

1 **References**

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19 **Table S1 Differentially expressed genes between WT and  $\Delta pde1\Delta pde2$  determined from**  
 20 **RNA-Seq analysis\*.**

<b>D39 Annotation</b>	<b>Fold change (Log<sub>2</sub>)</b>	<b>Description</b>
SPD_1529	-1.80	Mobile element protein
SPD_0058	-1.40	<i>purD</i> , Phosphoribosylamine-glycine ligase
SPD_0684	-1.40	BioY family biotin synthase
SPD_0913	-1.20	Hypothetical protein
SPD_1414	-1.10	Oxalate/formate antiporter
SPD_0267	-1.10	Xanthine/uracil permease
SPD_2068	-1.10	<i>htrA</i> , Serine protease
SPD_1526	-1.00	ABC transporter
SPD_1525	-1.00	ABC transporter ATP-binding protein
SPD_1087	-1.00	<i>fhs</i> , Formate-tetrahydrofolate ligase
SPD_1589	-1.00	Lipoprotein
SPD_1644	-0.90	Hypothetical protein
SPD_1506	-0.90	Acetyl xylan esterase
SPD_1590	-0.90	General stress protein 24
SPD_1588	-0.90	Hypothetical protein
SPD_1152	-0.90	<i>fld</i> , Flavodoxin
SPD_1524	-0.80	GntR transcriptional regulator
SPD_1375	-0.80	NADPH-dependent FMN reductase
SPD_2069	-0.80	<i>spoJ</i> , Chromosome partitioning protein
SPD_1100	-0.80	<i>zwf</i> , Glucose-6-phosphate 1-dehydrogenase
SPD_2054	0.81	<i>recF</i> , DNA replication and repair protein
SPD_1620	0.82	Glycosyl transferase
SPD_1619	0.83	Capsular polysaccharide biosynthesis protein
SPD_1170	0.83	ABC transporter, oligopeptide-binding protein
SPD_0594	0.84	Hypothetical protein
SPD_1868	0.85	<i>tgt</i> , queuine tRNA-rubosyltransferase
SPD_1357	0.85	<i>aliB</i> , ABC transporter, oligopeptide-binding protein
SPD_0472	0.93	<i>blpA</i> , Bacteriocin ABC transporter ATP-binding protein
SPD_0248	0.93	<i>glmS</i> , Glucosamine-fructose-6-phosphate aminotransferase
SPD_1587	0.95	Transcriptional activator
SPD_1341	0.97	<i>atpE</i> , ATP synthase F <sub>0</sub> , C subunit
SPD_1430	0.97	<i>fer</i> , Ferredoxin
SPD_0459	0.97	<i>grpE</i> , Heat shock protein/molecular chaperone
SPD_0817	0.98	CAAX amino terminal protease family protein
SPD_1828	0.99	Hypothetical protein
SPD_0460	0.99	<i>dnaK</i> , Heat shock protein/molecular chaperone
SPD_0890	1.00	<i>phtE</i> , Histidine triad protein E precursor
SPD_0608	1.01	<i>pyrF</i> , Orotidine 5'-phosphate decarboxylase

