1 **SUPLEMENTARY MATERIAL**

2 **Primers**

3 ftsIF1 NdeI	CAA CATATG TTGTTCTCGCGCGCCGGGCAGG
4 ftsIR1 EcoRI	CAA GAATTC GGGACGGTTGAACAGGTCGGACAG
5 ftsIdelF1 SpeI	CAAACTAGTACCTGGCCGAGGTGATCGAGC
6 ftsIdelR1 HindIII	CAAAAGCTTCCGGGACCCAGGTTCGAGAGG
7 ftsIdelF2 HindIII	CAAAAGCTTTCGCCAACTCGCCCAAGAACG
8 ftsIdelR2 EcoRI	CAAGAATTCCGCCGAACACCGCGATCAGC
9 ftsIF3 SacI	CAAGAGCTCGCATGAGCCTCTCGAACCTGGGTCC
10 ftsNF1 NdeI	CAACATATGGATGGCGTGCGCGGTCCCAAC
11 ftsNR1 EcoRI	CAAGAATTCGGAAGGCGCGCCCATCAGAAGG
12 delftsNF1 EcoRI	CAAGAATTCCAACAACCATGACGTCGACGAC
13 delftsNR1 BamHI	CAAGGATCCGGACATCTCAGCTAGGCTCC
14 delftsNF2 BamHI	CAAGGATCCTTCGTAAAGTGAGCCTTCTGATGG
15 delftsNR2 HindIII	CAAAAGCTTGATCTTCTTGATCGCCTTCTTCG
16 ftsNF2 NdeI	CAACATATGTCCGATCCGCACCGCGGGGCCTATAC
17 ftsNF1 SacI	CAAGAGCTCCGTCCGATCCGCACCGCGGGGCC
18 ftsNR1 NheI	CAAGCTAGCGGCTCACTTACGAAGCAGGATTTGC
19 ftsWF2 SacI	CAA GAGCTCCGGCCTCCAACGCGACCCACG
20 ftsWR2 NheI	CAAGCTAGCTTCCCCCGGCGGCGACAACAGC
21 dipMmChF1 NdeI	CAACATATGAGGCAGTTGTGGACGCAAG
22 dipMmChR1 SacI	CAAGAGCTCCGCGGGGCAGCACCAGCGCCGGATC
23 murGF1 SacI	CAAGAGCTCGGTTGGCAATCCGGTGCGTCC
24 murGR1 EcoRI	CAAGAATTCCTGCGCGCCGTCTTCTCCACCAG
25 mreBF1 NdeI	CAACATATGTTCTCTTCCCTTTTCGGCGTGATC
26 ftsQR1 EcoRI	CAAGAATTCTTGCCACCTCAGCCCATCACGTCAC
27 ftsQF2 NdeI	CAACATATGGTTATGCTCGCGACCGGCCATC
28 delftsLF1 SpeI	CAAACTAGTTTGGGTTTGGCGGGGTTTAAG
29 delftsLR1 EcoRV	CAAGATATCCCGGACCCGGCGATTGAAGAC
30 delftsLF2 EcoRV	CAAGATATCGTCCAGGGGGCGTTGCGATGAG

