SUPPLEMENTARY APPENDIX

Caulobacter PopZ forms an intrinsically disordered hub in organizing bacterial cell poles Joshua A. Holmes¹, Shelby E. Follett², Haibi Wang¹, Christopher Meadows¹, Krisztina Varga^{2,3}, and Grant R. Bowman¹

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SUPPLEMENTARY METHODS

Culture conditions and strains

C. crescentus CB15N was grown at 30°C in PYE media (0.2% bactopeptone, 0.1% yeast extract,1 mM MgSO₄, 0.5 mM CaCl₂) and imaged during log phase growth. For microscopy and BACTH experiments, *E. coli* strain BL21(DE3) was grown overnight in Luria Bertani (LB) medium, diluted 1:100, and grown to an OD₆₀₀ of 0.3 at 37°C. To induce expression from plasmids, arabinose (0.002%-0.2%) and IPTG (10-50 μ M) were added during an additional 2 hours of growth at 30°C. *E. coli* strains DH5 α was used for molecular cloning. When appropriate, media were supplemented with antibiotics at the following concentrations (μ g/ml in liquid/solid medium for E. coli strains); for ampicillin (50;100), chloramphenicol (20;30), kanamycin (30;50). For C. crescentus (μ g/ml in liquid/solid medium); for ampicillin (5;50), chloramphenicol (1;1), kanamycin (5;25). *C. crescentus* strains CB15N was used for all experiments. When appropriate, media were supplemented with antibiotics at the following concentrations (μ g/ml in liquid/solid (μ g/ml in liquid/solid medium); for ampicillin (5;50), chloramphenicol (μ g/ml in liquid/solid medium); for ampicillin (5;50), chloramphenicol (μ g/ml in liquid/solid medium); for ampicillin (5;25).

Plasmid construction

To create the pBAD-msfGFP entry plasmid, pBADHisA (Invitrogen) was opened with HindIII and XhoI, and a PCR product encoding msfGFP (from primers JH60 and JH67) was inserted into this site by isothermal cloning. Next, the coding sequences of PopZ binding candidate proteins were amplified using PCR, and these were inserted into pBAD-msfGFP opened with NcoI and XhoI. The ITC cloning sequences were TTGGGCTAACAGGAGGAATTAACCA for the 5' overlap and ACTGCCGCCGCCGCCGCTCTCGAG for the 3' overlap. To make the DivIVA-ParB-msfGFP fusion, DivIVA was amplified using primers JH290 and JH291, and ParB was amplified using JH288 and JH289. These two fragments were then placed into open pBAD-msfGFP by ITC. mCherry-PopZ variants were placed into pACYC (Novagen) by opening the plasmid with NcoI and XhoI and inserting PCR-amplified variants of mCherry-PopZ by ITC. The ITC cloning sequences were 5' GTTTAACTTTAATAAGGAGATATACCATG and 3' TCGCAGCAGCGGTTTCTTTACCAGACTCGAG.

To create pBad + SpmX-msfGFP, we designed primers that would amplify the first 350 amino acids of SpmX. This produces a truncated form of SpmX that lacks the transmembrane region in amino acids 351-431, but retains PopZ-dependent polar localization in *Caulobacter* (1)(2). In all

other experiments that involved SpmX, such as the column binding assay and the bacterial 2hybrid assay, we employed the same truncated form of the protein (aa 1-350). To make pBad + SpmX-msfGFP we used the following ITC cloning primers: 5' primer

CGCGAACAGATTGGAGGTatgaaaccgcgtcatcag 3' GTGGCGGCCGCTCTATTAcagatcatcctggcgc, capital letters denote overlap with pETITE vector, lowercase letters are overlap with SpmX. The bacterial two-hybrid assay plasmids pUT18 and pKT25 (Euromedex) were modified as follows. For candidate PopZ binding proteins, pUT18 was opened with HindIII and EcoRI, and candidate protein coding sequences were amplified by PCR and inserted into the open plasmid by ITC. The ITC cloning sequences were 5' CTATGACCATGATTACGCCAAGCTT and 3' CGTGGCCTCGCTGGCGGCTGAATTC. To place PopZ variants into pKT25, the plasmid was opened with PstI and EcoRI, and PCR amplified popZ variants were inserted by ITC. The ITC cloning sequences were 5' CTGGCGGCGGGGCTGCAG and 3' ACGTTGTAAAACGACGGCCGAATTC.

To create a *Caulobacter* strain expressing McpA-GFP, the McpA coding sequence was fused to the coding sequence for GFP, and the combined sequence was cloned into plasmid pVMCS4. Following electroporative transformation, the plasmid inserted into the *Caulobacter* chromosome by homologous recombination at the P_{vanA} promoter (3). Due to the low level of constitutive expression and the high stability of McpA-GFP, the addition of vanillate inducer was not necessary for obtaining a visible fluorescent signal.

