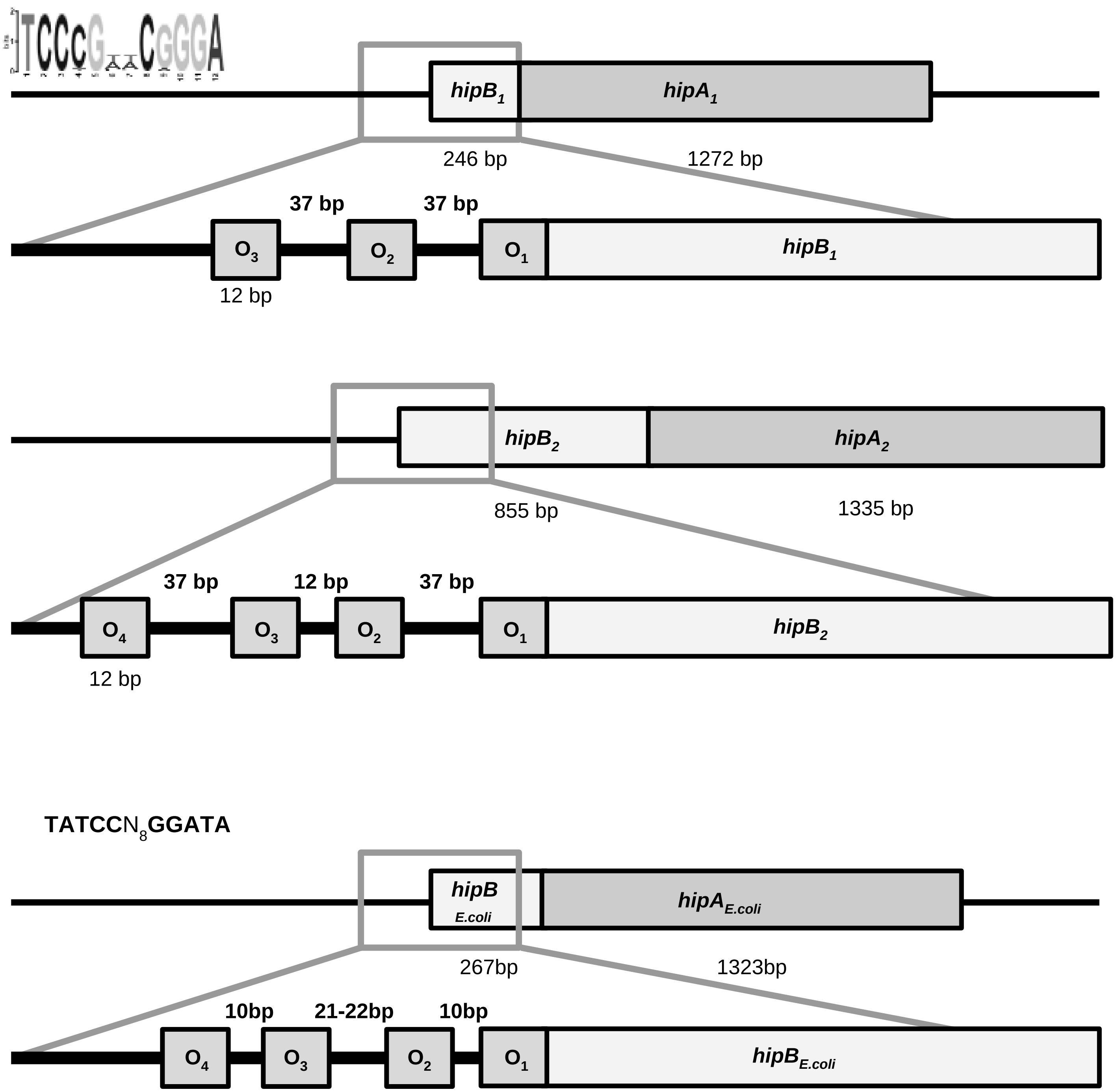
Supplementary Figure S5. The *C. crescentus* NA1000 *Δbla* strain displays persistence toward carbenicillin. The *Δbla* strain was grown to stationary phase in PYE and analyzed in a persistence assay using 100 µg/ml carbenicillin as described in Methods. The MIC of carbenicillin toward NA1000 *Δbla* was measured at 10-20 µg/ml.