SPD_1499	1.01	<i>nanB</i> , Sialidase B precursor
SPD_0458	1.04	<i>hrcA</i> , Heat-inducible transcription repressor
SPD_1502	1.06	ABC transporter, multiple sugar binding protein
SPD_0609	1.09	<i>pyrE</i> , Orotate phosphoribosyltransferase
intergenic SPD_1179/		Hypothetical protein
SPD_1180	1.11	
SPD_0470	1.12	<i>blpC</i> , Peptide pheromone
SPD_0029	1.13	<i>radA</i> , DNA repair protein
SPD_1498	1.13	Oxidoreductase
SPD_0994	1.13	<i>ribF</i> , Riboflavin biosynthesis protein
SPD_0048	1.16	Hypothetical protein
SPD_1618	1.16	Hypothetical protein
SPD_0028	1.17	Phosphoglycerate mutase family protein
intergenic SPD_0844/		Hypothetical protein
SPD_0845	1.21	
SPD_0027	1.23	<i>dut</i> , Deoxyuridine 5'-triphosphate nucleotidohydrolase
SPD_0591	1.23	Hypothetical protein
SPD_1380	1.26	Hypothetical protein
SPD_0308	1.27	<i>clpL</i> , ATP-dependent Clp protease ATP-binding subunit
SPD_1381	1.29	<i>def-2</i> , Polypeptide deformylase
SPD_0845	1.38	ABC transporter, ATP-binding protein
SPD_1740	1.40	<i>cinA</i> , Competence nicotinamide-nucleotide amidase
SPD_0471	1.40	<i>blpB</i> , ABC transporter of <i>blpC</i>
SPD_1965	1.44	<i>pcpA</i> , Choline binding protein
SPD_0846	1.45	ABC transport permease protein
SPD_1842	1.48	<i>araD</i> , L-ribulose-5-phosphate 4-epimerase
SPD_1503	1.49	Hypothetical protein
SPD_1593	1.55	<i>ccIA</i> , Type IV prepilin peptidase
SPD_1633	1.57	<i>galT-2</i> , Galactose-1-phosphate uridylyltransferase
SPD_0249	1.58	Hypothetical protein
SPD_1841	1.58	BglG family transcriptional regulator
SPD_1678	1.59	<i>agaN</i> , Alpha-galactosidase
SPD_1632	1.65	Acyl-CoA thioesterase
SPD_1676	1.66	<i>raff</i> , Sugar ABC transporter permease protein
SPD_1984	1.69	Hypersensitive-induced reaction protein 4
SPD_0392	1.71	Hypothetical protein
intergenic SPD_0393/		Hypothetical protein
SPD_0392	1.73	
SPD_1634	1.74	<i>galK</i> , Galactokinase
SPD_0035- 36	1.80	<i>ccs16</i> , Competence-induced protein
SPD_1675	1.84	<i>rafG</i> , Sugar ABC transporter permease
SPD_1673	1.85	<i>gtfA</i> , Sucrose phosphorylase

SPD_0037	1.90	Hypothetical protein
SPD_1677	1.95	<i>rafE</i> , Sugar ABC transporter, sugar-binding protein
SPD_1982	1.95	Hypothetical protein
intergenic		Hypothetical protein
SPD_0131/ SPD_0132	2.01	
intergenic		BOX elements
SPD_0048/ SPD_0049	2.08	
SPD_2028	2.22	<i>cbpD</i> , Choline binding protein
SPD_0898	2.27	Membrane protein
SPD_0024	2.28	<i>purA</i> , Adenylosuccinate synthetase
SPD_0047	2.29	Hypothetical protein
SPD_0097	2.30	Cyanate transporter
SPD_1124	2.38	<i>licB</i> , Choline transport protein
SPD_1123	2.39	<i>licC</i> , CTP-phosphocholine cytidylyltransferase
SPD_1126	2.42	Alcohol dehydrogenase
SPD_0391	2.42	Hypothetical protein
SPD_1125	2.49	<i>pck</i> , Choline kinase
SPD_1127	2.50	<i>ispD</i> , 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase
SPD_0046	2.81	<i>blpU</i> , Bacteriocin
SPD_1122	3.04	<i>dprA</i> , DNA processing protein
SPD_0865	3.39	<i>coiA</i> , Late competence protein
SPD_1744	3.79	<i>comM</i> , Immunity protein
SPD_0466	4.12	Hypothetical protein
SPD_0843	4.13	<i>comEA</i> , Late competence protein, DNA receptor
SPD_0844	4.22	<i>comEC</i> , Late competence protein, DNA transport
SPD_0014	4.34	<i>comX1</i> , Competence-specific sigma factor
SPD_1855	4.53	Hypothetical protein
SPD_1818	4.54	<i>comX2</i> , Competence-specific sigma factor
SPD_2063	4.72	<i>comE</i> , Response regulator
SPD_1711	4.81	<i>ssbB</i> , Single-strand DNA-binding protein
SPD_1856	4.82	Hypothetical protein
SPD_0049	4.88	<i>comA</i> , CSP ABC transporter ATP-binding protein/permease
SPD_2064	5.00	<i>comD</i> , Histidine sensor kinase
SPD_0050	5.02	<i>comB</i> , CSP ABC transporter permease
SPD_2065	5.08	<i>comC</i> , CSP precursor
SPD_1858	5.10	<i>comGF</i> , Late competence protein, DNA uptake
SPD_1859	5.16	Hypothetical protein
SPD_0473	5.17	<i>blpY</i> , Immunity protein
SPD_0475	5.19	CAAX amino terminal protease family protein
SPD_0023	5.21	<i>comW</i> , Competence positive regulator
SPD_1857	5.22	<i>comGG</i> , Hypothetical protein
SPD_1861	5.24	<i>comGC</i> , Late competence protein, DNA uptake
SPD_1860	5.44	<i>comGD</i> , Late competence protein, DNA uptake