31	delftsLR2 SpeI	CAAACTAGTGGGACGGTTGAACAGGTCGGACAG
32	ftsLF1 NdeI	CAACATATGACGGCGGCTGGCGTCTTCAATCG
33	ftsLR1 EcoRI	CAAGAATTCGGACCCAGGTTCGAGAGGCTCATCG
34	ftsbR1 EcoRI	CAAGAATTCAGCGACAGATCAGCGCGAACG
35	ftsLF2 NdeI	CAACATATGAAGACCTTCGCCGGTCGCGAG
36	ftsBF2 NdeI	CAACATATGCACGCTCTGACGGGTGATCG
37	mreBswF1 HindIII	CAAAAGCTTGATGCTTGGCGGGTCCCATCG
38	mreBswR1	CAAGCATGCGCTCGAGCCAGAACCGGGCGCGCGG
39	SphIXhoI	GCGGTGCCGATTTC
40	mreBswF2	CAAGCATGCTCTGGCGCGCCGGCCGACGGCG
41	SphIAscI	AAGGTCTGTCG
42	mrebr2 EcoRI	GAAGAATTCTCAAGGCCCAGGCCCGTCCAG
43	mCherryF2 XhoI	CAACTCGAGCATGGTCTCCAAGGGCGAGGAGGATAAC
44	mCherryR2 AscI	CAAGGCGCGCCCGACTTGTACAGCTCGTCCATGC
45	tmRNAF1 EcoRI	CAAGAATTCGGCGGCCAACGATAACTTCGCTGAAGAG
46	tmRNAR1 XbaI	CAATCTAGACTTAAGAGGCGAATTCACCTGC
47	CC3721 F3 NdeI	CAACATATGCGGTCAAAAACAGAACAAGGTTC
48	3721mchRI EcoRI	CAAGAATTCCGTTTGCGACACTGAACGGCCGGATC
49	CC3721 F3 NdeI	CAACATATGCGGTCAAAAACAGAACAAGGTTC
50	CC3721 F1 HindIII	CAAAAGCTTCGGGCCAGCATCTGGTCGAGG
51	CC3721 R1 BamHI	CAAGGATCCCGTCAAACCCATTCGAGACGAACC
52	CC3721 F2 BamHI	CAAGGATCCGGATCCGGCCGTTCAGTGTCG
53	CC3721 R2 EcoRI	CAAGAATTCCCTACGATCCATCAGGGGCCAGC
54	3721F6 NdeI	CAACATATGCAGGGTCCCCGCGTCACGCCCTCC
55		GGCCTGGAG
56	3721R6 EcoRI	CAAGAATTCCCCGATCCCAAGCCGACCTATCG
57	3721mchF1 HindIII	CAAAAGCTTGAGGTGCCGCGCTACGTTTCG

58

59 **Plasmid construction**

60 pNPTΔdipIΩ

61 The PCR products obtained using CB15N chromosomal DNA as template and the
62 primer pairs CC3721F1 HindIII/CC3721R1 BamHI and CC3721F2
63 BamHI/CC3721R2 EcoRI were digested with BamHI and ligated. An aliquot of the
64 ligase reaction mixture was used as template for a second PCR with the primers
65 CC3721F1 HindIII and CC3721 R2 EcoRI. The PCR product was cloned in the
66 HindIII/EcoRI sites of pNPTS138. The resulting plasmid was digested with BamHI
67 and ligated with the 2 Kb BamHI fragment containing the Ω^{Spc} from pBOR.
68
69 pXdipItm5
70 The PCR products obtained CB15N chromosomal DNA as template and the primer
71 pairs tmRNAF1 EcoRI/ tmRNAR1 XbaI and CC3721 F3 NdeI/3721mchRI EcoRI
72 were digested with EcoRI and ligated, this resulted in the addition of the following
73 sequence after the last coding codon of *dipI*:
74 AATTCGGCGGCCAACGATAACTTCGCTGAAGAGTTCGCCGTCGCTGCGTAAT
75 GCGGTGCAGGTGAATTCGCCTCTTAAGTCTAGA. After translation the following
76 amino acids were added to the sequence of DipI: N S A A N D N F A E E F
77 A V A A. An aliquot of the ligase reaction mixture was used as template for a
78 second PCR with the primers CC3721 F3 NdeI and tmRNAR1 XbaI. The PCR
79 product was cloned in pCR2.1TOPO the correct orientation of the fragment was then
80 selected and the resultant plasmid was digested with NdeI and SacI. The 600 bp
81 fragment was purified and cloned in pXTCYC-5 digested with the same restriction
82 enzymes.
83
84 pXdipI5
85 The PCR product obtained using CB15N chromosomal DNA as template and the
86 primers 3721mchRI EcoRI and CC3721 F3 NdeI was digested with EcoRI and NdeI
87 and cloned in the NdeI/EcoRI sites of pXTCYC-5. No additional amino acids were
88 added to que sequence of DipI.
89
90 pNPTΔftsN
91 The PCR products obtained using CB15N chromosomal DNA as template and the
92 primer pairs delftsNF1 EcoRI / delftsNR1 BamHI and delftsNF2 BamHI / delftsNR2
93 HindIII were digested with BamHI and ligated. An aliquot of the ligase reaction
94 mixture was used as template for a second PCR with the primers delftsNF1 EcoRI
95 and delftsNR2 HindIII. The PCR product was cloned in the HindIII/EcoRI sites of
96 pNPTS138.
97
98 pXftsN5
99 The PCR product obtained using CB15N chromosomal DNA as template and the
100 primers ftsNF2 NdeI and ftsNR1 EcoRI was digested with EcoRI and NdeI and
101 cloned in the NdeI/EcoRI sites of pXTCYC-5. No additional amino acids were added
102 to the sequence of FtsN.
103
104
105 pNPTΔftsI

