

## SUPPLEMENTARY MATERIAL

### **Reciprocal control of motility and biofilm formation by the PdhS2**

#### **two-component sensor kinase of *Agrobacterium tumefaciens***

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Running title: *Agrobacterium PdhS2 regulates motility and biofilms*

1) Supplementary Figures – S1-S8

2) Supplementary Figure Legends

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**Figure S1. A combined kinase- and phosphatase-null PdhS2 mutant allele has little effect on biofilm formation or swimming motility.** The ability of plasmid-borne expression of a kinase- and phosphatase- null allele of *pdhS2* (*p-pdhS2* (K<sup>-</sup>P<sup>-</sup>)) to complement the  $\Delta pdhS2$  phenotypes was compared against the wild-type *pdhS2* allele (*p-pdhS2*). Biofilm formation (black bars) and swimming motility (white bars) were evaluated as in Figure 2. (a) =  $P < 0.05$  compared to the wild-type background with vector only; (b) =  $P < 0.05$  compared to the  $\Delta pdhS2$  background with vector only. Statistical significance was determined using Student's *t* test.

**Figure S2. Morphology of WT,  $\Delta divK$ ,  $\Delta pdhS2$ , and  $\Delta divK \Delta pdhS2$  strains.** Strains were grown to exponential phase in ATGN. Aliquots of cells were placed on top of an ATGN/1% agarose pad and imaged using phase contrast microscopy. (A), WT; (B)  $\Delta divK$ ; (C)  $\Delta pdhS2$ ; (D)  $\Delta divK \Delta pdhS2$ . Representative images are shown. Scale bar = 10  $\mu$ m.

**Figure S3. PleD regulation of swimming motility is epistatic to PleC.** Swimming motility of the wild-type (WT) and indicated mutant strains was evaluated as described in Figure 2. (\*)  $P < 0.05$  compared to all strains.

**Figure S4. Predicted CtrA-dependent promoters bearing one or more CtrA binding motifs.** Promoter regions from select genes whose expression is upregulated (red) or downregulated (black) in the  $\Delta pdhS2$  mutant background relative to the wild-type background. Predicted CtrA binding sites, as defined in the main text, are

indicated. Only those genes with a predicted full CtrA binding site are shown.

Expression levels were determined either via microarray expression profiling or beta-galactosidase translational reporters (Tables 1 and 2 in the main text).

**Figure S5. PdhS2 regulation of swimming motility is independent of diguanylate cyclase activity.** Swimming motility of the wild-type (WT) and indicated mutant strains was evaluated as described in Figure 2.  $P < 0.05$  compared to WT <sup>(a)</sup>,  $\Delta pdhS2$  <sup>(b)</sup>, or corresponding diguanylate cyclase mutant <sup>(c)</sup>.

**Figure S6. PdhS2 does not affect global levels of cyclic-di-GMP.** Cyclic-di-GMP levels were measured in whole cell extracts from equivalent ODs of the indicated strains. Data are from three independent experiments (N = 3).

**Figure S7. A catalytically inactive DgcB modestly affects swimming motility.** The effect on swimming motility of plasmid-borne expression of wild-type *dgcB* (p-*dgcB*) or a catalytic mutant allele of *dgcB* (p-*dgcB*<sup>\*</sup>) was evaluated. Expression of each *dgcB* allele was driven by the  $P_{lac}$  promoter. Swimming motility was evaluated as described in Figure 2. (\*) =  $P < 0.05$  compared to vector alone.

**Figure S8. PdhS2 and DivJ are polarly localized in *A. tumefaciens*.** Time-lapse microscopy of a C-terminal green fluorescent protein fusion to PdhS2 (A) and DivJ (B). Overlaid phase and fluorescent images, acquired sequentially on Nikon E800

fluorescence microscope with a CCD camera using the 100 X objective. Time between panels is 40 minutes. To the right of each image is a cartoon interpretation of the image.

**Table S1. Strains used in this study**

<b>Species</b>	<b>Strain</b>	<b>Relevant Characteristics</b>	<b>Source</b>
<i>A. tumefaciens</i>	C58	Nopaline type strain, pAtC58, pTiC58	[1]
<i>A. tumefaciens</i>	C58-JE001	$\Delta dgcB \Delta pdhS2 \Delta pleD$ ( $\Delta Atu1691 \Delta Atu1888 \Delta Atu1297$ )	This study
<i>A. tumefaciens</i>	C58-JE002	$\Delta dgcB \Delta pleD$ ( $\Delta Atu1691 \Delta Atu1297$ )	This study
<i>A. tumefaciens</i>	C58-JEH076	$\Delta pdhS2$ ( $\Delta Atu1888$ )	[2]
<i>A. tumefaciens</i>	C58-JEH128	$\Delta pdhS2 \Delta pleD$ ( $\Delta Atu1888 \Delta Atu1297$ )	This study
<i>A. tumefaciens</i>	C58-JEH130	$\Delta pdhS2 \Delta dgcA$ ( $\Delta Atu1888 \Delta Atu1257$ )	This study
<i>A. tumefaciens</i>	C58-JEH131	$\Delta pdhS2 \Delta dgcB$ ( $\Delta Atu1888 \Delta Atu1691$ )	This study
<i>A. tumefaciens</i>	C58-JEH132	$\Delta pdhS2 \Delta dgcC$ ( $\Delta Atu1888 \Delta Atu2179$ )	This study
<i>A. tumefaciens</i>	C58-JEH145	$\Delta pdhS2 \Delta pleC$ ( $\Delta Atu1888 \Delta Atu0982$ )	This study
<i>A. tumefaciens</i>	C58-JEH146	$\Delta pdhS2 \Delta crdS \Delta chvAB \Delta cel \Delta upp \Delta exoA$ ( $\Delta Atu1888 \Delta Atu3055-3057 \Delta Atu2728-2730 \Delta Atu3302-8187 \Delta Atu1235-1240 \Delta Atu4053$ )	This study