SPD_1862	5.45	<i>comGB</i> , Late competence protein, DNA uptake
SPD_1863	5.46	<i>comGA</i> , Late competence protein, DNA uptake
SPD_0474	5.54	<i>blpZ</i> , Immunity protein
SPD_2035	5.55	<i>comFA</i> , Late competence protein, DNA uptake
SPD_2034	5.61	<i>comFC</i> , Late competence protein, DNA uptake
SPD_0133	6.00	<i>cibA</i> , Bacteriocin
SPD_0132	7.03	<i>cibB</i> , Bacteriocin

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21 \*Gene expression was analyzed by RNA-Seq from RNA purified from three biological replicates  
22 of WT and  $\Delta pde1\Delta pde2$  strains. Fold change indicates the mean RNA reads of the  
23  $\Delta pde1\Delta pde2$  strain compared to those of WT.

24 **Table S2 Regulated genes in  $\Delta pde1\Delta pde2$  compared to CSP-induced genes determined**  
 25 **from a previous microarray study.<sup>a</sup>**

<b>TIGR4 Annotation</b>	<b>Regulation<sup>b</sup></b>	<b>Description</b>
<i>Early</i>		
SP0014	↑	<i>comX1</i> , Competence-specific sigma factor
SP0018	↑	<i>comW</i> , Competence positive regulator
SP0019	↑	<i>purA</i> , Adenylosuccinate synthetase
SP0042	↑	<i>comA</i> , CSP ABC transporter ATP-binding protein/permease
SP0043	↑	<i>comB</i> , CSP ABC transporter permease
SP0530	↑	<i>blpA</i> , Bacteriocin ABC transporter ATP-binding protein
SP0545	↑	<i>blpY</i> , Immunity protein
SP0546	↑	<i>blpZ</i> , Immunity protein
SP0547	↑	CAAX amino terminal protease family protein
SP1110	↑	<i>ribC</i> , Riboflavin kinase/FMN adenylyltransferase
SP1549	↑	Peptide deformylase
SP1548	↑	Hypothetical protein
SP1717	-	ABC transporter ATP-binding protein
SP1716	-	Hypothetical protein
SP1918	-	ABC transporter ATP-binding protein
SP1945	↑	<i>comM</i> , Immunity protein
SP1944	-	<i>tsaE</i> , tRNA threonylcarbamoyladenosine biosynthesis protein
SP1943	-	GNAT family acetyltransferase
SP1942	-	LytR family transcriptional regulator
SP2006	↑	<i>comX2</i> , Competence-specific sigma factor
SP2156	↑	SPFH domain/Band 7 family
SP2237	↑	<i>comC</i> , CSP precursor
SP2236	↑	<i>comD</i> , Histidine sensor kinase
SP2235	↑	<i>comE</i> , Response regulator
<i>Delayed</i>		
SP0338	↑	<i>clpL</i> , ATP-dependent Clp protease ATP-binding subunit
SP0515	↑	<i>hrcA</i> , Heat-inducible transcription repressor
SP0516	↑	<i>grpE</i> , Heat shock protein/molecular chaperone
SP0517	↑	<i>dnaK</i> , Heat shock protein/molecular chaperone
SP0519	-	<i>dnaJ</i> , Heat shock protein/molecular chaperone
SP0785	-	HlyD family secretion protein
SP0786	-	ABC transport system ATP-binding protein
SP0787	-	ABC transport system permease protein
SP0798	-	<i>ciaR</i> , Response regulator
SP0799	-	<i>ciaH</i> , Histidine sensor kinase
SP1027	↓	Hypothetical protein
SP1029	-	TrmA family RNA methyltransferase