To create a *Caulobacter* strain expressing mCherry-PopZ, the mCherry-PopZ coding sequence was cloned into the plasmid pBXMCS-4, enabling plasmid-based expression from the P_{van}A promoter in *Caulobacter* cells (3). Due to the low level of constitutive expression and the high stability of mCherry-PopZ, the addition of vanillate inducer was not necessary for obtaining a visible fluorescent signal.

Strains using CckA-CFP and PopZ truncations were made using a PopZ KO strain with CckA-CFP integrated into the chromosome JH442. pBXMCS-2 was digested using NdeI and SacI, this was combined by PCR fragments of various PopZ truncations and via ITC combined. The plasmids were then introduced into JH442 via electroporative transformation.

Plasmid creation for in-vitro pull down assay. For SpmX-his tagged protein a commercial kit (Lucigen) was used. SpmX was PCR amplified and combined with commercial pre-processed pETite vector transformed directly into competent HI-Control 10G cells (Lucigen). For Strep tagged PopZ pASK-IB3+ plasmid was digested using NcoI and XhoI. PCR amplified popZ was inserted by ITC.

PopZ PED protein sequences

To create various PED linkers ITC was done by combining mCherry-PopZ n-terminal with C-term PopZ and pACYC backbone. pACYC plasmid was prepared by digesting with Ncol and Ball enzymes. 5' primer for all constructs mCherry-PopZ n-terminal fragments was TAACTTTAATAAGGAGATATACCATGGatgagcaagggcgag capital letters denote pACYC overlap and lowercase is mCherry. 3' for all constructs of C-terminal PopZ-pACYC overlap was CACCAGTTTTGATTTAAACGTGGCCatatggacaacttcttc, uppercase is pACYC overlap with vector and lowercase is pACYC fragment amplified with c-terminal PopZ. Univerisal 5' mCherry primer was used with 3' primer

CGGCGCCGCCGCATCCGCCGGCGGCGGATCTTCTTCctccgagatgatgcgt was used to create first half

of the scrambled PED linker; EEDPPPADAAAPPAPEAVPPEPPE and 5'

CCGGCGCCGGAAGCGGTGCCGCGGAACCGCCGGAAcggacgctggaagacg was used with universal 3' c-term pACYC primer to create second half.

Univerisal 5' mCherry primer was used with 3' primer

GCTCGCCGCCGCATCCGCGCTGCTGCTATCGCCGCCctccgagatgatgcgt to create first half of GS PED linker; GGDSSSADAAASSASGAVSSGSSG and 5'

Univerisal 5' mCherry primer was used with 3'

Univerisal 5' mCherry primer was used with 3' primer

CGGCGCCGCCGCATCCGCCGGCGGCGGCGGATCTTCTTCctccgagatgatgcgt was used to create first half of the ½ Scrambled PED linke; EEDPPPADAAAP and 5'

GAAGAAGATCCGCCGCCGGCGGATGCGGCGCGCGCGCGcggacgctggaagacg was used with universal 3' c-term pACYC primer to create second half.

Widefield Fluorescence Microscopy

Cells were immobilized on a 1% agarose pad containing LB media for *E. coli* cells and M2G for *C. crescentus*. Live-cell imaging was performed using a Zeiss Axio Imager Z2 epifluorescence microscope equipped with a Hamamatsu Orca-Flash4.0 sCMOS camera and a Plan-Apochromat 100x/1.46 Oil Ph3 objective. Zeiss Filter sets 47HE, 38HE, 46HE, and 63HE were used to acquire fluorescent images of CFP, msfGFP, eYFP and mCherry, respectively. Images were collected and processed with Zen Blue software.

Fluorescent Image Analysis

Fluorescent microscopy images were analyzed via the MicrobeTracker (4) suite running on MATLAB 2015b. E. *coli* cell meshes were obtained using MicrobeTracker algorithm 4. The MATLAB graythresh method was used to determine a threshold for fluorescence localization within the cell meshes. Percent localization was then calculated as the sum of the fluorescent signal which exceeded the threshold for background fluorescence divided by the sum of the fluorescent signal within each cell mesh.

Bacterial 2-hybrid assay

The Euromedex BACTH Bacterial Adenylate Cyclase Two-Hybrid System Kit was used with assay methods developed by Park and Jiao (5). Two negative controls were utilized: *E. coli* lacking CyaA components (CyaA null) and CyaA T25-PopZ with GFP-CyaA T18. To test for protein interaction, CyaA T25-PopZ variants were tested with candidate proteins expressed as N-terminal fusions to CyaA T18. All experiments were performed in a cyaA mutant background.