<i>A. tumefaciens</i>	C58-JEH147	$\Delta pdhS2 \Delta upp$ ( $\Delta Atu1888 \Delta Atu1235-1240$ )	This study
<i>A. tumefaciens</i>	C58-JEH148	$\Delta pdhS2 \Delta cel$ ( $\Delta Atu1888 \Delta Atu3302-8187$ )	This study
<i>A. tumefaciens</i>	C58-JEH149	$\Delta pdhS2 \Delta crdS$ ( $\Delta Atu1888 \Delta Atu3055-3057$ )	This study
<i>A. tumefaciens</i>	C58-JEH150	$\Delta pdhS2 \Delta chvAB$ ( $\Delta Atu1888 \Delta Atu2728-Atu2730$ )	This study
<i>A. tumefaciens</i>	C58-JEH151	$\Delta pdhS2 \Delta exoA$ ( $\Delta Atu1888 \Delta Atu4053$ )	This study
<i>A. tumefaciens</i>	C58-JEH153	$\Delta divK \Delta pdhS2$ ( $\Delta Atu1296 \Delta Atu1888$ )	This study
<i>A. tumefaciens</i>	C58-JW2	$\Delta pleC$ ( $\Delta Atu0982$ )	[2]
<i>A. tumefaciens</i>	C58-JW7	$\Delta divK$ ( $\Delta Atu1296$ )	[2]
<i>A. tumefaciens</i>	C58-JW8	$\Delta pleD$ ( $\Delta Atu1297$ )	[2]
<i>A. tumefaciens</i>	C58-JX100	$\Delta crdS$ ( $\Delta Atu3055-3057$ )	[3]
<i>A. tumefaciens</i>	C58-JX101	$\Delta chvAB$ ( $\Delta Atu2728-2730$ )	[3]
<i>A. tumefaciens</i>	C58-JX102	$\Delta cel$ ( $\Delta Atu3302-8187$ )	[3]
<i>A. tumefaciens</i>	C58-JX111	$\Delta crdS \Delta chvAB \Delta cel \Delta upp \Delta exoA$ ( $\Delta Atu3055-3057 \Delta Atu2728-2730$ )	[3]

		$\Delta$ Atu3302-8187 $\Delta$ Atu1235-1240 $\Delta$ Atu4053; "EPS-")	
<i>A. tumefaciens</i>	C58-JX125	$\Delta$ dgcA ( $\Delta$ Atu1257)	[4]
<i>A. tumefaciens</i>	C58-JX187	$\Delta$ dgcB ( $\Delta$ Atu1691)	[4]
<i>A. tumefaciens</i>	C58-MLL2 A	$\Delta$ exoA ( $\Delta$ Atu4053)	[5]
<i>A. tumefaciens</i>	C58-PMM26	$\Delta$ upp ( $\Delta$ Atu1235-1240)	[3]
<i>A. tumefaciens</i>	C58-YW010	$\Delta$ dgcC ( $\Delta$ Atu2179)	[4]
<i>E. coli</i>	S17-1 $\lambda$ pir	RK2 <i>tra</i> regulon, <i>pir</i> , host for <i>pir</i> -dependent plasmids	[6]
<i>E. coli</i>	TOP10 F'	F' <i>lacI</i> <sup>q</sup> Tn10 (Tet <sup>R</sup> ) <i>mcrA</i> $\Delta$ ( <i>mrr</i> - <i>hsdRMS</i> - <i>mcrBC</i> ) $\Phi$ 80 <i>lacZ</i> $\Delta$ M15 $\Delta$ <i>lacX74</i> <i>recA1</i> <i>araD139</i> $\Delta$ ( <i>ara-leu</i> )7697 <i>galU</i> <i>rpsL</i> <i>endA1</i> <i>nupG</i>	Thermo Fisher Scientific

**Table S2. Plasmids used in this study**

<b>Plasmid name</b>	<b>Relevant characteristics</b>	<b>Source</b>
pGEM-T Easy	PCR cloning vector; Amp <sup>R</sup>	Promega
<i>placZ/290</i>	Broad host range plasmid carrying promoterless <i>lacZ</i> for transcriptional fusions; Tet <sup>R</sup>	[7]
pNPTS138	ColE1 origin; <i>sacB</i> ; Km <sup>R</sup>	gift of M. Alley
pRA301	Broad host range plasmid carrying promoterless <i>lacZ</i> for translational fusions; Spec <sup>R</sup>	[8]
pSRKGm	Broad host range vector containing P <sub><i>lac</i></sub> ; <i>lacI<sup>q</sup></i> ; <i>lacZα<sup>+</sup></i> ; Gm <sup>R</sup>	[9]
<i>pctrA290</i>	<i>placZ/290</i> derivative with <i>C. crescentus ctrA</i> promoter	[10]
pDC001	pGEM-T Easy with full-length <i>pdhS2</i> <sup>(CA811-812GC, A823G)</sup> ( <i>PdhS2</i> <sup>His271A,Thr275Ala</sup> mutant)	This study



