

1 **Supplemental Text**

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3 **Carbon starvation induces the expression of PprB-regulated**  
4 **genes in *Pseudomonas aeruginosa***

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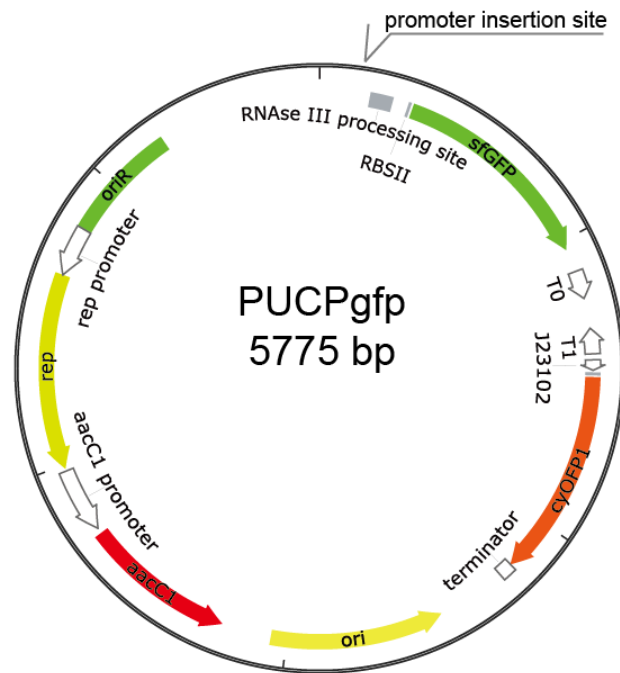
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23 **Growth curve measurement.** Overnight cultures of PAO1-pJN105 cells in FABSgen  
24 or pprBpJN-PAO1 cells in FABSgen + 0.02% L-arabinose were 100× diluted into the  
25 same media and were grown for 10 hours with shaking at 37 °C. OD<sub>600</sub> values were  
26 recorded every hour. Three parallel experiments were performed for each strain. Due  
27 to the enhanced cell-cell adhesion of PprB expressed cells, overnight culture of this  
28 strain contains lots of cell clusters at the bottom of the culture tube, this results in a  
29 relatively fewer cells in the supernatant compared to that in wild type. Thus, when we  
30 took a certain volume (10 μL) of overnight culture for extended culture and growth  
31 curve measurement, the initial cell number of PprB overexpressed strain is less. As the  
32 resolution of our absorption spectrophotometer is poor at OD<sub>600</sub><0.05, the resulting  
33 growth curve of PprB overexpression strain displayed a lag phase in the first three hours  
34 (Fig. S4).

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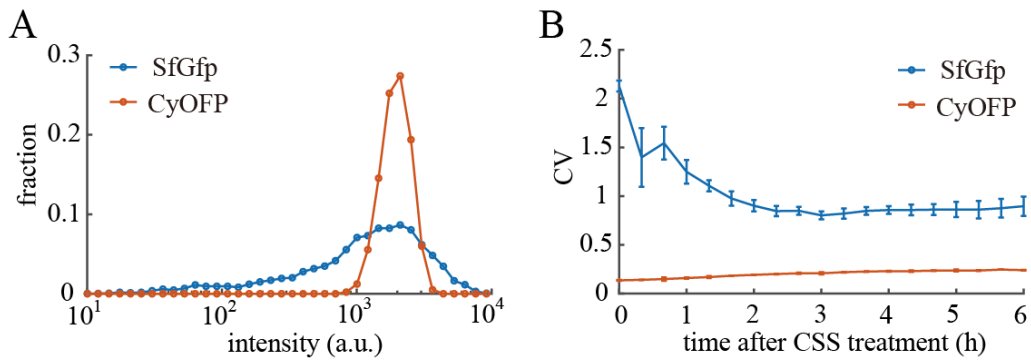


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38 **Figure S1: Functional map of plasmid PUCPgfp.** PUCPgfp is derived from PUCP20,

39 the promoter sequences were inserted before the RNase III processing site.