In vitro column binding assay

6His-Sumo-tagged proteins were expressed in *E. coli* strain BL21 Rosetta (EMD Millipore), purified under native conditions by cobalt affinity chromatography (Thermo), and eluted in buffer HMK (20mM HEPES pH 7.5, 2 mM MgCl2, 100 mM KCl) + 250 mM imidazole. The PopZ affinity column was prepared by expressing PopZ-streptag in *E. coli* strain DH5 α , passing the clarified lysate through a 0.2ml bed volume column containing Streptacin XL resin (IBA Life Sciences), and washing in buffer HMK. Lysates were prepared in by pulsed sonication (Branson). His-tagged protein samples were diluted 4-fold in Buffer HMK, passed through the PopZ affinity column, washed twice with 0.8 ml of buffer, and eluted with biotin.

Fluorescence loss in photobleaching (FLIP) assays

FLIP experiments were performed using confocal illumination, provided by an LMM5 laser launch (Spectral Applied research). Integration of all imaging systems components was provided by Biovision Technologies (Exton, PA). All image acquisition and analysis was performed using Metamorph 7.7 software (Molecular Devices). Images were acquired using Olympus objective 60 x/1.49 Oil DIC. ILAS² equipment was used to control laser power (at 10%) and position.

PopZ Purification for NMR experiments

The PopZ∆134-177 coding sequence was inserted into plasmid pET28a (Novagen) at the Ncol and Xhol restriction sites and transformed into Novagen® RosettaTM (Novagen). To express the 6xHis-tagged protein, a stationary culture was diluted 1:100-1:200 into 2 l fresh LB medium and grown at 37 °C with shaking at 200 rpm. At OD600 of 0.4, the cells were spun down at 7,700 $\times q$ for 15 minutes at 4 °C, resuspended in ¼ volume of M9 media (48 mM Na₂HPO₄, 22 mM KH₂PO₄, 9 mM NaCl, 2 mM MgSO₄, 0.1 mM CaCl₂, 0.4% ¹³C-glucose, 18 mM ¹⁵NH₄Cl with kanamycin at 30 μg/mL and chloramphenicol at 20 μg/mL), and incubated overnight with 0.8 mM IPTG and shaking at 200 rpm. ¹⁵NH₄Cl was purchased from Cambridge Isotope Laboratories, Inc. Frozen cell pellets were resuspended with ice cold binding buffer (25 mM Tris, 150 mM NaCl, 20 mM imidazole at pH 7.5) supplemented with EDTA free protease inhibitor and Benzonase nuclease. Cells were lysed using a French press, and the cell debris was removed by centrifugation (20 minutes at 27,200 ×g and 4 °C). The supernatant was filtered through a 0.22 µm syringe filter (Millipore) and concentrated. The protein was purified using a Fast Protein Liquid Chromatography instrument (FPLC; GE Healthcare ÄKTA purifier 900) equipped with a Ni-NTA column (GE Healthcare HisTrap HP 1 ml column). The sample was loaded onto the Ni-NTA column pre-equilibrated with binding buffer, washed, and eluted in the same buffer with a gradient of up to 500 mM imidazole. The peak protein-containing fractions were then reloaded onto an Ni-NTA column for an additional round of washing and elution. This was repeated for a total of three rounds of Ni-NTA purification. Peak fractions were pooled and dialyzed in 25 mM Tris, 150 mM NaCl pH 7.5 for 2 hours, then switched to fresh buffer and dialyzed overnight. The purity of the sample was analyzed by 12.5% SDS-PAGE and staining with Coomassie blue.

High Field NMR spectroscopy

For High field NMR experiments, the purified popZ was buffer exchanged into a citratephosphate buffer (50 mM phosphate, 50 mM citric acid, 20 mM NaCl, and 3 mM NaN₃ at pH 5.5). The sample was concentrated to 300 μ L for NMR experiments, and D2O, NaN3, and DSS were added to a 10% v/v, 4 mM and 1 mM final concentration, respectively. The concentration was estimated as 420 μ M using UV-Visible spectrophotometry (ϵ 280 =2,980 cm-1M-1). The NMR sample was packed into a Shigemi NMR tube. The ¹H-¹⁵N HSQC (Heteronuclear Single Quantum Correlation) NMR spectrum was collected at 25 °C on a VNMRS 800-MHz spectrometer equipped with and HCN z-axis gradient cold probe.

Circular Dichroism

 $PopZ^{\Delta 134-177}$ was purified as described for NMR and exchanged into 20 mM Na₂HPO₄ pH 7.5 at a final concentration of 18µM. The sample was analyzed in an Aviv 430 spetropolarimeter equipped with Peltier temperature control.