pDC002	pSRKGm with full-length <i>pdhS2</i> <sup>(CA811-812GC, A823G)</sup> (PdhS2 <sup>His271A,Thr275Ala</sup> mutant)	This study
pGZ22	<i>lacZ</i> /290 derivative with <i>C. crescentus ccrM</i> promoter	[11]
pJEH010	pSRKGm with full-length wild-type <i>cckA</i>	[2]
pJEH021	pGEM-T Easy with full-length <i>pdhS2</i>	[2]
pJEH026	pSRKGm with full-length <i>pdhS2</i>	[2]
pJEH030	pSRKGm with full-length Y674D <i>cckA</i> allele	[2]
pJEH040	pNPTS138 derivative with <i>pdhS2</i> SOE deletion fragment	[2]
pJEH052	pGEM-T Easy with <i>pdhS2</i> lacking a stop codon	This study
pJEH053	pGEM-T Easy with <i>gfpmut3</i>	This study

pJEH054	pGEM-T Easy with <i>divJ</i> lacking a stop codon	This study
pJEH060	pSRKGm with a <i>pdhS2::gfpmut3</i> translational fusion	This study
pJEH078	pSRKGm with a <i>divJ::gfpmut3</i> translational fusion	This study
pJEH091	pGEM-T Easy with full-length <i>pdhS2</i> <sup>(CA811-812GC)</sup> (PdhS2 <sup>His271Ala</sup> mutant)	This study
pJEH092	pSRKGm with full-length <i>pdhS2</i> <sup>(CA811-812GC)</sup> (PdhS2 <sup>His271Ala</sup> mutant)	This study
pJEH099	pGEM-T Easy with full-length <i>pdhS2</i> <sup>(A823G)</sup> (PdhS2 <sup>Thr275Ala</sup> mutant)	This study
pJEH102	pSRKGm with full-length <i>pdhS2</i> <sup>(A823G)</sup> (PdhS2 <sup>Thr275Ala</sup> mutant)	This study
pJEH113	pGEM-T Easy with A. <i>tumefaciens ccrM</i> promoter	This study

pJEH115	pGEM-T Easy with <i>A. tumefaciens ctrA</i> promoter	This study
pJEH119	pGEM-T Easy with <i>A. tumefaciens pdhS1</i> promoter	This study
pJEH121	pRA301 with <i>A. tumefaciens ccrM</i> promoter	This study
pJEH122	pRA301 with <i>A. tumefaciens ctrA</i> promoter	This study
pJEH124	pRA301 with <i>A. tumefaciens pdhS1</i> promoter	This study
pJEH141	pRA301 with 5'-TTAA-3' → 5'-AATT-3' mutation of <i>A. tumefaciens dgcB</i> promoter	This study
pJFP006	pRA301 with 5'-TTAA-3' → 5'-AATT-3' mutation of <i>A. tumefaciens</i> Atu3318 promoter	This study

pJW109	pNPTS138 derivative with <i>pleD</i> SOE deletion fragment	[2]
pJS70	<i>placZ</i> /290 derivative with <i>C. crescentus pilA</i> promoter	[12]
pJX158	pRA301 with <i>A.</i> <i>tumefaciens</i> Atu3318 promoter	[4]
pJX162	pRA301 with <i>A.</i> <i>tumefaciens dgcb</i> promoter	[4]
pJX520	pSRKGm with full-length <i>dgcb</i>	[4]
pJX521	pSRKGm with full-length <i>dgcb</i> <sup>A767C, A770C</sup> ( <i>DgcB</i> <sup>EE256- 257AA</sup> mutant)	[4]
pJX802	pNPTS138 derivative with <i>dgcb</i> SOE deletion fragment	[4]
pJZ383	pPZP201 derivative with <i>P<sub>tac</sub>::gfpmut3</i> ; Spec <sup>R</sup>	[13]

**Table S3. Primers used in this study**

Primer	Sequence (5' – 3')	Use
JEH65	GAAGA <u>ACATATGAGT</u> AAAAGCGTCAGCA	cloning <i>pdhS2</i> with NdeI site
JEH85	GATTCGCGCGATCCCTTCGA	Internal primer for <i>pdhS2</i> locus
JEH87	GAGCAGATGCTGGCCGGA	Internal primer for <i>pdhS2</i> locus
JEH100	GCTCTGTTGAAGGCGGCCAA	External primer for <i>pdhS2</i> locus
JEH113	GCCGGTTTCATGCACACGCA	External primer for <i>pdhS2</i> locus
JEH146	GAAGAAGCTAGCGGCGAAAGACCGCGG	cloning <i>pdhS2</i> w/o STOP and with NheI site
JEH147	GAAGA <u>ACATATGAGAG</u> AAAAAGCGGTCGCA	cloning <i>divJ</i> with NdeI site
JEH148	GAAGAAGCTAGCGGCGATTTTCGCTTTCGCGG	cloning <i>divJ</i> w/o STOP and with NheI site
JEH149	GAAGAAGGTACCTTATTTGTATAGTTCATCCATGCCA	cloning <i>gfpmut3</i> with KpnI site
JEH150	GAAGAAGCTAGCATGAGTAAAGGAGAAGAACTT	cloning <i>gfpmut3</i> with NheI site
JEH245	CGTGCGCAGCTCGgcCGACATGGAA GCG	<i>pdhS2</i> <sup>CA811-812GC</sup> mutagenesis
JEH246	CGCTTCCATGTTCGgcCGAGCTGCGCACG	<i>pdhS2</i> <sup>CA811-812GC</sup> mutagenesis
JEH261	CGCACGAGCTGCGCgCGCCGCTCAACGC	<i>pdhS2</i> <sup>A823G</sup> mutagenesis
JEH262	GCGTTGAGCGGCGcGCGCAGCTCGTGCG	<i>pdhS2</i> <sup>A823G</sup> mutagenesis
JEH282	<u>GGTACCTGCCAGAATCGTTGCT</u>	cloning <i>ccrM</i> promoter region, +222 bp to -9 bp from translational start, with KpnI site
JEH284	<u>AAGCTTTGCTGCCATTGGTACT</u>	cloning <i>ccrM</i> promoter region, +222 bp to -9 bp from translational start, with HindIII site
JEH285	<u>GGTACCTTAACCTTTGTTTACGGGC</u> A	cloning <i>ctrA</i> promoter region, +328 bp to -9 bp from translational start, with KpnI site
JEH287	<u>CTGCAGA</u> ACCCGCATAATTATCCCCTTT	cloning <i>ctrA</i> promoter region, +328 bp to -9 bp from

