SUPPLEMENTARY MATERIAL

Absence of the polar organizing protein PopZ results in reduced and asymmetric cell division in *Agrobacterium tumefaciens*

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SUPPLEMENTARY METHODS

Western Blot Analysis of PopZ-GFP Fusions. To detect PopZ-GFP fusions 3 ml cultures were grown in the absence of inducer for 8 hours, followed by overnight growth in the presence of 1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) as the inducer. The overnight cultures were diluted to an OD_{600} of 0.3 and grown for an additional 4 hours in the presence of inducer. 2 ml of culture was spun for 5 min at 7000 x g in a desktop centrifuge. Cell pellets were concentrated in GoldBio Bacterial Protein Extraction Lysis Buffer (GoldBio) with containing a protease inhibitor (ProBlock Gold) to an OD_{600} of ~25 and lysed following the recommended protocol (GoldBio). Whole-cell lysates were cleared by centrifugation at 13,000 x g for 10 min. 1X Laemmli Sample Buffer was added to the cleared supernatents. The supernatents were boiled at 100°C prior to loading on an SDS 4-20% PAGE gel (GenScript) with recommended electrophoresis conditions. Proteins were electroblotted onto a PVDF membrane (Biorad) and blocked in 5% non-fat dry milk solubilized in TBST (1X TBS, 1% Tween-20) overnight. The blocked membranes were probed using anti-GFP monoclonal antibody (1:3000) in 2.5% nonfat milk solubilized in TBST for 2.5 hours. After washing, the membranes were incubated with anti-mouse HRP conjugated secondary antibody (1:3000) in 2.5% milk/TBST for 2.5 hours. The secondary antibody was detected using Clarity Western ECL Substrate (BioRad) for 10 min followed by SuperSignal West Pico Substrate (Thermo-Scientific) for an additional 10 min.

Swim Plate Assay. A fresh colony was picked with a pipette tip and stabbed into ATGN plates containing 0.3% agar followed by incubation in a sealed humid chamber for 7

47 days. Swim ring diameters were then measured. Each strain was tested using 8
48 independent colonies.
49
50 **Dual Labeling with NADA and DAPI.** Cells were grown in ATGN to exponential phase
51 and labeled with a green FDAA (NBD-amino-D-alanine; NADA) for 5 minutes and then
52 ethanol fixed. The cells were subsequently labeled with DAPI and imaged using phase
53 contrast and epifluorescence microscopy with an inverted Nikon Eclipse TiE and a
54 QImaging Rolera em-c^2 1K EMCCD camera.
55
56 **SUPPLEMENTARY TABLES**
57
58 **Table S1.** Bacterial strains and plasmids used in this study.