SP1380	-	ABC transport system permease protein
SP1714	↓	GntR transcriptional regulator
SP1715	↓	ATP transporter ATP-binding protein
SP1907	-	<i>groES</i> , Chaperonin
SP1906	-	<i>groEL</i> , Chaperonin
SP2239	↓	<i>htrA</i> , Serine protease
SP2240	↓	<i>spoJ</i> , Chromosome partitioning protein
<i>Late</i>		
SP0021	↑	dUTP pyrophosphatase
SP0022	↑	Hypothetical protein
SP0023	↑	<i>radA</i> , DNA repair protein
SP0024	-	Carbonic anhydrase
SP0025	-	Hypothetical protein
SP0026	-	Hypothetical protein
SP0031	↑	Hypothetical protein
SP0030	↑	<i>ccs16</i> , Competence-induced protein
SP0029	↑	Hypothetical protein
SP0125	↑	<i>cibA</i> , Bacteriocin
SP0124	↑	<i>cibB</i> , Bacteriocin
SP0200	-	<i>ccs4</i> , Competence-induced protein
SP0201	-	Hypothetical protein
SP0782	-	Hypothetical protein
SP0954	↑	<i>ceIA</i> , Competence-related DNA transporter
SP0955	↑	<i>ceIB</i> , Competence-related DNA transporter
SP0956	↑	Hypothetical protein
SP0957	↑	ABC transporter ATP-binding protein
SP0958	↑	ABC transport permease protein
SP0978	↑	<i>coiA</i> , Late competence protein
SP0979	-	<i>pepF</i> , Oligoendopeptidase
SP0980	-	O-methyltransferase
SP0981	-	<i>cpoA</i> , Glycosyl transferase
SP1065	-	Hypothetical protein
SP1071	-	<i>dnaG</i> , DNA primase
SP1073	-	<i>rpoD</i> , Sigma factor
SP1074	-	Hypothetical protein
SP1096	-	Hypothetical protein
SP1095	-	<i>prsA2</i> , Ribose-phosphate pyrophosphokinase
SP1094	-	Cysteine desulfurase
SP1093	-	Hypothetical protein
SP1092	-	Hypothetical protein
SP1090	-	Redox-sensing transcriptional repressor
SP1089	-	Glutamine amidotransferase
SP1088	-	<i>radC</i> , DNA repair protein
SP1097	-	GTP pyrophosphokinase
SP1098	-	NAD <sup>+</sup> kinase

SP1099	-	Ribosomal large subunit pseudouridine synthase
SP1100	-	<i>ptA</i> , Phosphate acetyltransferase
SP1266	↑	<i>dprA</i> , DNA processing protein
SP1264	-	Hypothetical protein
SP1480	-	Hypothetical protein
SP1479	-	<i>pgdA</i> , Peptidoglycan N-acetylglucosamine deacetylase A
SP1478	-	Oxidoreductase
SP1808	↑	<i>cclA</i> , Type IV prepilin peptidase
SP1809	-	Transcriptional regulator
SP1810	-	Hypothetical protein
SP1811	-	<i>trpA</i> , Tryptophan synthase alpha chain
SP1897	↑	<i>rafE</i> , Sugar ABC transporter, sugar-binding protein
SP1908	↑	<i>ssbB</i> , Single-strand DNA-binding protein
SP1941	↑	<i>cinA</i> , Competence nicotinamide-nucleotide amidase
SP1940	-	<i>recA</i> , Recombinase
SP1939	-	<i>dinF</i> , MATE efflux family protein
SP1937	-	<i>lytA</i> , Autolysin/N-acetylmuramoyl-L-alanine amidase
SP1981	-	<i>ccs50</i> , DNA recombination protein
SP1980	-	<i>cbf1</i> , 3'-5' exoribonuclease
SP2019	↑	ABC transporter ATP-binding protein
SP2018	-	Transposase
SP2017	-	Membrane protein
SP2016	-	<i>nadC</i> , Nicotinate-nucleotide pyrophosphorylase
SP2015	-	Transposase
SP2014	-	Transposase
SP2013	-	Hypothetical protein
SP2053	↑	<i>cgIA</i> , Late competence protein, DNA uptake
SP2052	↑	<i>cgIB</i> , Late competence protein, DNA uptake
SP2051	↑	<i>cgIC</i> , Late competence protein, DNA uptake
SP2050	↑	<i>cgID</i> , Late competence protein, DNA uptake
SP2049	↑	<i>cgIE</i> , Hypothetical protein
SP2048	↑	<i>comGF</i> , Late competence protein, DNA uptake
SP2047	↑	<i>comGG</i> , Hypothetical protein
SP2046	↑	Pseudogene
SP2045	↑	DNA-methyltransferase
SP2201	↑	<i>cbpD</i> , Choline binding protein
SP2200	-	Hypothetical protein
SP2199	-	Hypothetical protein
SP2198	-	ABC transporter permease protein
SP2197	-	ABC transporter substrate-binding protein
SP2196	-	ABC transporter ATP-binding protein
SP2208	↑	<i>cflA</i> , Late competence protein, helicase
SP2207	↑	<i>cflB</i> , Late competence protein, DNA uptake
SP2206	-	<i>yfiA</i> , Ribosomal subunit interface protein