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42 **Figure S2: Heterogeneity of flp transcription among cells.** (A), Distribution of

43 SfGFP (expressed by *flp* promoter) and CyOFP (expressed by J23102 promoter)

44 fluorescence intensity among cells after 4-hour CSS treatment. B, Coefficient of

45 variations (CV) of the distribution of SfGFP and CyOFP fluorescence intensity after

46 CSS treatment, data are from three independent experiments and shown as the

47 mean  $\pm$  s.d.

48

*flp*  
-83 TGCAAAACGCCATCATCGGCCTAGCCTCAGCGTGGAGGCAGGGCTC

*cupE*  
-198 CAGAGGCTTATTCGCGCTGCTATATAGCCAGTCAAGCATGACTGGC

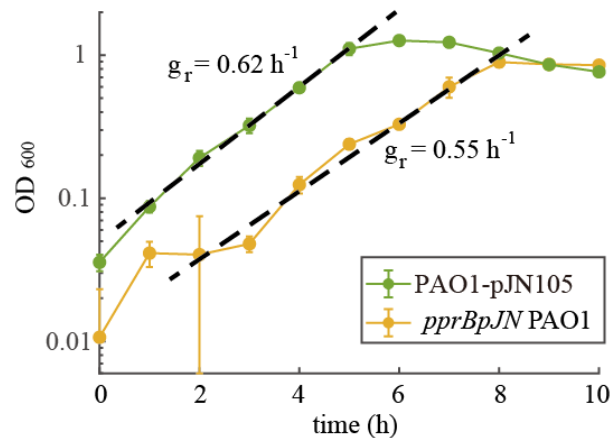
*rcpC*  
-280 GGCGGGTTACAAGGCTTGGACTACATCTAATGACAAATTGACATTGAT

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50 **Figure S3: Promoter regions of the *cupE*, *rcpC* and *flp* genes.** Putative RpoS binding sites

51 are framed.

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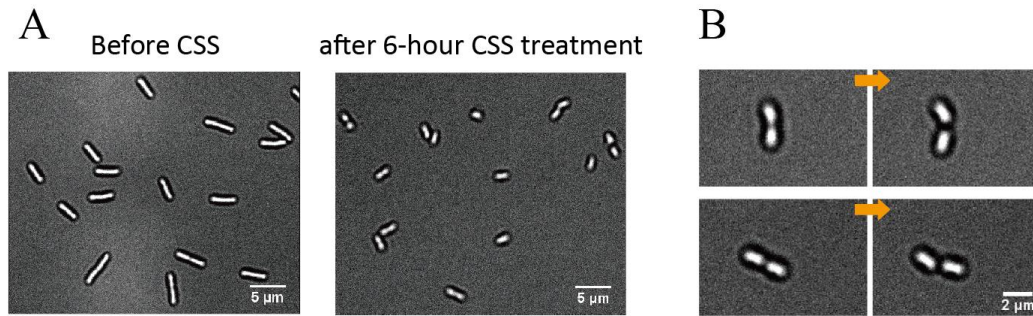
54 **Figure S4: Growth curves of wild type and the PprB overexpression strains.** Data

55 are from three independent experiments and shown as the mean  $\pm$  s.d. Growth rates ( $g_r$ )

56 are fitted from data at exponential phase using linear fit method, the resulting  $g_r$  are

57 indicated in the graph.