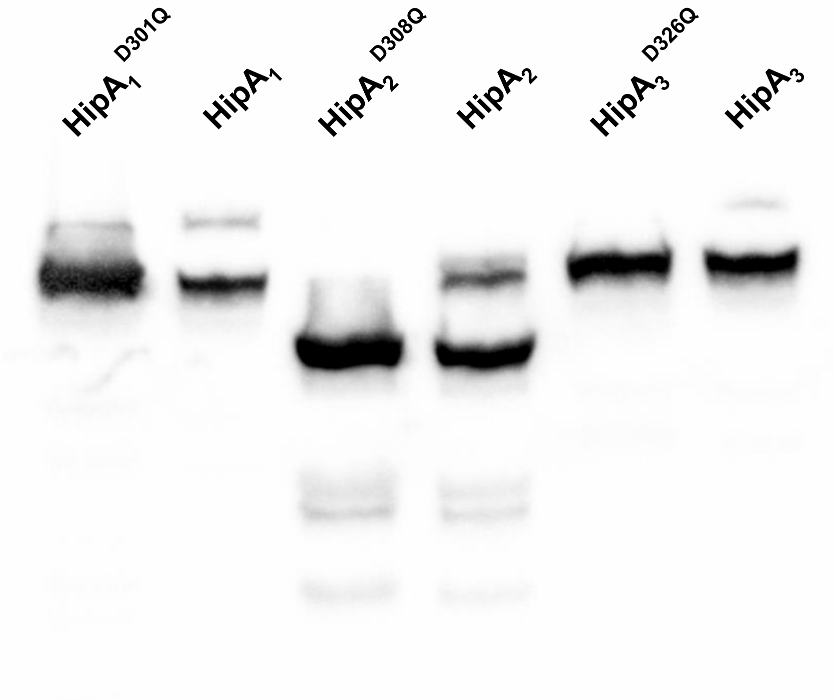
Supplementary Figure S6. NA1000 *ΔhipBA* strains do not have a growth defect. Growth curves of NA1000 and the Δ*hipBA* operon knockout strains in PYE medium. Exponential phase cultures were diluted to OD_{660}= 0.02 at time zero. At the indicated times, samples were withdrawn for CFU enumeration. Data points are the mean and standard deviation of three independent biological replicates.



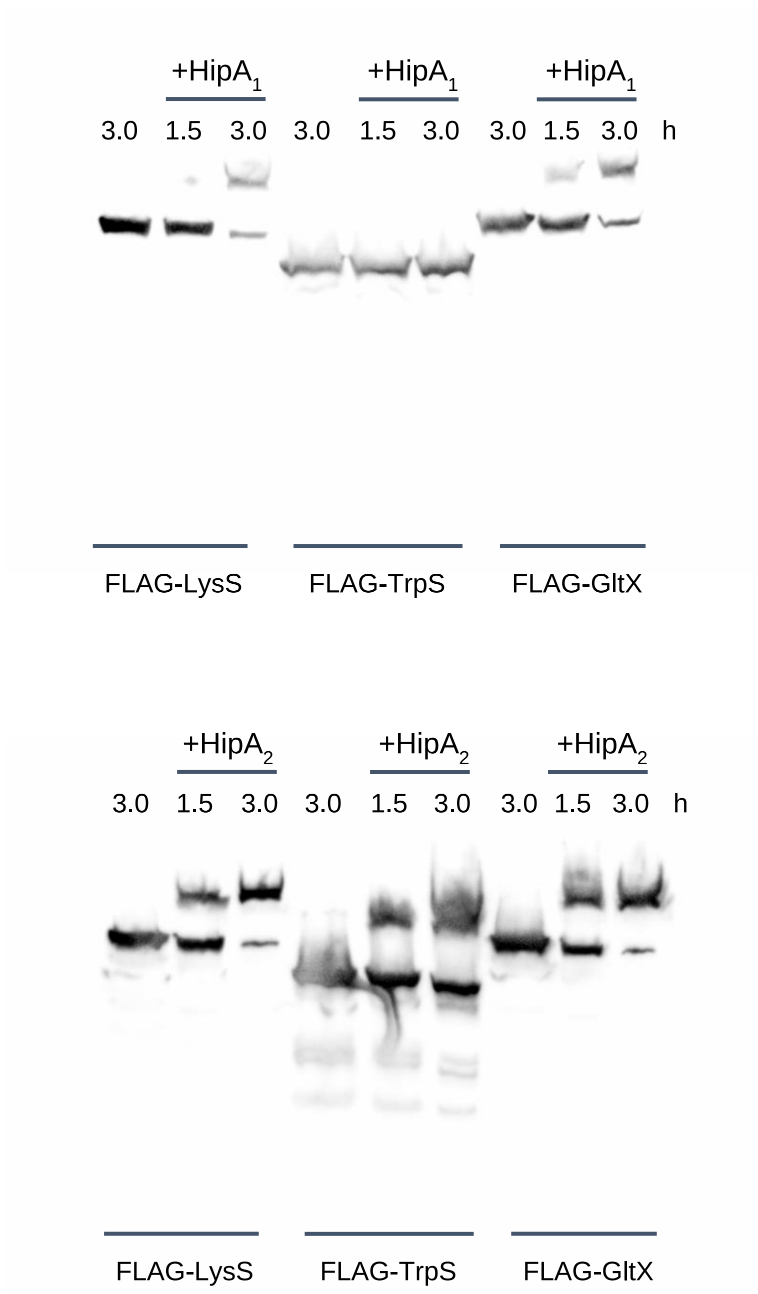
Supplementary Figure S7. The *ΔspoT* strain has reduced tolerance to the conditions of stationary phase in PYE. Exponential phase cultures of WT and *ΔspoT* strains were diluted to OD_{660}= 0.02 and grown to stationary phase in PYE medium. Samples were withdrawn for CFU enumeration at the indicated times. Data points are the means of two independent biological replicates, each with six technical replicates.