Purification of RcdA, ChpT, and His-SUMO-DivK for NMR

The coding sequences of *rcdA* and *chpT* were amplified by PCR and co-transformed into Lucigen HI-ControlTM 10G cells with pETiteTM N-His SUMO. The resulting plasmids were transformed into Lucigen HI-Control[™] BL21(DE3), generating strains HW#293/294. To express protein, cultures were grown at 37°C in LB until OD_{600nm} 0.5, and induced overnight with 1mM IPTG at 21°C with shaking at 200rpm. Frozen cell pellets were resuspended with ice cold binding buffer (20 mM HEPES, 100 mM KCl, 2 mM MgCl₂, 20 mM imidazole at pH 7.5 adjusted with KOH) supplemented with EDTA free protease inhibitor and Benzonase nuclease. Cells were lysed using a French press, and the cell debris was removed by centrifugation (20 minutes at 27,200 × g and 4 °C). The supernatant was filtered through a 0.22 μ m syringe filter (Millipore) and concentrated. The protein was purified using a Fast Protein Liquid Chromatography instrument (FPLC; GE Healthcare ÄKTA purifier 900) equipped with a Ni-NTA column (GE Healthcare HisTrap HP 1 ml column). The sample was loaded onto the Ni-NTA column pre-equilibrated with binding buffer, washed, and eluted in the same buffer with a gradient of up to 500 mM imidazole. The peak protein-containing fractions were then re-loaded onto a Ni-NTA column for an additional round of washing and elution after removing high concentration of imidazole. The purity of the samples were analyzed by 12.5% SDS-PAGE and staining with Coomassie blue.

His-SUMO-DivK was buffer exchanged (Zeba spin desalting columns 7K MWCO, 5 mL) to 20 mM HEPES, 100 mM KCl, 2 mM MgCl₂ at pH 7.5 (adjusted with KOH) for NMR experiments.

After the initial 2 step purification, RcdA and ChpT were buffer exchanged to 25 mM Tris, 150 mM NaCl pH 7.5 buffer and diluted to approximately 1mg/mL concentration. SUMO Express Protease (Lucigen) was then added at a concentration of 1 unit per 50-100 μg protein, along with 2 mM final concentration of fresh DTT. Digests were incubated at 4°C overnight, followed by additional incubation at 30°C for another day after the addition of 1 mM fresh DTT.

After cleavage, the proteins were passed through an additional Ni-NTA column and the flow through was collected. The flow through contained the cleaved proteins which were buffer exchanged to 20 mM HEPES, 100 mM KCl, 2 mM MgCl₂ at pH 7.5 (adjusted with KOH) for NMR experiments. The purity of the samples were analyzed by 12.5% SDS-PAGE and staining with Coomassie blue.

NMR sample conditions for testing protein interactions

For NMR experiments, the purified PopZ was buffer exchanged into 20 mM HEPES, 100 mM KCl, 2 mM MgCl₂ at pH 7.5 (adjusted with KOH). The concentration for each sample was estimated using UV-Visible spectrophotometry and final concentrations were calculated (see table below). Each sample was prepared by mixing PopZ with one of the binding partners (in the necessary volumes needed to obtain the final concentrations) and then concentrating to 300 μ L. For NMR experiments D₂O, NaN₃ and DSS were added to a 10% v/v, 1 mM and 1 mM final concentration, respectively. The NMR sample was packed into a Shigemi NMR tube. The 2D ¹H-¹⁵N HSQC (Heteronuclear Single Quantum Correlation) NMR spectra were collected at 25 °C on a Bruker 600 MHz spectrometer equipped with a 5 mm SmartProbeTM.

Sample #	[PopZ] (μM) (ε ₂₈₀ =2,980 cm ⁻¹ M ⁻¹)	Binding Partner	[Binding Partner] (µM)
1	146	-	-
2	146	RcdA ($\epsilon_{280} = 18,450 \text{ cm}^{-1}\text{M}^{-1}$)	1150
3	146	ChpT ($\epsilon_{280} = 17,990 \text{ cm}^{-1}\text{M}^{-1}$)	614
4	157	DivK ($\epsilon_{280} = 9,970 \text{ cm}^{-1}\text{M}^{-1}$)	596
F	157	DivK ($\epsilon_{280} = 9,970 \text{ cm}^{-1}\text{M}^{-1}$)	596
5	107	RcdA (ϵ_{280} = 18,450 cm ⁻¹ M ⁻¹)	442
6	157	BSA ($\epsilon_{280} = 43,824 \text{ cm}^{-1}\text{M}^{-1}$)	416