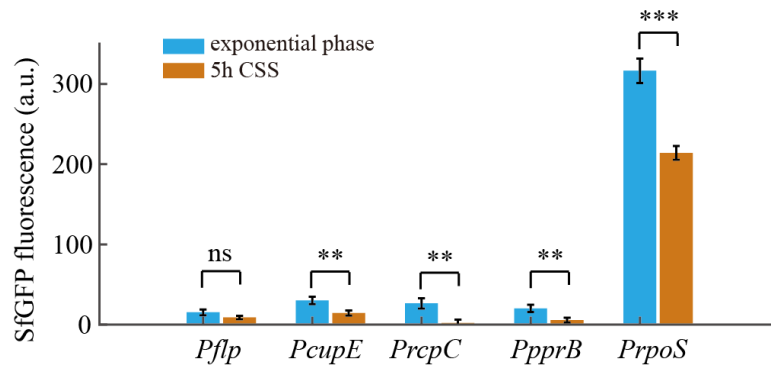
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60 **Fig. S5: Bacteria can still divide after carbon deprivation.** A, Bright-field  
 61 microscopy image of *pprB* mutant cells before and after 6 hours of CSS treatment. B,  
 62 Two division events of *pprB* mutant cells under CSS.

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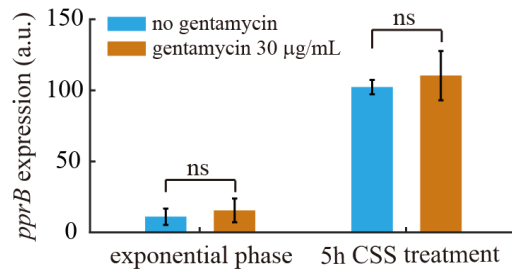


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65 **Figure S6: Expression values of *flp*, *cupE*, *rcpC*, *pprB*, and *rpoS* transcriptional**  
 66 **reporters in *relA* mutant strain under exponential phase or after 5 hours of carbon**  
 67 **deprivation.** Statistical analysis used pairwise comparisons (t-test) of data in control  
 68 experiment to data in stress conditions. \*\*\*P < 0.001, \*\*P < 0.01, ns P > 0.05.

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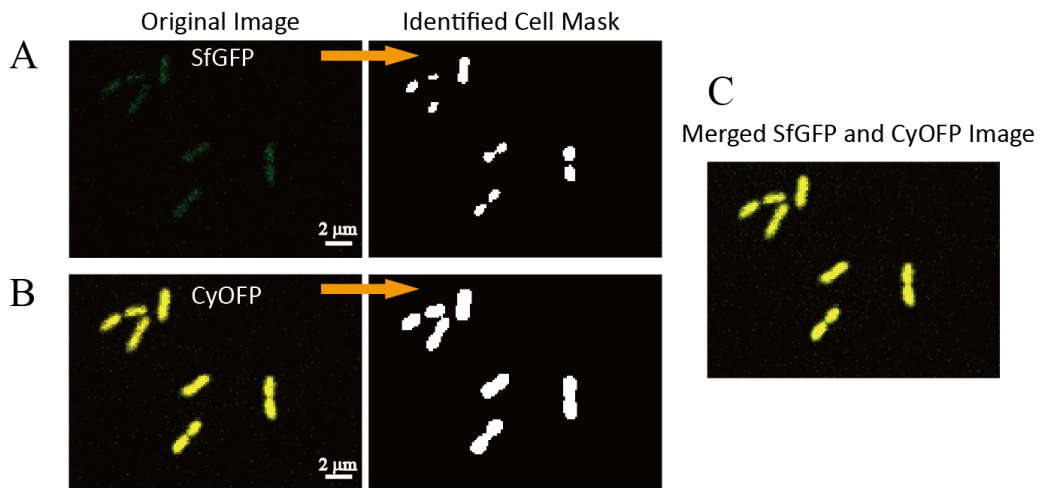
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72 **Fig. S7: Gentamycin addition does not affect the induction of PprB-regulated gene**

73 **expression under CSS.** Expression values of *pprB* transcriptional reporter with or

74 without gentamycin addition at exponential phase or after 5 hours of CSS treatment.

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77 **Fig. S8: Bacterial cell identification from fluorescent images.** (A) Cell mask

78 determination using SfGFP image of PprB reporter under carbon abundant condition.

79 (B) Cell mask determination using CyOFP image of PprB reporter. (C) Merged SfGFP

80 and CyOFP image of PprB reporter.