Supplementary Figure S8. Identical putative binding motifs with the indicated spacing are observed in the promoter regions upstream of *hipBA$_1$* and *hipBA$_2$*. Repetitive inverted repeats were identified using the EMBOSS bioinformatics suite[2]. The *E. coli hipBA* operon and HipB-binding operator sequence are included for comparison.



Supplementary Figure S9. Full gel image corresponding to Fig. 1d. Whole-cell lysates were analyzed by Phos-tag mobility shift and Western blotting with anti-FLAG antibodies. Molecular size standards are omitted because they do not migrate at the correct sizes in Phos-tag gels, according to manufacturer's information (APExBIO).



Supplementary Figure S10. Full gel images corresponding to Fig. 4. Molecular size standards are omitted because they do not migrate at the correct sizes in Phos-tag gels, according to manufacturer's information (APExBIO).

Supplementary Table S2. Strains used in this study.

Number	Description	Expanded genotype	Reference
KR3707	NA1000 or wild-type (WT)	*Caulobacter crescentus* NA1000	[3]
KR4280	WT + pFLAG-*hipA*$_1$		This study
KR4281	WT + pFLAG-*hipA*$_2$		This study
KR4282	WT + pFLAG-*hipA*$_3$		This study
KR658	*Δbla*		[4]
KR3744	*ΔhipBA*$_1$		This study
KR3714	*ΔhipBA*$_2$		This study
KR3631	*ΔhipBA*$_3$		This study
KR3794	*ΔhipBA*$_{1,2}$	*ΔhipBA*$_1$ *ΔhipBA*$_2$	This study
KR3750	*ΔhipBA*$_{1,2,3}$	*ΔhipBA*$_1$ *ΔhipBA*$_2$ *ΔhipBA*$_3$	This study
KR4283	*ΔhipBA*$_{1,2,3}$ + pMR20		This study
KR4284	*ΔhipBA*$_{1,2,3}$ + pJS71 + pMR20		This study
KR4285	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_1$		This study
KR4286	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_2$		This study
KR4287	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_3$		This study
KR4288	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_1$ + pJS71		This study
KR4289	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_2$ + pJS71		This study
KR4290	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_3$ + pJS71		This study
KR4291	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_1$D301Q		This study
KR4292	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_2$D308Q		This study
KR4293	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_3$D326Q		This study
KR4294	*ΔhipBA*$_{1,2,3}$ + pMR20 + pFLAG-*hipB*$_1$		This study
KR4295	*ΔhipBA*$_{1,2,3}$ + pMR20 + pFLAG-*hipB*$_2$		This study
KR4296	*ΔhipBA*$_{1,2,3}$ + pMR20 + pFLAG-*hipB*$_3$		This study
KR4297	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_1$ + pFLAG-*hipB*$_1$		This study
KR4298	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_2$ + pFLAG-*hipB*$_1$		This study
KR4299	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_3$ + pFLAG-*hipB*$_1$		This study
KR4300	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_1$ + pFLAG-*hipB*$_2$		This study