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26 <sup>a</sup>In a previous study performed by Peterson *et al.* (S.N. Peterson, C.K. Sung, R. Cline, B.V.  
27 Desai, E.C. Snesrud, Luo P, J. Walling, H. Li, M. Mintz, G. Tsegaye, P.C. Burr, Y. Do, S. Ahn, J.  
28 Gilbert, R.D. Fleischmann, D.A. Morrison. *Mol Microbiol* 51:1051-70, 2004), upregulated genes  
29 were detected by microarray in *S. pneumoniae* TIGR4 after CSP induction. These genes were  
30 annotated as early, delayed, or late competence genes.

31 <sup>b</sup>From our study, genes that were significantly upregulated (↑), downregulated (↓), or not  
32 regulated (-) in  $\Delta pde1\Delta pde2$  strain compared to WT are shown.

33 **Table S3 Transformation efficiencies of WT and  $\Delta trkH$  strains with a modified**  
 34 **transformation protocol<sup>a</sup>.**

[KCl] (mM)	WT			$\Delta trkH$		
	Input	Output	Efficiency <sup>b</sup>	Input	Output	Efficiency
<b>0</b>	$4.0 \times 10^7$	UD	$< 2.5 \times 10^{-8}$	$2.3 \times 10^7$	UD	$< 4.3 \times 10^{-8}$
<b>1</b>	$8.1 \times 10^7$	$5.6 \times 10^3$	$6.9 \times 10^{-5}$	$2.5 \times 10^7$	UD	$< 4.0 \times 10^{-8}$
<b>10</b>	$6.8 \times 10^7$	$6.8 \times 10^3$	$1.0 \times 10^{-4}$	$6.2 \times 10^7$	$3.3 \times 10^3$	$5.3 \times 10^{-5}$

35 <sup>a</sup>Pneumococcal stocks were inoculated in THY media instead of THYI. Bacteria were washed in  
 36 K<sup>+</sup>-free CDM and then transformed with plasmid pVA838 in CDM with indicated concentration of  
 37 KCl as described in Methods.

38 <sup>b</sup>Transformation efficiency for each individual experiment was calculated as output/input. The  
 39 values shown in the table are the averages of three independent biological replicates. UD,  
 40 Under the level of detection limit. In experiments that yielded no transformants, the efficiency  
 41 limits were calculated using the output as one.