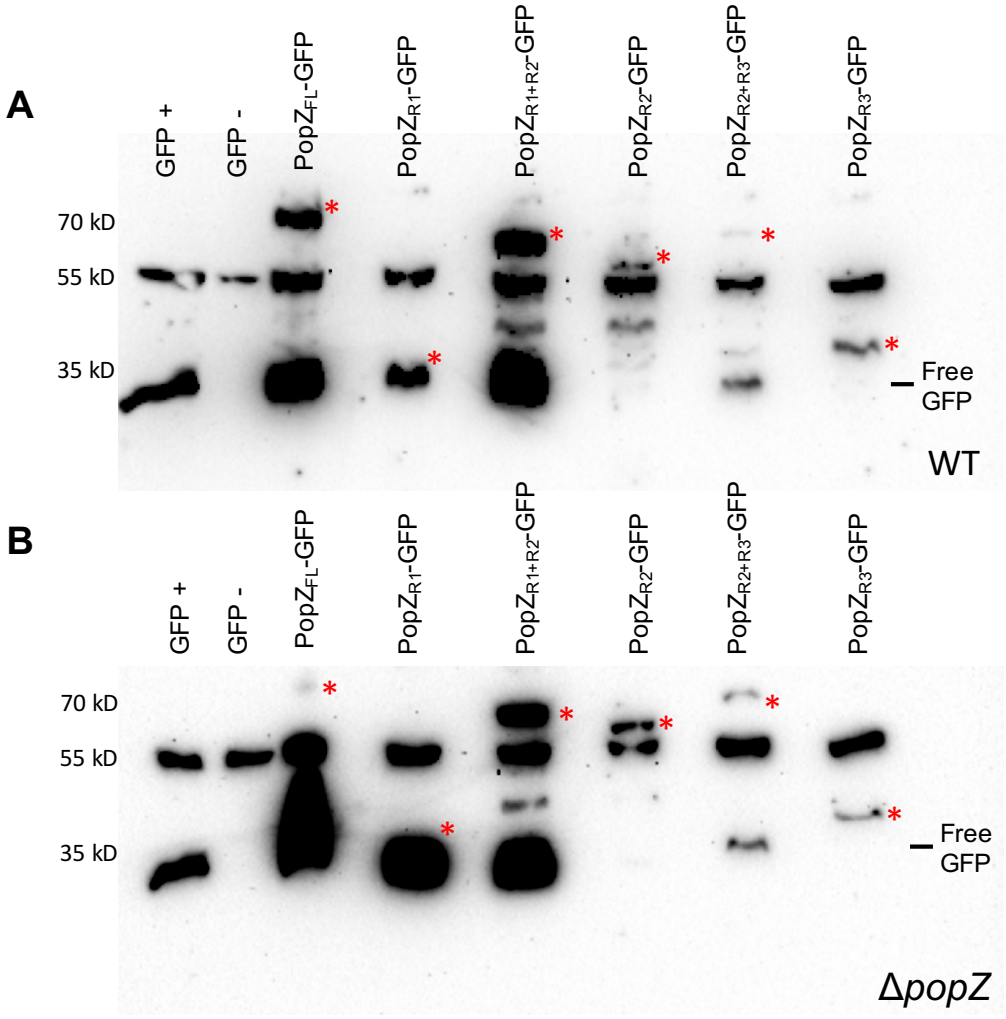
Strain or plasmid	Relevant characteristics	Reference/Source
Plasmids		
pSRKKm-Plac	Km^r; broad host range vector containing $lacI^q$ and *lac* promoter	15
pKC129	Source of sfGFP	KC Huang Lab
pSRKKm-Plac-sf*gfp*	pSRKKm vector containing $lacI^q$ and *lac* promoter with sfGFP	13
pSRKKM-Plac-*popZR1*-sf*gfp*	Km^r; vector containing *popZR1-sfgfp* (bp 1-75).	This study
pSRKKM-Plac-*popZR2*-sf*gfp*	Km^r; vector containing *popZR2-sfgfp* (bp 76-774).	This study
pSRKKM-Plac-*popZR3*-sf*gfp*	Km^r; vector containing *popZR3-sfgfp* (bp 775-999).	This study
pSRKKM-Plac-*popZR1R2*-sf*gfp*	Km^r; vector containing *popZR1R2-sfgfp* (bp 1-774).	This study
pSRKKM-Plac-*popZR2R3*-sf*gfp*	Km^r; vector containing *popZR2R3-sfgfp* (bp 76-999).	This study
pSRKKM-Plac-*popZ*-sf*gfp*	Km^r; vector containing *popZ-sfgfp* (full length).	This study
pGB1246	Km^r; vector containing *fliM-eyfp*	This study
pSRKKM-P*qaz-sfgfp*	Km^r; vector containing *A. tumefaciens* native *QAZ* promoter.	This study
pSRKKM-P*qaz-ftsA*-sf*gfp*	Km^r; vector containing *A. tumefaciens* native *QAZ* promoter followed by *ftsA-sfGFP* coding sequence.	This study
pRVGFPC-2	Km^r; vector containing vanillate promoter which provides low constitutive expression in *A. tumefaciens*	14
pSRKKM-Plac-*ftsZ*-sf*gfp*	Km^r; Source of *ftsZ*-sf*gfp*.	This study
pRV-*ftsZ*-sf*gfp*	Km^r; Constitutive expression of *ftsZ*-sf*gfp*..	This study
E. coli strains		
DH5α	Cloning strain	Life

		Technologies
A. tumefaciens strains		
C58C1	C58 strain lacking pTiC58 plasmid	Bowman Lab
GB1163 C58C1 Δ*popZ*	C58C1 with *popZ* replaced with spec resistance cassette	17
GB1158 C58C1 Δ*popZ::mchy-popZ*	C58C1 Δ*popZ* with *mchy-popZ* integrated into the native locus	17
C58C1 pSRKKM *popZR1*-sf*gfp*	C58C1 with pSRKKM expressing *popZR1*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM *popZR2*-sf*gfp*	C58C1 with pSRKKM expressing *popZR2*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM *popZR3*-sf*gfp*	C58C1 with pSRKKM expressing *popZR3*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM *popZR1R2*-sf*gfp*	C58C1 with pSRKKM expressing *popZR1R2*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM *popZR2R3*-sf*gfp*	C58C1 with pSRKKM expressing *popZR2R3*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM *popZ*-sf*gfp*	C58C1 with pSRKKM expressing *popZ*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZR1*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZR1*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZR2*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZR2*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZR3*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZR3*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZR1R2*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZR1R2*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZR2R3*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZR2R3*-sf*gfp* under the *lac* promoter.	This study
C58C1 Δ*popZ* pSRKKM *popZ*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *popZ*-sf*gfp* under the *lac* promoter.	This study
C58C1 pSRKKM-Pqaz-*ftsA*-sf*gfp*	C58C1 with pSRKKM expressing *ftsA*-sf*gfp* under the lac native *qaz* promoter.	This study
C58C1 Δ*popZ* pSRKKM-Pqaz-*ftsA*-sf*gfp*	C58C1 Δ*popZ* with pSRKKM expressing *ftsA*-sf*gfp* under the native *qaz* promoter.	This study
C58C1 pRV-*ftsZ*-sf*gfp*	C58C1 with pRV expressing *ftsZ*-sf*gfp* under the *van* promoter.	This study
C58C1 Δ*popZ* pRV-*ftsZ*-sf*gfp*	C58C1 Δ*popZ* with pRV expressing *ftsZ*-sf*gfp* under the vanillate promoter.	This study
GB1250 C58C1 Δ*popZ::mchy-popZ* pGB1246	C58C1 Δ*popZ::mchy-popZ* with pSRKKM expressing YFP-FliM under the *lac* promoter	This study
GB1261 C58C1 Δ*popZ* pGB1246	C58C1 Δ*popZ* pSRKKM expressing YFP-FliM under the *lac* promoter	This study