KR4301	*ΔhipBA*$_{1,2,3}$+ pFLAG-*hipA*$_{2}$ + pFLAG-*hipB*$_{2}$		This study
KR4302	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_{3}$ + pFLAG-*hipB*$_{2}$		This study
KR4303	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_{1}$ + pFLAG-*hipB*$_{3}$		This study
KR4304	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_{2}$ + pFLAG-*hipB*$_{3}$		This study
KR4305	*ΔhipBA*$_{1,2,3}$ + pFLAG-*hipA*$_{3}$ + pFLAG-*hipB*$_{3}$		This study
KR3063	*ΔspoT*		[5]
KR4306	*ΔspoT* + pMR20		This study
KR4307	*ΔspoT* + pFLAG-*hipA*$_{1}$		This study
KR4308	*ΔspoT* + pFLAG-*hipA*$_{2}$		This study
KR4309	*ΔspoT* + pFLAG-*hipA*$_{3}$		This study
KR4310	WT + pFLAG-*metG*		This study
KR4311	WT + pFLAG-*lysS*		This study
KR4312	WT + pFLAG-*trpS*		This study
KR4313	WT + pFLAG-*gltX*		This study
KR4314	WT + pMR20 + pFLAG-*lysS*		This study
KR4315	WT + pMR20 + pFLAG-*trpS*		This study
KR4316	WT + pMR20 + pFLAG-*gltX*		This study
KR4317	WT + p*hipA*$_{1}$ + pFLAG-*lysS*		This study
KR4318	WT + p*hipA*$_{1}$ + pFLAG-*trpS*		This study
KR4319	WT + p*hipA*$_{1}$ + pFLAG-*gltX*		This study
KR4320	WT + p*hipA*$_{2}$ + pFLAG-*lysS*		This study
KR4321	WT + p*hipA*$_{2}$ + pFLAG-*trpS*		This study
KR4322	WT + p*hipA*$_{2}$ + pFLAG-*gltX*		This study
KR4323	WT + p*hipA*$_{3}$ + pFLAG-*lysS*		This study
KR4324	WT + p*hipA*$_{3}$ + pFLAG-*trpS*		This study
KR4325	WT + p*hipA*$_{3}$ + pFLAG-*gltX*		This study

Supplementary Table S3. Plasmids used in this study.

Name	Description	Reference
pMR20	Broad-host-range, low-copy-number vector; *tetAR*	[6]
pJS71	Broad host-range cloning vector; high copy; chlor^{R}; pBBR1MCS derivative with unique EcoRI site	[7]
pNPTS138	kan^{R}; *sacB*-containing integration vector	GenBank Accession Number MK533795.1
pCYH1	pNPTS138-$hipBA_1$KO (*CCNA_00481-00482*)	This study
pCYH2	pNPTS138-$hipBA_2$KO (*CCNA_02821-02822*)	This study
pCYH3	pNPTS138-$hipBA_3$KO (*CCNA_02858-02859*)	This study
p$hipA_1$	pMR20 - PriboA - $hipA_1$(*CCNA_00482*)	This study
p$hipA_2$	pMR20 - PriboA - $hipA_2$(*CCNA_02821*)	This study
p$hipA_3$	pMR20 - PriboA - $hipA_3$(*CCNA_02858*)	This study
pFLAG-$hipA_1$	pMR20 - PriboA - FLAG-$hipA_1$	This study
pFLAG-$hipA_2$	pMR20 - PriboA - FLAG-$hipA_2$	This study
pFLAG-$hipA_3$	pMR20 - PriboA - FLAG-$hipA_3$	This study
pFLAG-$hipA_1^{D301Q}$	pMR20 - PriboA - FLAG-$hipA_1$D301Q	This study
pFLAG-$hipA_2^{D308Q}$	pMR20 - PriboA - FLAG-$hipA_2$D308Q	This study
pFLAG-$hipA_3^{D326Q}$	pMR20 - PriboA -pFLAG-$hipA_3$D326Q	This study
pFLAG-$hipB_1$	JS71 - PriboA - FLAG-$hipB_1$(*CCNA_00481*)	This study
pFLAG-$hipB_2$	JS71 - PriboA - FLAG-$hipB_2$(*CCNA_02822*)	This study
pFLAG-$hipB_3$	JS71 - PriboA - FLAG-$hipB_3$(*CCNA_02859*)	This study
pFLAG-*metG*	JS71 - PriboA - FLAG-*metG*(*CCNA_01547*)	This study
pFLAG-*lysS*	JS71 - PriboA - FLAG-*lysS*(*CCNA_00082*)	This study
pFLAG-*trpS*	JS71 - PriboA - FLAG-*trpS*(*CCNA_00062*)	This study
pFLAG-*gltX*	JS71 - PriboA - FLAG-*gltX* (*CCNA_01982*)	This study
p$hipBA_1$	pMR20 - $hipBA_1$	This study
p$hipBA_2$	pMR20 - $hipBA_2$	This study
p$hipBA_3$	pMR20 - $hipBA_3$	This study
p$hipBA_{1,2}$	pMR20 - $hipBA_{1,2}$	This study