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JH50dwk-mstypmcherry-pap2This studyJH51jele-mstypmcherry-pap2This studyJH51cok4-mstypmcherry-pap2This studyJH61cok4-mstypmcherry-pap2This studyJH62mstypmcherry-pap2This studyJH63mstypmcherry-pap2This studyJH76dwi-mstypmcherry-pap2This studyJH77mstypmcherry-pap2This studyJH78dwi-mstypmcherry-pap2This studyJH78pap4-mstypmcherry-pap2This studyJH78dwi-mstypmcherry-pap2This studyJH78pap4-mstypmcherry-pap2This studyJH78dwi-mstypmcherry-pap2This studyJH78dwi-mstypmcherry-pap2This studyJH78dwi-mstypmcherry-pap2This studyJH78dwi-mstypmcherr	JH42		mcherry-popZ				This study
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divLmsfgfpmcherny-popZThis studyJH205msfgfpmcherny-popZThis studyJH207divLAr23-769msfgfpmcherny-popZJH206parB-msfgfpmcherny-popZThis studyJH207divLAr1-138msfgfpmcherny-popZJH208cckA-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH209parB-msfgfpmcherny-popZAn-termThis studyJH210rcdA-msfgfpmcherny-popZA24-81This studyJH211cpdR-msfgfpmcherny-popZA24-81This studyJH212chpT-msfgfpmcherny-popZA24-81This studyJH213divLA1-138-msfgfpmcherny-popZA24-81This studyJH213divLA1-138-msfgfpmcherny-popZA24-81This study	JH179	popZ-msfgfp ,, Δ1-138 Δ723-769					This study
JH207dlvL $\Delta^{723-769}$ msfgfpmcherry-popZThis studyJH206parB-msfgfpmcherry-popZThis studyJH207dlvL Δ^{1-138} -msfgfpmcherry-popZThis studyJH208cckA-msfgfpmcherry-popZThis studyJH209parB-msfgfpmcherry-popZThis studyJH209parB-msfgfpmcherry-popZThis studyJH209parB-msfgfpmcherry-popZThis studyJH210rcdA-msfgfpmcherry-popZThis studyJH211cpdR-msfgfpmcherry-popZThis studyJH212chpT-msfgfpmcherry-popZThis studyJH213dlvL Δ^{1-138} -msfgfpmcherry-popZThis studyJH213dlvL Δ^{1-138} -msfgfpmcherry-popZThis study	JH205	aivL msfgfp	mcherry-popZ				This study
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JH207 $dlvL^{\Delta 1-138}$ -msfgfpmcherny-popZ^{An-term}This studyJH208cckA-msfgfpmcherny-popZ^{An-term}This studyJH209parB-msfgfpmcherny-popZ^{Aped}This studyJH209parB-msfgfpmcherny-popZ^{A24-81}This studyJH210rcdA-msfgfpmcherny-popZ^{A24-81}This studyJH211cpdR-msfgfpmcherny-popZ^{A24-81}This studyJH212chpT-msfgfpmcherny-popZ^{A24-81}This studyJH213dlvL Δ^{1-138} -msfgfpmcherny-popZ^{A24-81}This study	JH206	parB-msfgfp	_Δn-term mcherry-popZ				This study
JH208cckA-msfgfpmcherny-popZ^An-termThis studyJH209parB-msfgfpmcherny-popZ^ApedThis studyJH209parB-msfgfpmcherny-popZ^A24-81This studyJH210rcdA-msfgfpmcherny-popZ^A24-81This studyJH211cpdR-msfgfpmcherny-popZ^A24-81This studyJH212chpT-msfgfpmcherny-popZ^A24-81This studyJH213dlvL Δ^{1-138} -msfgfpmcherny-popZ^A24-81This study	JH207	$dlvL \Delta^{1-138}$ -msfgfp	ے mcherry-popZ				This study
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JH209parB-msfgfpmcherry-pop2^{\Delta24-81}This studyJH210rcdA-msfgfpmcherry-pop2^{\Delta24-81}This studyJH211cpdR-msfgfpmcherry-pop2^{\Delta24-81}This studyJH212chpT-msfgfpmcherry-pop2^{\Delta24-81}This studyJH213dlvL $^{\Delta1-138}$ -msfgfpmcherry-pop2^{\Delta24-81}This study	JH209	parB-msfgfp	$mcherry-popZ^{\Delta ped}$				This study
JH210rcdA-msfgfpmcherry-pop2^{224-81}This studyJH211cpdR-msfgfpmcherry-pop2^{224-81}This studyJH212chpT-msfgfpmcherry-pop2^{224-81}This studyJH213dlvL ^{Δ1-138} -msfgfpmcherry-pop2^{24-81}This study	JH209	parB-msfgfp	mcherry-popZ ^{$\Delta 24-81$}				This study
JH211 $cpdR$ -msfgfpmcherry-popZ^{\Delta 24-81}This studyJH212 $chpT$ -msfgfpmcherry-popZ^{\Delta 24-81}This studyJH213 $dlvL^{\Delta 1-138}$ -msfgfpmcherry-popZ^{\Delta 24-81}This study	JH210	rcdA-msfgfp	mcherry-popZ ^{$\Delta 24-81$}				This study
JH212 $chpT$ -msfgfp mcherry-pop $Z^{\Delta 24-81}$ This study JH213 $dlvL^{\Delta 1-138}$ -msfgfp mcherry-pop $Z^{\Delta 24-81}$ This study	JH211	cpdR-msfgfp	mcherry-popZ ^{$\Delta 24-81$}				This study
JH213 dlvL -msfgfp mcherry-popZ This study	JH212	chpT-msfgfp	mcherry-popZ ^{$\Delta 24-81$}				This study
	JH213	$dIvL \frac{\Delta 1-138}{}$ -msfgfp	mcherry-popZ ^{$\Delta 24-81$}				This study