		translational start, with PstI site
JEH291	<u>GGTACC</u> ATTTGCAAGTGCCTCTT	cloning <i>pdhS1</i> promoter region, +264 bp to -9 bp from translational start, with KpnI site
JEH293	<u>AAGCTT</u> GGCGGGCATGTCGAAA	cloning <i>pdhS1</i> promoter region, +264 bp to -9 bp from translational start, with HindIII site

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Figure S1

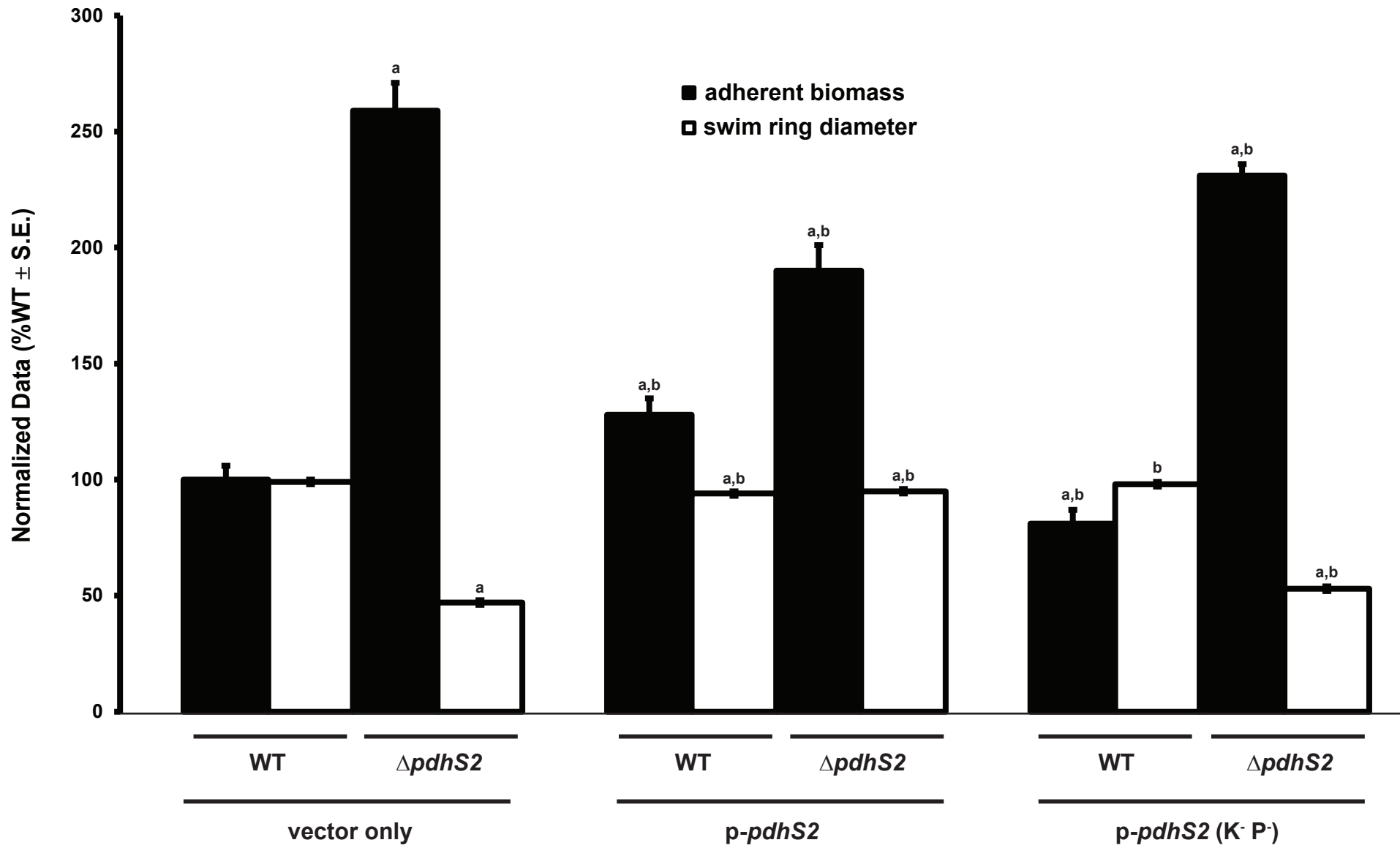


Figure S2

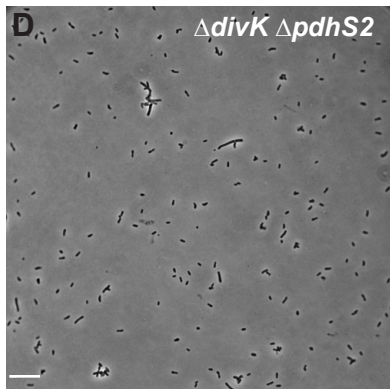
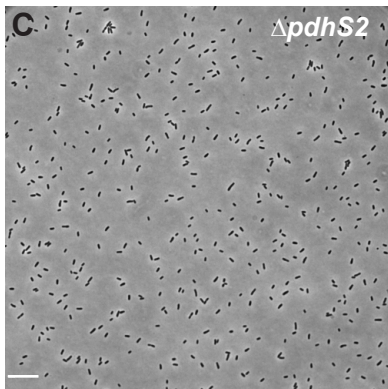
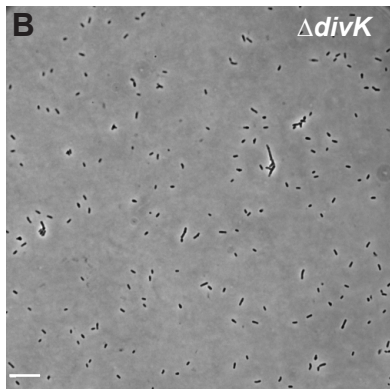
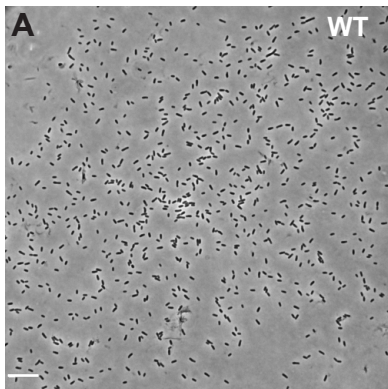


