Supplementary material including legends of supplementary figures and movies, and plasmid and strain constructions

Legends of supplementary figures

Fig. S1: Time-lapse microscopy sequence of FtsZ-YFP localization in the Δ*dipM* mutant. Cells of strain CJW3430 were grown in the presence of 250 μM of vanillic acid for 3 h to induce the expression of *ftsZ-yfp* prior to imaging. The arrows and white lines indicate the stalled FtsZ rings and unstable accumulation of FtsZ, respectively. The arrowhead shows a condensed FtsZ ring following unstable FtsZ accumulation.

Fig S2: Western blot analysis of various mCherry fusions using anti-RFP antibody. Protein extracts were generated from cultures grown under the conditions described in the main text. (A) Lane 1, CJW3116 carrying mCherry-dipM$_{\Delta 1\text{-}30}$; lane 2, CJW3117 carrying mCherry-DipM$_{\Delta 1\text{-}236;}$ lane 3, CJW3121 carrying DipM$_{\Delta 297\text{-}609}$-mCherry; lane 4, CJW3124 carrying DipM-mCherry; lane 5, CJW3439 carrying DipM $_{\Delta 501\text{-}609}$-mCherry; lane 6, CJW3526 carrying DipM$_{1-296\Delta 121-167}$-mCherry; lane 7, CJW3528 carrying DipM$_{1-296\Delta 175-223}$-mCherry. Cultures were grown in the presence of 250 μM of vanillic acid. Samples were taken when the cultures reached an $OD_{660} \approx 0.3$. (B) Lanes 1-3, CJW3446 carrying DipM$_{\Delta 501\text{-}609}$-mCherry grown in the presence of 0.3% xylose and 250 μM vanillic acid (lane 1), only 250 μM vanillic acid (lane 2), or only 0.3 % xylose (lane 3); lane 4, CJW3121 carrying DipM$_{\Delta 297\text{-}609}$-mCherry; lane 5, CJW3530 carrying DipM$_{\Delta 297\text{-}609}$-mCherry; lane 6, CJW3117 carrying DipM$_{\Delta 297\text{-}609}$-mCherry; lane 7, CJW2959 carrying periplasmic mCherry. Induction conditions were the same as in (A).

(C) Lane 1, CJW3440 carrying DipM-mCherry; lane 2, CJW3444 carrying $DipM_{\Delta 54-419}$-mCherry. (D) Schematics of the different mCherry fusions examined.

Fig. S3. Localization of periplasmic mCherry and $DipM_{\Delta 501-609}$-mCherry in different genetic backgrounds. *Top left panel*, fluorescent micrographs of $DipM_{\Delta 501-609}$-mCherry (left) in DipM-depleted cells (CJW3446); depletion of DipM was achieved by subculturing CJW3446 without vanillic acid and with 0.3% xylose for 24 h. The culture was at an OD_{660} of 0.2 prior to imaging. *Top right panel*, fluorescent micrograh of periplasmic mCherry in Δ*dipM* mutant cells (strain CJW3551). Cells were grown in the presence of 250 μM vanillic acid until the culture reached an OD_{660} of ~ 0.2. Arrows show shed vesicles containing mCherry signal. *Bottom left panel*, fluorescent micrograph of $DipM_{\Delta 501-609}$-mCherry in FtsZ-depleted cells (CJW3446) grown without xylose and with 250 mM vanillic acid for 5 h. *Bottom right panel*, fluorescent micrograph of periplasmic mCherry in FtsZ-depleted cells (CJW3550) grown without xylose and with 250 mM vanillic acid.for 5 h. The bars represent 1 μm.

Movie legends

Movie S1. A Δ*dipM* mutation is associated with vesicle shedding. Δ*dipM* cells (strain CJW3137) growing in M2G liquid medium were spotted on an agarose-padded microscope slide containing M2G medium and were imaged at room temperature by time-lapse DIC microscopy. The arrow indicates a division site that recedes. Time is in h:min.

Movie S2. A Δ*dipM* mutation is associated with cell pole enlargement over time. Δ*dipM* cells (strain CJW3137) growing in M2G liquid medium were spotted on an agarose-

padded microscope slide containing M2G medium and were imaged at room temperature by time-lapse DIC microscopy. The arrowhead shows an example of a cell pole enlarging as it ages. Time is in h:min.