E. coli strains

This	study
This	study

		A24-81
JH214	parB-msfgfp	mcherry-popZ
JH215	parB-msfgfp	mcherry-popZ
JH226	parB-msfgfp	divIVA-mcherry-popZ
JH227	cckA-msfgfp	mcherry-popZ
JH229	parB-msfgfp	mcherry-popZ
JH237	chpT-msfgfp	∆n-term mcherry-popZ
JH241	grcA-msfgfp	mcherry-popZ
JH242	vanA-msfgfp	mcherry-popZ
JH252	۵351-431 Spmx-msfgfp	mcherry-popZ
JH253	rcdA-msfgfp	Δn-term mcherry-popZ
JH254	cpdR-msfgfp	Δn-term mcherry-popZ
JH260	∆351-431 Spmx-msfgfp	$\Delta 24-81$ mcherry-popZ
JH262	parB-msfgfp	$\Delta h2$ mcherry-popZ
JH266	parB-msfgfp	∆124-177 divIVA-mcherry-popZ
JH266	$dlvL \overset{\Delta 1-138}{}$ -msfgfp	Δh^2 mcherry-popZ
JH267	∆351-431 Spmx-msfgfp	_Δn-term mcherry-popZ
JH274	rcdA-msfgfp	_∆h2 mcherry-popZ
JH275	cckA-msfgfp	Δ124-177 divIVA-mcherry-popZ
JH276	cpdR-msfgfp	Δh^2 mcherry-popZ
JH278	chpT-msfgfp	Δ124-177 divIVA-mcherry-popZ
JH279	cpdR-msfgfp	Δ124-177 divIVA-mcherry-popZ
JH280	rcdA-msfgfp	∆124-177 divIVA-mcherry-popZ
JH281	∆351-431 Spmx-msfgfp	∆124-177 divIVA-mcherry-popZ
JH282	$dlvL \overset{\Delta 1-138}{}$ -msfgfp	۵124-177 divIVA-mcherry-popZ
JH283	parB-msfgfp	∆134-177 divIVA-mcherry-popZ
JH284	cckA-msfgfp	۵134-177 divIVA-mcherry-popZ
JH285	chpT-msfgfp	∆134-177 divIVA-mcherry-popZ
JH286	cpdR-msfgfp	۵134-177 divIVA-mcherry-popZ
JH287	rcdA-msfgfp	∆134-177 divIVA-mcherry-popZ
JH288	∆351-431 Spmx-msfgfp	∆134-177 divIVA-mcherry-popZ
JH289	$dlvL \overset{\Delta 1-138}{}$ -msfgfp	∆134-177 divIVA-mcherry-popZ
JH301	chpT-msfgfp	Δ48-102 mcherry-popZ
JH302	cckA-msfgfp	Δ48-102 mcherry-popZ
JH303	rcdA-msfgfp	Δ48-102 mcherry-popZ
JH304	parB-msfgfp	Δ48-102 mcherry-popZ
JH305	cpdR-msfgfp	$\Delta 48-102$ mcherry-popZ
JH306	$dlvL \overset{\Delta 1-138}{}$ -msfgfp	$\Delta 48-102$ mcherry-popZ
JH307	∆351-431 Spmx-msfgfp	Δ48-102 mcherry-popZ
JH321	cckA-msfgfp	Δh^2 mcherry-popZ
JH322	chpT-msfgfp	Δh^2 mcherry-popZ
JH323	chpT-msfgfp	Δped mcherry-popZ
JH324	cpdR-msfgfp	Δped mcherry-popZ
JH325	rcdA-msfgfp	_∆ped mcherry-popZ