Figure S3

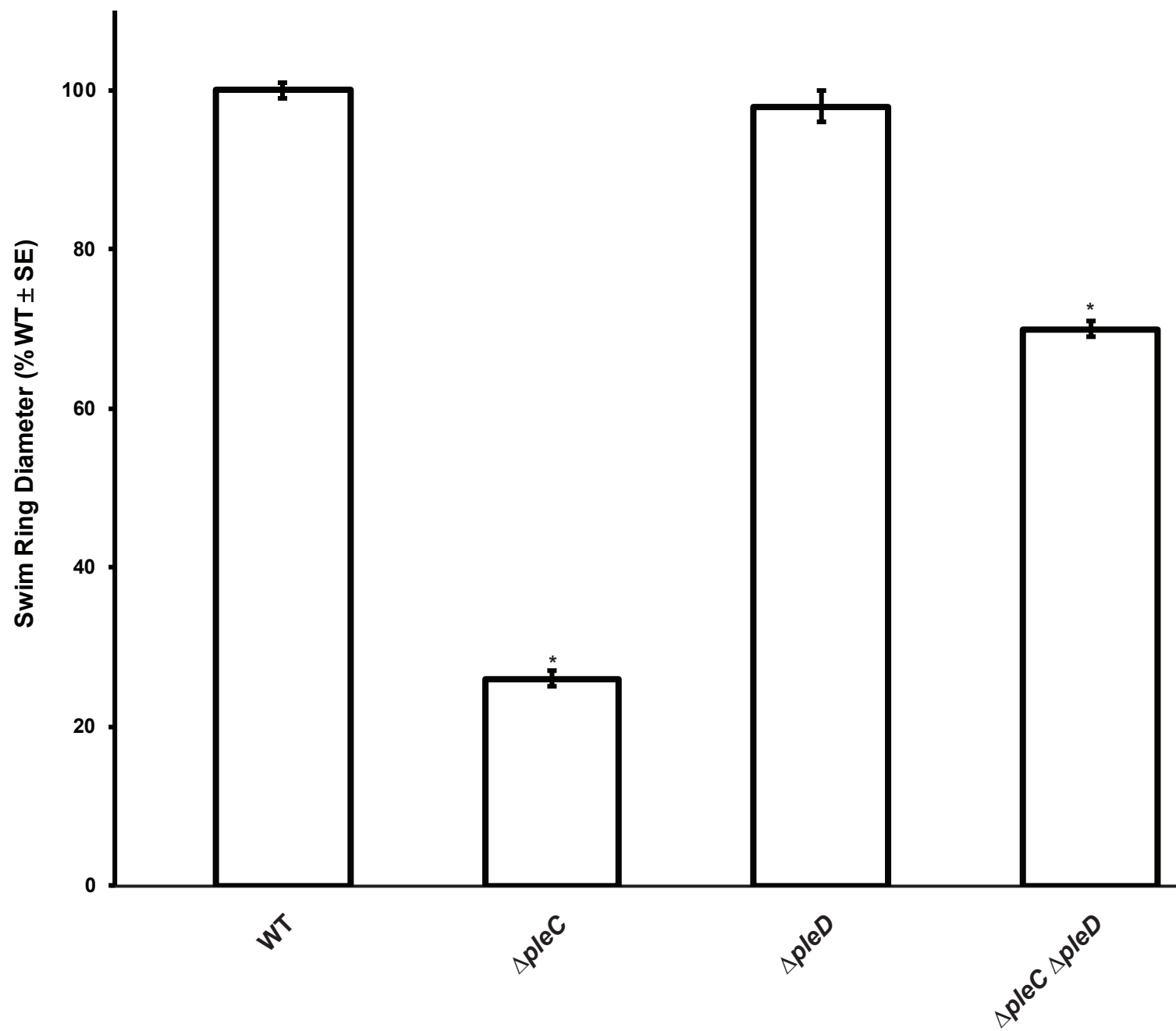


Figure S4

CtrA Binding Site Consensus