Movie S3. A Δ*dipM* mutation is occasionally associated with cell branching from the poles. Δ*dipM* cells (strain CJW3137) growing in M2G liquid medium were spotted on an agarose-padded microscope slide containing M2G medium and were imaged at room temperature by time-lapse phase-contrast microscopy. Time is in min.

Movie S4. A Δ*dipM* mutation affects FtsZ dynamics. Δ*dipM* cells (strain CJW3430) were grown in the presence of 250μM vanillic acid for 3 h to induce the synthesis of FtsZ-YFP (in red) prior to time-lapse microscopy at room temperature on agarose-padded slides containing M2G medium (but no vanillic acid). Arrows follow the position of an unstable FtsZ ring shown here as an example. Time is in h:min:sec.

Movie S5. Division can occur a relatively long time after the FtsZ ring has disappeared in Δ*dipM* cells. Δ*dipM* cells (strain CJW3430) were grown in the presence of 250μM vanillic acid for 3 h to induce the synthesis of FtsZ-YFP (in red) prior to time-lapse microscopy at room temperature on agarose-padded slides containing M2G medium (but no vanillic acid). The arrowheads show sites where cell division occurs well after the FtsZ ring has finished constricting and has disassembled. Time is in h:min:sec.

Movie S6. DipM-mCherry changes its localization during the cell cycle. A synchronized population of cells producing DipM-mCherry (in red) from the native chromosomal *dipM* locus (strain CJW3124) were spotted on an agarose-padded slide

containing M2G medium where they resumed growth at room temperature. The time-lapse microscopy sequence of phase contrast and fluorescent images is shown as an overlay. The arrowhead in the first time point indicates the old pole where a stalk forms at later time points. Time is in min.

Plasmid construction

pNPTSΔdipM::Ω: The flanking regions of *dipM* were amplified using CB15N chromosomal DNA as template and the primer pairs CAACTAGTCCATGCCCAAGCCGCAGCTTC with CAAAGCTTTGGTGCTGCCGCGCTGATCAG and CAGAATTCATGAGCGGAGGGACGACGAAG with CAAAGCTTGCGTCCACAACTGCCTCATAG. The PCR fragments were digested with HindIII and SpeI and with EcoRI and HindIII, respectively. The two PCRs were triple ligated into pNPTS134 digested with EcoRI and SpeI. The resulting plasmid was purified, digested with HindIII and used as recipient of the Ω–Spc (Alexeyev *et al.*, 1995).

pXMCS2ftsA: A PCR fragment of the 3´-end of the *ftsA* gene was obtained with primers CACATATGTCGCGAATGGAGGATCGGAAACAG and CAGCTAGCCGAACATGGCGCGGGGATCAC and using CB15N chromosomal DNA as template. The PCR product was digested with NheI and NdeI and cloned into pXMCS2 digested with the same restriction enzymes.

pdipMCHY: A PCR of the 5´-end of *dipM* was obtained with primers CAGAATTCAGCGCCGCCGCAGCGTCGACG and

CAACCGGTGGCGGGGCAGCACCAGCGCCGGATC, digested with EcoRI and AgeI, and cloned into pCHYC-4.

pVdipM$_{\Delta 501\text{-}609}$CHY: The PCR product obtained with primers CACATATGATGAGGCAGTTGTGGACGCAAG and CAGAATTCTCGCCCGTGCCCTTCACGCCAAAG was digested with EcoRI and NdeI, and cloned into pVCHYC-4.

pVdipM$_{\Delta 297\text{-}609}$CHY: The PCR product obtained with primers CACATATGATGAGGCAGTTGTGGACGCAAG and CAGAATTCTCGACCTGACGCGGCCCCGCCAC was digested with EcoRI and NdeI, and cloned into pVCHYC-4.

pVspCHYdipM$_{\Delta 1\text{-}30}$: The PCR product obtained with primers CAGAATTCCATCCTGATCAGCGCGGCAGC and CAGGTACCCGCTTCACGCCGAACTTCCCG was digested with EcoRI and KpnI, and cloned into plasmid pVsigpepCHYNMCS-4.

pVspCHYdipM$_{\Delta 1\text{-}236}$: The PCR product obtained with primers CAGAATTCCATCCTGATCAGCGCGGCAGC and CAGGTACCACGATGGTCGCCGAGGCCGAG was digested with EcoRI and KpnI, and cloned into plasmid pVsigpepCHYNMCS-4.

pTOPOdipM: The PCR product obtained with primers GCGGATCCAGGAGGAATTAACCATGGGGCAGTTGTGGACGCAAGCGGCGGT