42 **Table S4 Bacterial strains and plasmids used in this study.**

Strain	Description <sup>a</sup>	Source
<i>S. pneumoniae</i>		
D39	<i>S. pneumoniae</i> serotype 2	(1)
ST581	D39 derivative; Strep <sup>r</sup>	(2)
ST2729	ST581 $\Delta pde1$ ; Strep <sup>r</sup>	(2)
ST2733	ST581 $\Delta pde2$ ; Strep <sup>r</sup>	(2)
ST2734	ST581 $\Delta pde1 \Delta pde2$ ; Strep <sup>r</sup>	(2)
ST3275	ST581 $\Delta comA::Janus$ ; Kan <sup>r</sup>	This study
ST3293	ST581 $\Delta bgaA::cdaA^+$ Janus (pST3286); Kan <sup>r</sup>	This study
ST3313	ST3293 $\Delta bgaA::cdaA^+$ ; Strep <sup>r</sup>	This study
ST3334	ST3313 $\Delta cdaA::Janus$ ; Kan <sup>r</sup>	This study
ST3405	ST3334 <i>cdaA*</i> ; Strep <sup>r</sup>	This study
ST3406	ST3405 $\Delta bgaA::Janus$ ; Kan <sup>r</sup>	This study
ST3409	ST3406 <i>bgaA</i> ; Strep <sup>r</sup>	This study
ST3444	ST2734 <i>cdaA-32</i> ; Strep <sup>r</sup>	(3)
ST3466	ST3409 $\Delta bgaA::Janus$ ; Kan <sup>r</sup>	This study
ST3467	ST3409 $\Delta bgaA::cdaA^+$ Janus (pST3286); Kan <sup>r</sup>	This study
ST3478	ST581 $\Delta comD::Janus$ ; Kan <sup>r</sup>	This study
ST3479	ST3409 $\Delta comD::Janus$ ; Kan <sup>r</sup>	This study
ST3480	ST3409 $\Delta comA::Janus$ ; Kan <sup>r</sup>	This study
ST3519	ST3409 $\Delta trkH \Delta cabP::Janus$ ; Kan <sup>r</sup>	This study
ST3520	ST3409 $\Delta trkA \Delta trkG::Janus$ ; Kan <sup>r</sup>	This study
HSR1	ST2734 <i>cdaA*</i> (suppressor); Strep <sup>r</sup>	(3)
<i>E. coli</i>		
DH5 $\alpha$	<i>E. coli</i> strain used for cloning	Laboratory stock
<b>Plasmid</b>		
<i>Transformation efficiency</i>		
pVA838	<i>S. pneumoniae</i> expression vector; Erm <sup>r</sup>	(4)
<i>Constructing cdaA*</i>		
pBluescript SK (-)	Vector used for cloning; Ap <sup>r</sup>	Stratagene
pUC19	Vector used for cloning; Ap <sup>r</sup>	NEB
pST3167	pBluescript + upstream <i>bgaA</i> ; Ap <sup>r</sup>	This study
pST3172	pST3167 + downstream <i>bgaA</i> ; Ap <sup>r</sup>	This study
pST3222	pST3172 + Janus; Ap <sup>r</sup> Kan <sup>r</sup>	This study
pST3277	pUC19 + pBluescript <i>bgaA</i> + Janus; Ap <sup>r</sup> Kan <sup>r</sup>	This study
pST3286	pST3277 + <i>cdaA</i> ; Ap <sup>r</sup> Kan <sup>r</sup>	This study
<i>Promoter-reporter</i>		
pST1556	pPP2; Ap <sup>r</sup>	(5)
pST2868	pPP2-erm; Ap <sup>r</sup> Erm <sup>r</sup>	(3)
pST2885	pPP2-erm: P' <i>gyrA</i> ; Ap <sup>r</sup> Erm <sup>r</sup>	(3)
pST3302	pPP2-erm: P' <i>ssbB</i> ; Ap <sup>r</sup> Erm <sup>r</sup>	This study

43 <sup>a</sup>Ap<sup>r</sup>, ampicillin resistance; Erm<sup>r</sup>, erythromycin resistance; Kan<sup>r</sup>, kanamycin resistance; Strep<sup>r</sup>,

44 streptomycin resistance.