TTAANNNNNNNGTTAAC

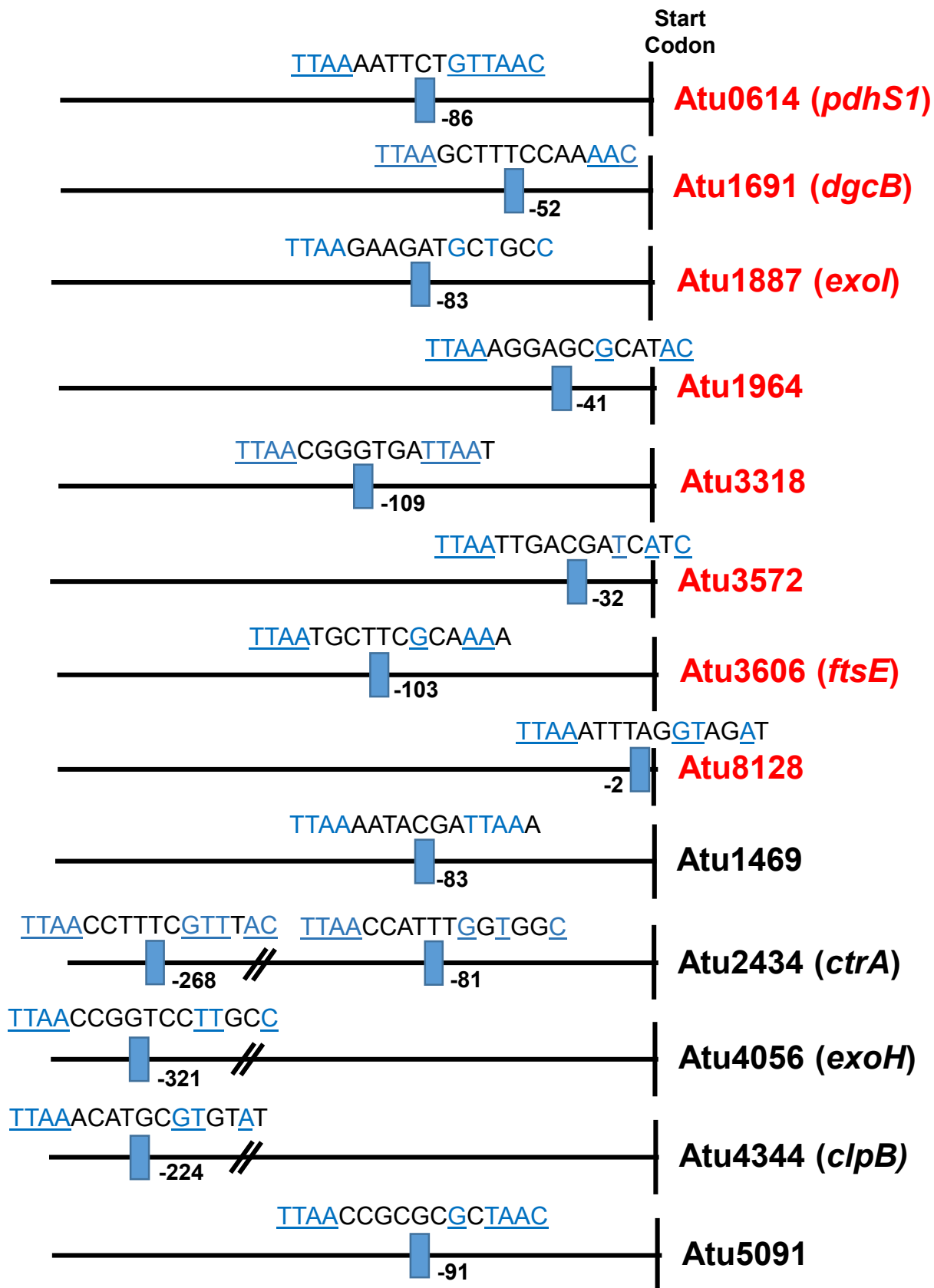


Figure S5

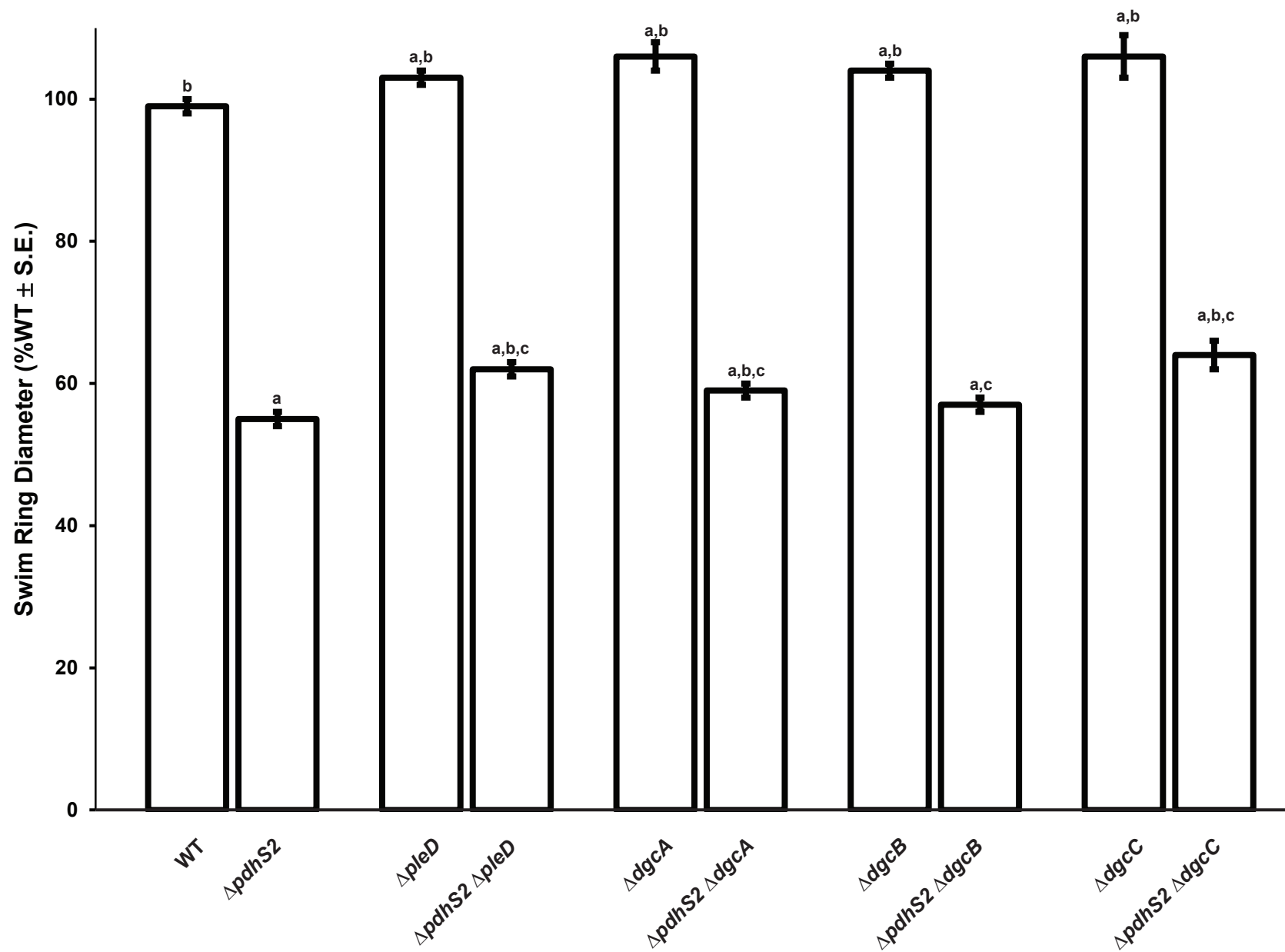