123 G and GGAGCGGGGCAGCACCAGCGCCGGATC was cloned in plasmid pCR2.1-
124 TOPO following the manufacturer instructions.
125
126 **pRVdipMCHY**: Plasmid pTOPO*dipM* was used as a template to obtain a PCR with
127 primers CAGAATTCTCGCGGGGCAGCACCAGCGCCGGATC and
128 CACATATGATGAGGCAGTTGTGGACGCAAG that was digested with NdeI and
129 EcoRI, and then was cloned into pRVCHYCMCS-5.
130
131 **pRVdipM$_{\Delta 54\text{-}419}$CHY:** Plasmid pTOPOdipM was used as template for an inverse PCR
132 reaction (Ochman *et al.*, 1988) with primers
133 AGGTACCCTGGGCCTGGATCGTCTCACC and
134 CAGGTACCGCCAACACCTATGCCCGCGTG. The PCR product was digested with
135 KpnI and re-ligated. The resulting plasmid was used as a template in a PCR reaction
136 with primers CAGAATTCTCGCGGGGCAGCACCAGCGCCGGATC and
137 CACATATGATGAGGCAGTTGTGGACGCAAG. The PCR product was digested
138 with NdeI and EcoRI and cloned into pRVCHYCMCS-5.
139
140 **pVLysM1ACHY:** Plasmid pVdipM$_{\Delta 296\text{-}609}$CHY was used as template in an inverse
141 PCR reaction with primers CAGGTACCACGACCGCCAAGGCCTATGTC and
142 CAGGTACCGGGCTTGCCCGCGACTTCGAC. The product was digested with KpnI,
143 ligated and electroporated. The sequence of the resultant plasmid was verified.
144
145 **pVLysM1BCHY:** Plasmid pVdipM$_{\Delta 296\text{-}609}$CHY was used as template in an inverse
146 PCR reaction with primers CAGGTACCGACATAGGCCTTGGCGGTCGTC and

CAGGTACCCCGATCAAGACCACCCAGGTG. The product was digested with KpnI, ligated and electroporated. The sequence of the resultant plasmid was verified.

Strain construction

CJW2959: Plasmid pVspCHYNMCS-4 was electroporated into CB15N and insertion into the chromosome was selected by plating cells on PYE plates containing gentamycin (Gm).

CJW3116: Plasmid pVspCHYdipM was electroporated into CB15N and insertion into the chromosome was selected by plating cells on PYE plates containing Gm.

CJW3117: Plasmid pVspCHYdipM$\Delta_{1\text{-}236}$ was electroporated into CB15N and insertion into the chromosome was selected by plating cells on PYE plates containing Gm.

CJW3118: Plasmid pVspCHYdipM$\Delta_{1\text{-}412}$ was electroporated into CB15N.

CJW3121: Plasmid pVdipM$_{\Delta 297\text{-}609}$CHY was electroporated into CB15N and insertion into the chromosome was selected by plating cells in PYE plates containing Gm. Insertion at the *vanA* locus was identified by a wild type phenotype of the colonies when grown in M2G liquid medium in the absence of 250mM vanillic acid. The site of insertion was verified by PCR.

CJW3122: Plasmid pVdipM$_{\Delta 501\text{-}609}$CHY was electroporated into CB15N and insertion into the chromosome was selected by plating cells on PYE plates containing Gm. Insertion at the *dipM* locus was identified through by a Δ*dipM* phenotype of the colonies

when grown in M2G liquid medium in the absence of 250mM vanillic acid. The site of insertion was verified by PCR.

CJW3124: Plasmid pdipMCHY was electroporated into CB15N.

CJW3137: Plasmid pNPTSΔdipM::Ω was electroporated into CB15N. Colonies in which the plasmid integrated into the chromosome were selected by plating cells on PYE plates containing spectomycin (Spc) and kanamycin (Kan). Second crossover events were selected through negative selection of the *sacB* gene (RiedandCollmer, 1987) by plating cells on PYE plates containing 3% sucrose and Spc. Deletion of *dipM* was verified by PCR.

CJW3187: Plasmid pXMCS2ftsA was electroporated into CB15N cells and the transformants were grown in PYE plates containing 0.3% xylose and kanamycin.