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JH326	Δ351-431 Spmx-msfgfp	Δped mcherry-popZ				This study
JH327	$dlvl L^{\Delta 1-138}$ -msfgfp					This study
JH328	cckA-msfgfp	_Δped mcherry-popZ				This study
JH337			суаА-Т25-рорZ 	parB-cyaA-T18		This study
JH340			177	parB-cyaA-T18		This study
JH343			суаА-Т25-рорZ 	msfgfp-cyaA-T18		This study
JH344			177	msfgfp-cyaA-T18		This study
JH356			cyaA-T25-popZ	cckA-cyaA-T18		This study
JH357			cyaA-T25-popZ	cckA ^{Δ1-72} -cyaA-T18		This study
JH365			cyaA-T25-popZ	Δ351-431 Spmx cyaA-T18		This study
JH366			cyaA-T25-popZ	cpdR-cyaA-T18		This study
JH367			cyaA-T25-popZ	rcdA-cyca-T18		This study
JH368			cyaA-T25-popZ	chpT-cyaA-T18		This study
JH369			cyaA-T25-popZ	dlvL ^{Δ1-138} -T18		This study
JH373	tipN-msfgfp	mcherry-popZ				This study
JH374	divIVA-parB-msfgfp	∆134-177 mcherry-popZ				This study
JH375	Δ351-431 Spmx-msfgfp	mcherry-popZ Δh^2				This study
			∆134- cyaA-T25-popZ	A1-128		
JH387			Δ134-	dlvL ²¹⁻¹³⁰ -T18		This study
111388			суаА-125- рор2 177	rcdA-cyca-T18		This study
011000			∆134- cyaA-T25- <u>p</u> opZ	loun cycu i ro		This study
JH389			Δ134- cyaA-T25- popZ	cpdR-cyaA-T18		This study
JH390			177 суаА-Т25- рорZ ^{Д134-}	chpT-cyaA-T18		This study
JH391			177 	Δ ³⁵¹⁻⁴³¹ Spmx cyaA-T18		This study
JH392			177	cckA-cyaA-T18		This study
JH414	parB-msfgfp	A PED:scr linker mcherry-popZ				This study
JH415	parB-msfgfp	△ PED:gs linker mcherry-popZ mcherry-popZ mcherry-popZ				This study
JH416	parB-msfgfp	linker mcherry-popΖ PED:1/2 scr				This study
JH417	parB-msfgfp	linker _ΔPED; divIVA-mcherry- <u>po</u> pZ				This study
JH425	parB-msfgfp	Δ134-177			<i>popZ</i> in	This study
JH470					pASK- IBA3+	This study
					Δ351- Spmx 431	
JH472					in pETite	This study
HW293					rcdA in pETite	This study
HW294					chpT in pETite	This study

Strain	Description	Source
GB050	vanA :: pVanA – mcpA-gfp	This study
LS3310	cckA :: cckA-gfp	(6)
GB438	pBXMCS4 + mCherry-popZ	(3)
JH443	mVenus-popZin pBXMCS-2, cckA :: ccka-cfp	This study
JH444	<i>mVenus-popZ</i> ^{Δ24-81} in pBXMCS-2, cckA :: ccka-cfp	This study
JH445	mVenus-pop $Z^{448-102}$ in pBXMCS-2, cckA :: ccka-cfp	This study
JH446	<i>mVenus-popZ</i> ^{Δn-term} in pBXMCS-2, cckA :: ccka-cfp	This study
JH447	<i>mVenus-pop</i> Z^{h2} in pBXMCS-2, cckA :: ccka-cfp	This study
JH448	$mVenus-popZ^{\Delta PED}$ in pBXMCS-2, cckA :: ccka-cfp	This study

Caulobacter strains

			Localization		
			Localization	(with co-expressed mCherry	
CCNA	Protein	Description	(without mCherry PopZ)	PopZ)	-
CCNA_00010	DnaK	Polar/Midline	Foci	Partial co-localization	
CCNA_00781	CpdR	Polar/Cell cycle	Diffuse	Co-localized	
CCNA_00852	TipR	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_00852	ClpP	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_01116	DivJ	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_01132	CckA	Polar/Cell cycle	Diffuse	Co-localized	
CCNA_01552	TipN	Polar/Cell cycle	Foci	Partial co-localization	
CCNA_01918	PopA	Polar/Cell cycle	Foci	Partial co-localization	
CCNA_01926	DgcB	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_02039	ClpX	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_02125	PodJ	Polar/Cell cycle	Foci	Non-overlapping foci	1
CCNA_02246	MipZ	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_02255	SpmX	Polar/Cell cycle	Diffuse	Co-localized	
CCNA_02328	GcrA	Cell cycle	Diffuse	Diffuse	
CCNA_02334	StaR	Polar/Cell cycle	Diffuse	Diffuse	
CCNA_02476	VanA	Cytoplasmic	Diffuse	Diffuse	S
CCNA_02546	PleD	Polar/Cell cycle	Foci	Partial co-localization	р
CCNA_02547	DivK	Polar/Cell cycle	Diffuse	Diffuse	a ta
CCNA_02567	PleC	Polar/Cell cycle	Diffuse	Diffuse	w
CCNA_03130	CtrA	Polar/Cell cycle	Diffuse	Diffuse	s
CCNA_03333	CC_3225	Polar	Foci	Non-overlapping foci	S
CCNA_03404	RcdA	Polar/Cell cycle	Diffuse	Co-localized	s
CCNA_03507	PdeA	Polar	Diffuse	Partial co-localization	b
CCNA_03584	ChpT	Cell cycle	Diffuse	Co-localized	F
CCNA_03598	DivL	Polar/Cell cycle	Diffuse	Co-localized	(
CCNA_03868	ParB	Polar/Cell cycle	Diffuse	Co-localized	



Supplementary Figure S1: Candidate proteins use in *E. coli* co-expression assay (Table, left). Examples of GFP-tagged candidate proteins co-expressed with mCherry-PopZ (Right panels). PdeA shows partial co-localization, CC_3225 shows non-overlapping foci, and CtrA and StaR are two examples of diffuse signals. StaR and some other proteins appeared to be partly excluded from PopZ foci. Fluorescence signals (red and green) are overlayed on the phase contrast image (grayscale).