Figure S6

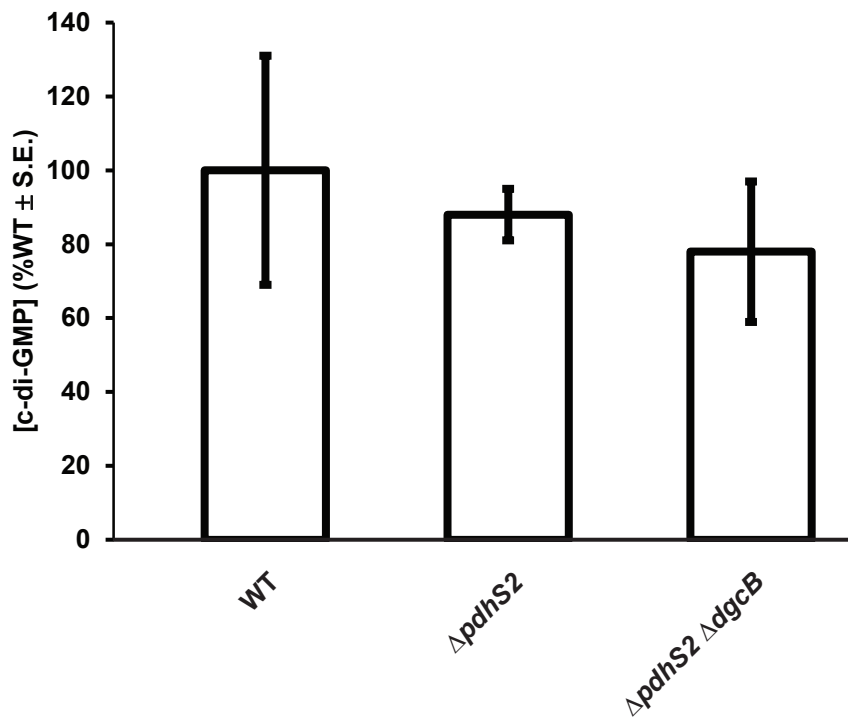


Figure S7