CJW3430: Plasmid pNPTSΔdipM::Ω was electroporated into MT196. Colonies in which the plasmid integrated into the chromosome were selected by plating cells on PYE plates containing spectomycin (Spc) and kanamycin (Kan). Second crossover events were selected through negative selection of the *sacB* gene (RiedandCollmer, 1987) by plating cells on PYE plates containing 3% sucrose and Spc. Deletion of *dipM* was verified by PCR.

CJW3436: Plasmid pdipMCHY was electroporated into MT196.

CJW3437: The *dipM*::pdipMCHY allele was transduced into strain CJW1715 with a ΦCR30 phage lysate obtained from strain CJW3124. Transductants were on PYE plates containing Gm.

CJW3438: The *dipM*::pdipMCHY allele was transduced into strain YB1585 with a ΦCR30 phage lysate obtained from strain CJW3124. Transductants were selected on PYE plates containing Gm.

CJW3439: Plasmid pVdipM$\Delta_{501\text{-}609}$CHY was electroporated into CB15N, insertion into the chromosme was selected by plating cells on PYE plates containing Gm. Insertion at the *vanA* locus was identified by a wild type phenotype of the colonies when grown in M2G liquid medium in the absence of 250mM vanillic acid. The site of insertion was verified by PCR.

CJW3440: Plasmid pRVdipMCHY was electroporated into CJW3137 and transformants were selected by repetitive subculture in liquid M2G media containing oxytetracyclin (OTc).

CJW3444: Plasmid pRVdipM$_{\Delta 50\text{-}412}$CHY was electroporated into CJW3137 and transformants were selected by repetitive subculture in liquid M2G media containing OTc.

CJW3445: Plasmid pVdipM$_{\Delta 296\text{–}609}$CHY was electroporated into CB15N. Integrants were selected on PYE plates containing Gm. Insertion at the *dipM* locus was identified

by a Δ*dipM* phenotype of the colonies when grown in M2G liquid medium in the absence of 250mM vanillic acid. The site of insertion was verified by PCR.

CJW3446: The *ftsZ*:pBJM allele was transduced into strain CJW3122 with a ΦCR30 phage lysate obtained from strain YB1585. Transductants were selected on PYE plates containing Kan and 0.3% xylose.

CJW3447: The *ftsA*::pXMCS2ftsA allele was transduced into strain MT196 with a ΦCR30 phage lysate obtained from strain CJW3187. Transductants were selected on PYE plates containing Kan and 0.3% xylose. The resultant strain was transformed with plasmid pCHYCdipM and integrants were selected by on PYE plates containing Gm.

CJW3448: Strain CJW3455 was transformed with plasmid pNPTSΔdipM::Ω. Integrants were selected on PYE plates containing Spc and Kan. Second crossover events were selected on PYE plates containing 3% sucrose and Spc. Deletion of *dipM* was verified by PCR.

CJW3449: Plasmid pXGFPMCS2 (Thanbichler *et al.*, 2007) was electroporated into CJW3137.

CJW3526: Plasmid pVLysM1ACHY was electroporated into CB15N. Integration of the plasmid was selected by plating in Gm-containing PYE plates. The site of insertion was verified by PCR.

CJW3528: Plasmid pVLysM1BCHY was electroporated into CB15N. Integration of the plasmid was selected by plating in Gm containing PYE plates. The site of insertion was verified by PCR.

CJW3530: The *ftsZ*:pBJM allele was transduced into strain CJW3121 with a ΦCR30 phage lysate obtained from strain YB1585. Transductants were selected on PYE plates containing Kan and 0.3% xylose.

CJW3550: Single *vanA*::pVsigpepCHYN-4 allele from strain CJW2959 was transduced into YB1585.

CJW3551: The *vanA*::pVsigpepCHYN-4 allele from strain CJW2959 was transduced into CB15N/pNPTSΔdipM::Ω using a ΦCR30 phage lysate obtained from strain CJW2959. Transductants were selected on PYE plates containing Gm. Second crossover events were selected through negative selection of the *sacB* gene by plating cells on PYE plates containing 3% sucrose and Spc. Deletion of *dipM* was verified by PCR.

CJW3591: Plasmid pdipMCHY was electroporated into PC8848 and insertion into the chromosome was selected on PYE plate containing Gm.

References

Alexeyev, M.F., Shokolenko, I.N.and Croughan, T.P., (1995) Improved antibiotic-resistance gene cassettes and omega elements for *Escherichia coli* vector construction and in vitro deletion/insertion mutagenesis. *Gene* **160**: 63-67.