Supplementary Table S4. Primers used in this study.

Name	Sequence (5'-3')	Purpose
hipBA1 Up F	attgaagccggctggcgccaTCGACCCGGGCGATCTTC	*hipBA*$_1$ chromosomal knockout
hipBA1 Up R	tctagcgctcGAGGGTCATGAAAATTCTCCCG	
hipBA1 Dn F	catgaccctcGAGCGCTAGACTCGATCCCGC	
hipBA1 Dn R	cgtcacggccgaagctagcgGCCCAGGACGGCGACACC	
hipBA2 Up F	attgaagccggctggcgccaTTGCCTCTTGAGGAGCCG	*hipBA*$_2$ chromosomal knockout
hipBA2 Up R	acacctcctaCAGGAGCATAAGTAGCTAACGAAG	
hipBA2 Dn F	tatgctcctgtagGAGGTGTTCGGCATGAGG	
hipBA2 Dn R	cgtcacggccgaagctagcgAAGATCACCCCGCTGTCG	
hipBA3 Up F	attgaagccggctggcgccaCCCGGGCGACAAGAAGGC	*hipBA*$_3$ chromosomal knockout
hipBA3 Up R	cctaccgcctAAATTTCATAAATCGCCTCTTGTGGCG	
hipBA3 Dn F	tatgaaatttAGGCGGTAGGCGGGGGAT	
hipBA3 Dn R	cgtcacggccgaagctagcgGACAGCGGCACCAGCCGTC	
riboA to pJS71 F	cggccgctctagaactagtgTGGTGCAAAACCTTTCGC	pJS71 protein expression vector
riboA to pMR10 or pMR20 F	ctatgaccatgattacgccaTGGTGCAAAACCTTTCGC	pMR10/pMR20 expression vector
riboA to FLAG R	cttgtcgtcatcgtctttgtagtcCATCTTGTTGATACCCCC	riboA promoter for protein expression
riboA to hipA1 R	tagtcatcgcCATCTTGTTGATACCCCC	
riboA to hipA2 R	cgggcccactCATCTTGTTGATACCCCC	
riboA to hipA3 R	cgacggtggtCATCTTGTTGATACCCCC	
hipA1 to riboA F	caacaagatgGCGATGACTAGAGTGCTGACG	*hipA*$_1$ for pMR20 expression vector
hipA1 F	gactacaaagacgatgacgacaagGCGATGACTAGAGTGCTGACG	FLAG-hipA1 for pMR20 expression vector
hipA1 R	ttgtaaaacgacggccagtgCTAGCGCTCGCCCTGACC	
hipA2 to riboA F	caacaagatgAGTGGGCCCGCAGGCCTG	*hipA*$_2$ for pMR20 expression vector
hipA2 F	gactacaaagacgatgacgacaagAGTGGGCCCGCAGGCCTG	FLAG-*hipA*$_2$ for pMR20 expression vector
hipA2 R	ttgtaaaacgacggccagtgTCAACTCGGCGCCAAGGCG	
hipA3 to riboA F	caacaagatgACCACCGTCGCCGAGGTT	*hipA*$_3$ for pMR20 expression vector
hipA3 F	gactacaaagacgatgacgacaagACCACCGTCGCCGAGGTT	FLAG-*hipA*$_3$ for pMR20 expression vector
hipA3 R	ttgtaaaacgacggccagtgCTACCGCCTGAGCTCCAG	
hipA1 D301Q F	tcttgccgtgagcctgggcgttgccgacg	*hipA*$_1$ D301Q mutagenic primers
hipA1 D301Q R	cgtcggcaacgcccaggctcacggcaaga	
hipA2 D308Q F	tggttcttggcgtggttctgagtattgccaatcagaagatt	*hipA*$_2$ D308Q mutagenic primers
hipA2 D308Q R	aatcttctgattggcaatactcagaaccacgccaagaacca	
hipA3 D326Q F	gttcttgacgtggtcctgctggtttcgcgccag	*hipA*$_3$ D326Q mutagenic primers
hipA3 D326Q R	ctggcgcgaaaccagcaggaccacgtcaagaac	
hipB1 F	gactacaaagacgatgacgacaagACCCTCCTCCGGCAAGGC	FLAG-*hipB*$_1$ for pJS71 expression vector
hipB1 R	cgaggtcgacggtatcgataCTAGTCATCGCCACCGCTCAC	
hipB2 F	gactacaaagacgatgacgacaagCTCCTGAGCGGGAGCGAAAAC	FLAG-*hipB*$_2$ for pJS71 expression vector
hipB2 R	cgaggtcgacggtatcgataTCATCTGGGCGCCGCCAT	
hipB3 F	gactacaaagacgatgacgacaagAAATTTGAATCTCTCCTGTCC	FLAG-*hipB*$_3$ for pJS71 expression vector
hipB3 R	cgaggtcgacggtatcgataTCATTTTTCATCGCCCCAC	