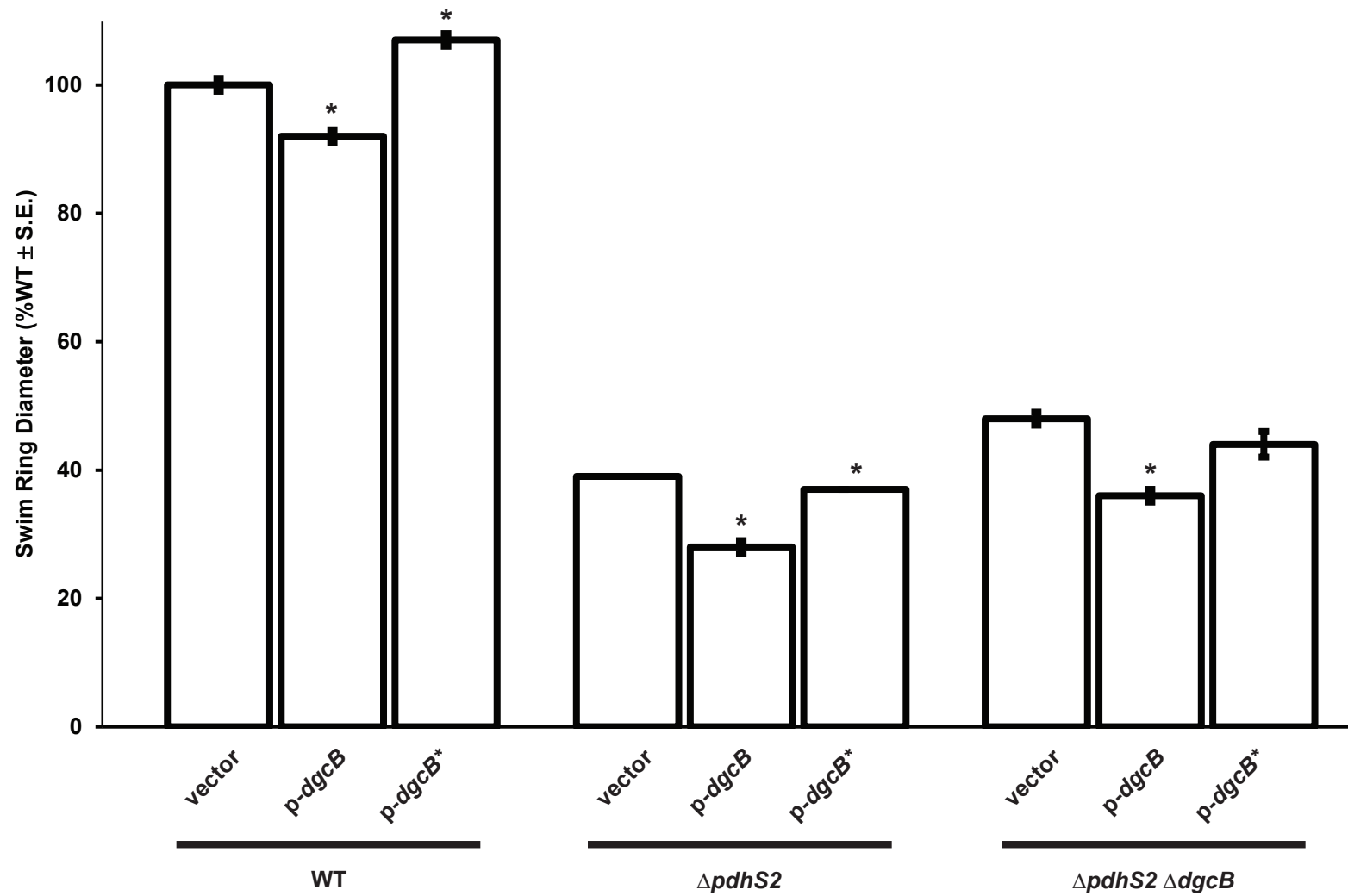
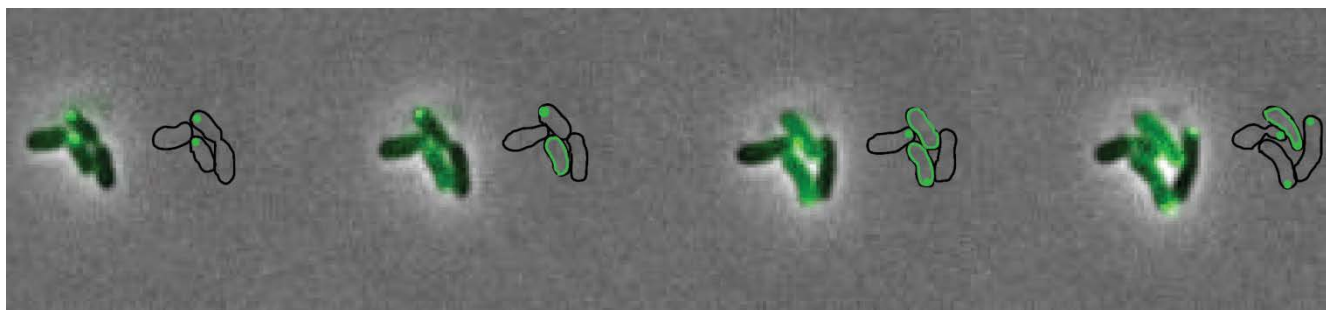


Figure S8

A



B