268 Ochman, H., Gerber, A.S.and Hartl, D.L., (1988) Genetic applications of an inverse
269 polymerase chain reaction. *Genetics* **120**: 621-623.
270 Ried, J.L.and Collmer, A., (1987) An *nptI-sacB-sacR* cartridge for constructing
271 directed, unmarked mutations in gram-negative bacteria by marker exchange-
272 eviction mutagenesis. *Gene* **57**: 239-246.
273 Thanbichler, M., Iniesta, A.A.and Shapiro, L., (2007) A comprehensive set of plasmids
274 for vanillate- and xylose-inducible gene expression in *Caulobacter crescentus*.
275 *Nucleic Acids Res* **35**: e137.
276
277

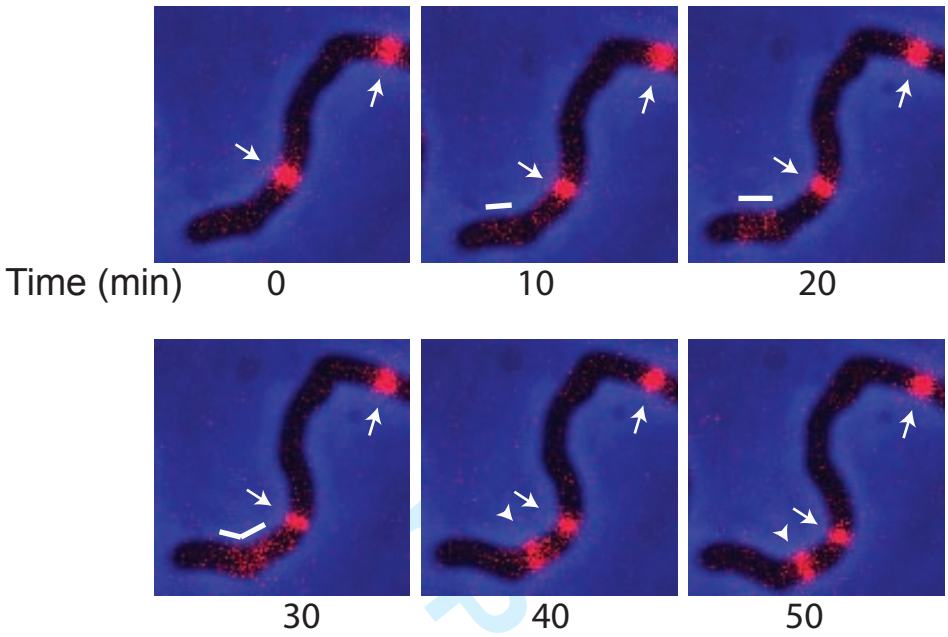

Fig. S1

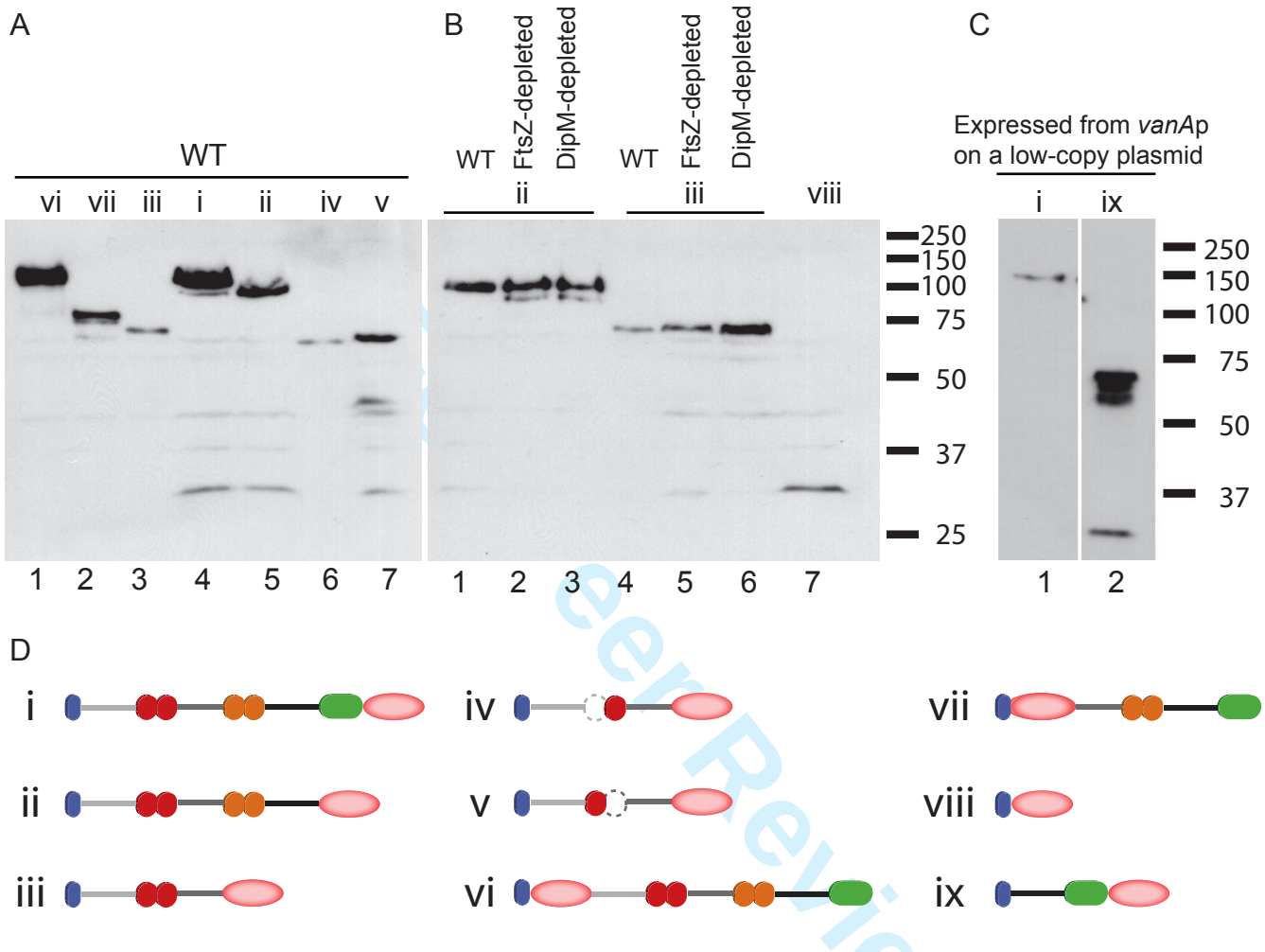

Fig. S2

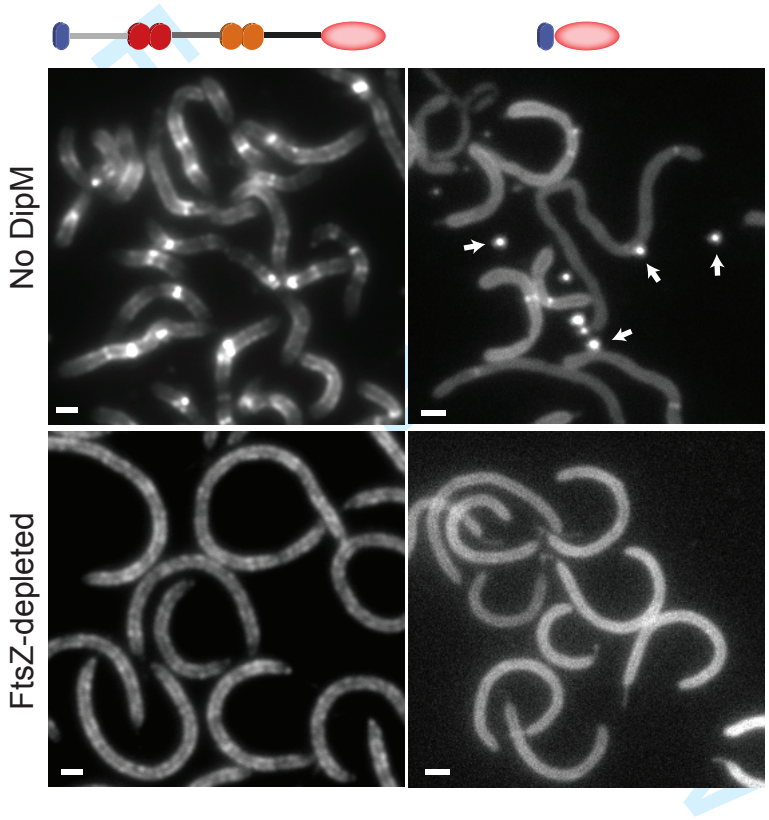

Fig. S3