gltX F	gactacaaagacgatgacgacaagTCGAACCCCACCCCTACC	FLAG-*gltX* for pJS71 expression vector
gltX R	cgaggtcgacggtatcgataTCATGCGCGAGGCGCTAG	
lysS F	gactacaaagacgatgacgacaagTTTGAAGGCCTCTCCCCC	FLAG-*lysS* for pJS71 expression vector
lysS R	cgaggtcgacggtatcgataTCAGCCCAGCTTTTCCTC	
metG F	gactacaaagacgatgacgacaagGCTCGCATCCTGATTACCTCCG	FLAG-*metG* for pJS71 expression vector
metG R	cgaggtcgacggtatcgataTTACTCCGCGCCGCCGAA	
trpS F	gactacaaagacgatgacgacaagACCGACCAAGCTCCCGTC	FLAG-*trpS* for pJS71 expressi2n vector
trpS R	cgaggtcgacggtatcgataCTACGCGCCCCAGAAGCC	
hipBA1 F	ctatgaccatgattacgccaGGGGCCCAGCCCCATGGC	*hipBA*$_1$ operon and promoter for pMR10 complementation vector
hipBA1 R	ttgtaaaacgacggccagtgCTAGCGCTCGCCCTGACCCAG	
hipBA2 F	ctatgaccatgattacgccaTCAACTCGGCGCCAAGGC	*hipBA*$_2$ operon and promoter for pMR10 complementation vector
hipBA2 R	ttgtaaaacgacggccagtgCTGCGGGGAGGGTGTGGG	
hipBA1 to hipBA2 R	gccgagttgaCTAGCGCTCGCCCTGACCCAG	*hipBA*$_1$ and *hipBA*$_2$ operons for pMR10 complementation vector
hipBA2 to hipBA1 F	cgagcgctagTCAACTCGGCGCCAAGGC	
CCNA_03445 qPCR F	CTGGATCTGTCTCGCCTTGG	RT-PCR oligonucleotides
CCNA_03445 qPCR R	GGTGGGATGGGGCTTCTG	
CCNA_03181 qPCR F	TTCAGACGCTTGAGACCACC	
CCNA_03181 qPCR R	CGTACATGGTTTTCGGCACG	
CCNA_01674 qPCR F	CAGACCCTAGCGGACACTCC	
CCNA_01674 qPCR R	GGCGTGAACATCTCGTCGTA	
CCNA_01596 qPCR F	ATGTTTCCCGACACCGACTC	
CCNA_01596 qPCR R	CTGCTCGGGGTTCAGGC	
CCNA_00520 qPCR F	GCGATCGCGGAATATCTCGAC	
CCNA_00520 qPCR R	CTGGTCGACTGGATGACGAA	
rho1 F	AGACACCGAAAACCAGGTTCC	
rho1 R	CGCTTCAGTGGTGATGTCGG	
rho2 F	ACCGAAGACACCGAAAACCAG	
rho2 R	GCCGCTTCAGTGGTGATGT	

References

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