45 Table S5 Primers used in this study\*.

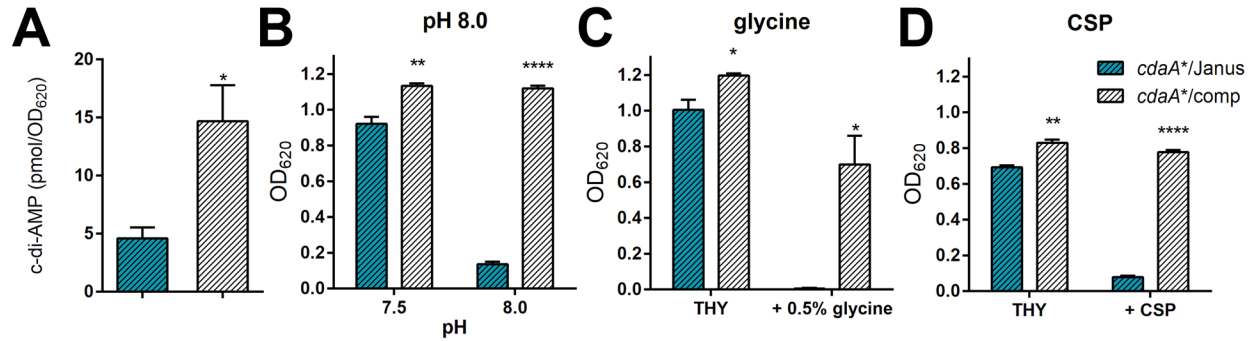
Primer	Sequence 5'→3'	Restriction site	Location*	Application
Pr1097	gagatctagaaccgtttgatttttaatggataatg	XbaI	F Janus	Generate mutant
Pr1098	gagactcgagcctttccttatgcttttgac	XhoI	R Janus	Generate mutant
Pr2084	ttgaaattatggatactgagatgc	-	up <i>comA</i>	Generate mutant
Pr2085	aactctagactaatgaagctacaccgcagtc	XbaI	up <i>comA</i>	Generate mutant
Pr2086	atcgctcgagggtcaatagctagaaagaggagag	XhoI	down <i>comA</i>	Generate mutant
Pr2087	gaagcaccatcctcact	-	down <i>comA</i>	Generate mutant
Pr2874	tgacgataatctgtgacgg	-	up <i>cdaA</i>	Generate <i>cdaA</i> *
Pr2877	ccctgaaatacgtgtgtcac	-	down <i>cdaA</i>	Generate <i>cdaA</i> *
Pr2879	tttgatccacaagcaaaaagagtgagg	BamHI	R <i>cdaA</i>	Generate <i>cdaA</i> *
Pr2973	tttctgacctgagaccgctcat	-	down <i>cabP</i>	Generate mutant
Pr2976	tttgatgaggagccagttacca	-	up <i>trkH</i>	Generate mutant
Pr2994	ggaacctggtgatagcc	-	up <i>trkA</i>	Generate mutant
Pr3001	cattctgtagagcgtatgatagac	-	down <i>trkG</i>	Generate mutant
Pr3181	gcagggtttctcctctacgc	-	<i>comGG</i>	qRT-PCR
Pr3182	tccccactttcttgctcaac	-	<i>comGG</i>	qRT-PCR
Pr3183	ggcagcaggcttgagtac	-	<i>comFC</i>	qRT-PCR
Pr3184	cggcttctccaacagtttc	-	<i>comFC</i>	qRT-PCR
Pr3187	ccggggtgaagttgactaaa	-	<i>comA</i>	qRT-PCR
Pr3188	agccacgctgcttactaat	-	<i>comA</i>	qRT-PCR
Pr3189	acagagcaagcagacagcaa	-	<i>comEA</i>	qRT-PCR
Pr3190	tgtcctgagctcgtttcct	-	<i>comEA</i>	qRT-PCR
Pr3195	tgccaagcgtactacgacag	-	<i>radA</i>	qRT-PCR
Pr3196	tctctctgtcaagcccagt	-	<i>radA</i>	qRT-PCR
Pr3197	ggacaacgtttcggtgagat	-	<i>rpoB</i>	qRT-PCR
Pr3198	tcggaaggattctggaacac	-	<i>rpoB</i>	qRT-PCR
Pr3199	gttcgcaattcctgcaaat	-	<i>htrA</i>	qRT-PCR
Pr3200	ccgaacgaacaattacacca	-	<i>htrA</i>	qRT-PCR
Pr3332	ttgggtaccaaattcaatatcgggtctgac	KpnI	up <i>bgaA</i>	Generate integrative plasmid
Pr3333	ggtctcgagcattgagtttatatttatagg	XhoI	up <i>bgaA</i>	Generate integrative

				plasmid
Pr3334	ttgcggccggttgatttttaatggataatg	EagI	F Janus	Generate integrative plasmid
Pr3335	gggccgcccccttccttatgcttttgac	SacII	R Janus	Generate integrative plasmid
Pr3336	agtcgcggttagataagactgacg	SacI	down <i>bgaA</i>	Generate integrative plasmid
Pr3337	ccggagctcctaggttcactttgttctg	SacII	down <i>bgaA</i>	Generate integrative plasmid
Pr3342	cttcaattgatgtcagatcgtagattgg	Sall	F P' <i>cdaA</i>	Generate <i>cdaA</i> *
Pr3404	ccggcatgcctaggttcactttgttctg	SphI	down <i>bgaA</i>	Generate integrative plasmid
Pr3421	ctcaggatattgcagacac	EcoRI	F P' <i>ssbB</i>	Promoter-reporter
Pr3422	ctggtgtagacgttaaacgc	BamHI	R P' <i>ssbB</i>	Promoter-reporter
Pr3434	ttccgcggaagcaaaaaagagtgagg	SacII	R <i>cdaA</i>	Generate <i>cdaA</i> *
Pr3470	cattatccattaaaaatcaaacggttgatagctatcgccatgg	-	F Janus- R up <i>cdaA</i>	Generate <i>cdaA</i> *
Pr3471	ccatggacgatagctatcaaccggttgatttttaatggataatg	-	R up <i>cdaA</i> -F Janus	Generate <i>cdaA</i> *
Pr3472	tcctagcaatcgctctttaaagagccttccttatgcttttgac	-	F down <i>cdaA</i> -R Janus	Generate <i>cdaA</i> *
Pr3473	gtccaaaagcataaggaaaggctctttaaagaacgattgctagga	-	R Janus- F down <i>cdaA</i>	Generate <i>cdaA</i> *
Pr3529	tgggggaagtttaggattgtc	-	up <i>comD</i>	Generate mutant
Pr3530	tggctagatccaataaatccattactcttcc	XbaI	up <i>comD</i>	Generate mutant
Pr3531	tggctcgagttaagaggaattgaaag	XhoI	down <i>comD</i>	Generate mutant
Pr3532	ccaaaaatgacttgtaggaga	-	down <i>comD</i>	Generate mutant