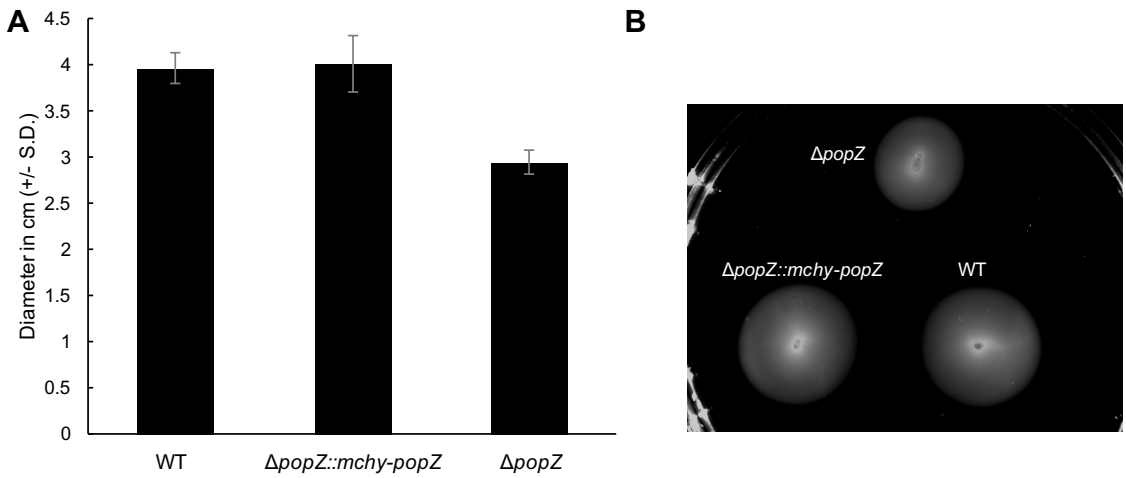
Table S2. Synthesized DNA primers and gene fragments used in this study.