Supplementary Figure S2: Candidate proteins that exhibit colocalization with PopZ in the E. *coli* co-expression assay. GFPtagged candidate proteins are co-expressed with mCherry-PopZ. Fluorescence signals (red and green) are overlayed on the phase contrast image (grayscale).

	CckA	ChpT	CpdR	DivL ^{∆1-138}	ParB	RcdA	SpmX
WT PopZ	10.2	43.6	15.2	19.4	26.5	16.6	15.7
PopZ ΔPED	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PopZ ΔN-term	Nd	Nd	Nd	Nd	Nd	Nd	Nd
PopZ Δ48-102	8.9	40.2	13.8	17.4	23.2	14.1	13.8
PopZ Δ24-81	8.1	41.4	14.3	17.3	24.2	14.7	14.2
ΡορΖ ΔΗ2	Nd	Nd	Nd	Nd	Nd	Nd	Nd
DiviVA-PopZ Δ 134-177	7.3	39.1	12.7	16.3	22.5	11.2	12.1
DiviVA-PopZ A124-177	Nd	Nd	Nd	Nd	Nd	Nd	Nd
50							
45 40 35 40 35 40 35 40 35 40 35 40 40 35 40 35 40 35 40 35 40 35 40 35 40 40 40 40 40 40 40 40 40 40					WT Po PopZ Z PopZ Z DiviVA	pZ Δ48-102 Δ24-81 -PopZΔ1	34-177

Supplementary Figure S3: Analysis of PopZ interactions using automated computational methods. (Top Panel): Measurement of the co-localization between GFP-tagged candidate binding proteins and variants of mCherry-tagged PopZ, using automated image quantitation. A minimum of 1000 cells was used for each data point, and the standard deviation for all reported values is less than 0.18 (not shown). Values under 2% were not discernable by eye and are reported as not detectable (ND). (Lower Panel): A graphical representation of the values reported in the upper panel. Notably, each of the mutations in PopZ affected interactions with all candidate binding partners to a roughly equivalent degree.

DivL

ParB

RcdA

SpmX

0

CpdR

ChpT

CckA



Supplementary Figure S4. The E. *coli* co-localization screen produces consistent results. The polar recruitment of GFP-tagged candidate proteins was assessed in three separate experiments, and the relative localization index was measured as described in the methods section of the main text. Error bars represent the standard deviation.



Supplementary Figure S5. A bacterial two-hybrid assay for assessing interactions between PopZ and candidate proteins. (A) Full length PopZ tested against positive candidates from the *E. coli* co-expression assay, with CyAnull and GFP as negative controls. (B) PopZ^{Δ 134-177}, a monomeric variant of PopZ with diffuse localization, tested against the same candidates as in A.



Supplementary Figure S6: Testing PopZ variants with mutations in the PED region in the *E. coli* co-expression assay. (A) The PED region was replaced with a scrambled sequence of amino acids from 24-47 (B) The PED region was replaced with a 24 amino acid gly/ser linker. (C) The PED region was replaced with 24 amino acid pro/ser linker. (D) The PED region was replaced with 12 amino acids from the insert in A. (E) DivIVA was fused with N-N-term of PopZ (1-23) and H2 (103-133). In all panels, the GFP-labeled protein is ParB-GFP. Fluorescence signals (red and green) are overlayed on the phase contrast image (grayscale).



Supplementary Figure S7. 2D ¹H-¹⁵N HSQC NMR spectrum of PopZ^{Δ134-177}, collected on a high field 800 MHz NMR spectrometer at 25 °C. The data shows well-defined peaks with roughly uniform intensities and line shapes for the majority of resonances. All of the backbone proton peaks were clustered in the 7.80-8.85 ppm region. This narrow dispersion of proton chemical shifts is a strong indicator of an intrinsically disordered protein, compared to well-ordered proteins, which exhibit more broadly distributed peaks in the proton dimension (7). The triplet outliers, connected by horizontal lines, are signals from the side chains of glutamine residues in this sequence and are not an indication of local structure.



Supplementary Figure S8: Computational prediction of structural features in PopZ. (A) The propensity for intrinsic disorder, as indicated by four independent prediction programs: Metadisorder MD2 (8), DnDisorder (9), MFDp2 (10), and SPINE-D (11). (B) The presence of MoRF-like characteristics, as indicated by three independent prediction programs: ANCHOR (12), MoRFpred (13), and MoRFCHiBi_WEB (14). The combined average score for these programs is shown in Figure 5A of the main text.

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