46 \*F, forward primer of the ORF; R, reverse primer of the ORF; up, upstream arm of the gene;

47 down, downstream arm of the gene.

48



49

50 **FIG S1 Complementation of the *cdaA\** strain by introduction of native *cdaA* at the *bgaA***

51 **locus.** The low c-di-AMP-producing strain, *cdaA\**, was transformed with integrative plasmids as

52 described in Methods to introduce either Janus cassette (*cdaA\*/Janus*) or with the native *cdaA*

53 (complemented, *cdaA\*/comp*) at the *bgaA* locus. (A) Determination of c-di-AMP levels in

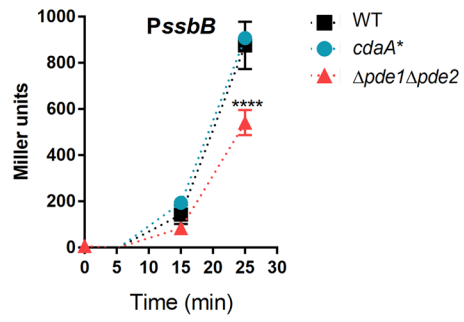
54 *cdaA\*/Janus* and *cdaA\*/comp*. These strains were assayed for growth in THY (pH 7.5) or THY

55 adjusted to pH 8.0 (B), with the addition of 0.5% glycine (C), or with the addition of 375 ng·mL<sup>-1</sup>

56 CSP (D). Bacterial growth was measured at OD<sub>620</sub> after 10 h. Data shown are the means and

57 SEMs from three independent replicate experiments.

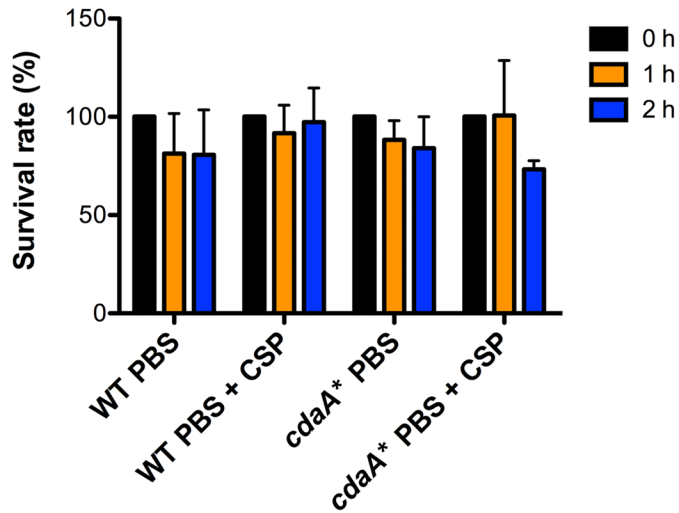
58



59

60 **FIG S2 Initiation of the competence state in WT,  $\Delta pde1\Delta pde2$ , and *cdaA\**.** Promoter activity  
61 of *ssbB* was monitored at 0, 5, 15, and 25 min after CSP addition. Data shown are the means  
62 and SEMs from three independent replicate experiments.

63



64

65 **FIG S3 Incubation of *cdaA\** in PBS in the presence or absence of 375 ng·mL<sup>-1</sup> CSP.** WT  
 66 and *cdaA\** were grown in THY an OD<sub>620</sub> of 0.05. Bacteria in 200 μL culture were harvested and  
 67 resuspended in 200 μL PBS either in the presence or absence of 375 ng·mL<sup>-1</sup> CSP followed by  
 68 incubation at 37°C. At 0, 1, and 2 h of incubation, dilutions were prepared and spread on TSA  
 69 blood agar plates to determined CFU. The survival rate at 0 h was set as 100%, which is used  
 70 to normalize the rate after 1 and 2 h. Data shown are the means and SEMs from three  
 71 independent replicate experiments.