Synthesized DNA	Sequence
Primers	
PopZ FL For NdeI	5’-CGC GAT CAT ATG GCT CAG CCA AGT GT -3’
PopZ R1 Rev BamHI	5’-GTC GCT GGA TCC GCT TTC GAT GAT CCG-3’
PopZ R2 For NdeI	5’-GTC GCT CAT ATG AAC GCG CCT GGA CCT-3’
PopZ R2 Rev BamHI	5’-GTC GCT GGA TCC ATC CGC AAT GTG CGC-3’
PopZ R3 For NdeI	5’-GTC GCT CAT ATG GGC CTG TCG CTC AAT-3’
PopZ FL Rev BamHI	5’-GTC GCT GGA TCC GCG GCG CGA GCC GCG-3’
FtsA For NdeI	5’-CGA CGC CAT ATG AGC TTT TTTGGT TC-3’
FtsA Rev BamHI	5’-CGG CAC GGA TCC TCA AAA ACT TTC TTT C-3’
FtsZ For NdeI	5’-CGA CGC CAT ATG ACG ATA CAG CTG C-3’
FtsZ Rev BamHI	5’-GCG TGA GGA TCC GTT GGA CTGGCG GCG C-3’
sfGFP Rev NheI	5’-GCT CAG GCT AGC CTA TTT GTA GAG TTC-3’
pSRK_FliM_FOR	5’-GAG CGG ATA ACA ATT TCA CAC AGG AAA CAG CAT ATG GCA AAA GCT GCA GCG C-3’
5’eYFP_ITCR_3’FliM	5’-GCC CTT GCT CAT GGA TCC AGA TCC ACC CAT CAA ATG TCG TAA GAT CTC-3’
3’FliM_ITC_5’eYFP	5’-TTG ATG GGT GGA TCT GGA TCC ATG AGC AAG GGC GAG GAG-3’
eYFP_pSRK_REV	5’-CCC TCG AGG TCG ACG GTA TCG ATA AGC TTT TAC TTG TAC AGC TCG TCC ATG C-3’
Gene Fragments	
QAZ promoter	5’-GGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAA TCTTCGAATTCGTAGCTGAGCTTGGACTCCTGTTGATAGATCCAGTAA TGACCTCAGAACTCCATCTGGATTTGTTCAGAACGCTCGGTTGCCGCC GGGCGTTTTTTATTGGTGAGAATCCAAGCTAGACTGCGATGAGTGGCA GGGGTAATGACTCTCTAGCTTGAGGCATCAAATAAAACGAAAGGCTC AGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCGGTGAACG CCTCCTGAGTAGGACAAATCCGCCGCTAGGAGCTTGCGGCCACTAGTG TTTGGGTTCCATTTCAGATGATTGGAATGTCATGCCTTGGAAGGCAGG GACTGCGTTAACGCAATTTTTGTTCCTATGGAATGCTAAAAAAGAAAT AATGCAAATAATGTGATTGTTGAACACCGCAGGCCTCGCAATGCACTG CCGGTCACGGTGCTGCGATTGAGAGACTGATTCAGGCCGCGGATTTAA AATGCTGAAACGCCAGATAATTTTGTCTGGGGAATAAGCCCGTCTGTT AACGTTTCGACCTTGCCTTCATCGCGAAGTGGCGAACCCGCCAGGGCG GTTTTATTTCCGATCATTGAAAACCTGCATGTTGCAATCCGTACAACTC TCTGATTCCAAGGAGGTAATCGGGATTGATGCTGATTCTTTAACGGAA CTCGGCATGTTGGACGCGGCGGTTAACGAAAGTAAACGATTCATTAA CCTTAATGGCGTTTAACTGATCTCAAGGATCATGTGCCAGAATCACCG TCCGTTTCATTCGGTCGGTTTGGACATGGTTCGTAATGGAGAGTTGGT GTCAGAGGGTTCGCATATGCCTGCAGGCGCCTTAATTAATATGCATGG TACCTTAACATCTCGAGCTCCGGGATCCGCTGGCTCCGCTGCTGGTTC TGGCAAGCTTGGCGTGTCTAAAGGTG -3’

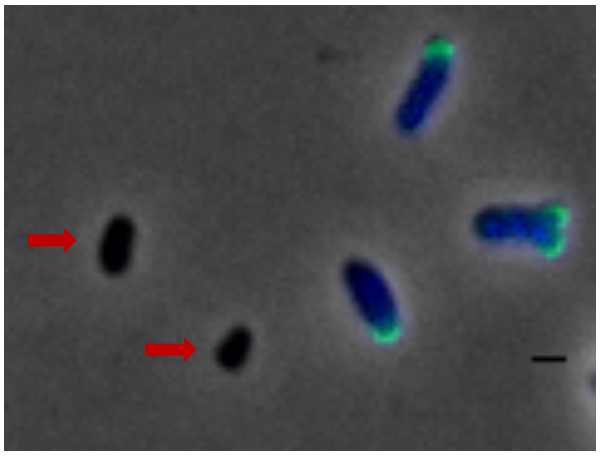
SUPPLEMENTAL FIGURES



Supplemental Figure S1. Western blot analysis of PopZ-GFP fusions. Western Blot analysis of PopZ-GFP fusion strains in wildtype (A) and Δ*popZ* (B) after overnight induction with IPTG using a monoclonal anti-GFP antibody. The position of each PopZ fragment fused with GFP is shown with a red asterisk. The position of free GFP on each blot is indicated. A cross reacting band is present in all samples near 55 kD.



Supplemental Figure S2. Δ*popZ* cells are motile. Indicated strains were assayed for motility on ATGN soft agar for 7 days. A. The average swim ring diameter and standard deviation was determined for each strain. Data were collected from 8 independent experiments. B) Representative image of swim rings formed by each strain after 7 days.



Supplemental Figure S3. Small cells lack peptidoglycan synthesis and DNA. Representative image of Δ*popZ* cells dual labeled with NADA (green) and DAPI (blue). Red arrows indicate two cells smaller than 1.5 μm. Scale bar is 1 μm. Quantitation of labeling patterns in small cells reveals that 95% of small cells that lack NADA labeling also do not label with DAPI (98/103).