The PCR products obtained using CB15N chromosomal DNA as template and the primer pairs ftsIdelF1 SpeI / ftsIdelR1 HindIII and ftsIdelF2 HindIII/ftsIdelR2 EcoRI were digested with HindIII and ligated. An aliquot of the ligase reaction mixture was used as template for a second PCR with primers ftsIdelF1 SpeI and ftsIdelR2 EcoRI. The PCR product was cloned in the SpeI/EcoRI sites of pNPTS138.

pXftsI5

The PCR product obtained using CB15N chromosomal DNA as template and the primers ftsIF4 NdeI and ftsIR1 EcoRI was digested with EcoRI and NdeI and cloned in the NdeI/EcoRI sites of pXTCYC-5. No additional amino acids were added to the sequence of FtsI.

pNPTΔftsL

The PCR products obtained using CB15N chromosomal DNA as template and the primer pairs delftsLF1 SpeI / delftsLR1 EcoRV and delftsLF2 EcoRV / delftsLR2 SpeI were digested with EcoRV and ligated. An aliquot of the ligase reaction mixture was used as template for a second PCR with delftsLF1 SpeI and delftsLR2 SpeI. The PCR product was cloned in the SpeI site of pNPTS138.

pXftsL5

The PCR product obtained using CB15N chromosomal DNA as template and the primers ftsLF1 NdeI and ftsLR1 EcoRI was digested with EcoRI and NdeI and cloned in the NdeI/EcoRI sites of pXTCYC-5. No additional amino acids were added to the sequence of FtsL.

pVdipMCHY2

The PCR product obtained using CB15N chromosomal DNA as template and the primers dipMmCHF1 NdeI and dipMmChR1 SacI was digested with NdeI and SacI and cloned in the NdeI/SacI sites of pVCHYC-2. This resulted in the fusion of DipM and mCherry by the following linker: RAPENSNVTRHRSAT.

pmurGCHY4

The PCR product obtained using CB15N chromosomal DNA as template and the primers murGF1 SacI and murGR1 EcoRI was digested with SacI and EcoRI and cloned in the SacI/EcoRI sites of pCHYC-4. This resulted in the fusion of MurG and mCherry by the following linker: RAPENSNVTRHRSAT.

pVCHYftsI4

The PCR product obtained using CB15N chromosomal DNA as template and the primers ftsIF3 SacI and ftsIR1 EcoRI was digested with SacI and EcoRI and cloned in the SacII/EcoRI sites of pVCHYN-4. This resulted in the fusion of mCherry and FtsI by the following linker: PAGALINMHGTLRSRAP.

153 pVCHYftsN6
154 The PCR product obtained using CB15N chromosomal DNA as template and the
155 primers ftsNF1 SacI and ftsNR1 NheI was digested with SacI and NheI and cloned
156 in the SacI/NheI sites of pVCHYN-6. This resulted in the fusion of mCherry and FtsN
157 by the following linker: PAGALINMHGTLRSRAP.
158
159 pXVENftsB2

160 The PCR product obtained using CB15N chromosomal DNA as template and the
161 primers ftsBF1 SacI and ftsBR1 NheI was digested with SacI and NheI and cloned
162 in the SacI/NheI sites of pXVENN-2. This resulted in the fusion of Venus and FtsB
163 by the following linker: PAGALINMHGTLRSRAP.
164

165 pXVENftsN2

166 The PCR product obtained using CB15N chromosomal DNA as template and the
167 primers ftsNF1 SacI and ftsNR1 NheI was digested with SacI and NheI and cloned
168 in the SacI/NheI sites of pXVENN-2. This resulted in the fusion of Venus and FtsN
169 by the following linker: PAGALINMHGTLRSRAP.
170
171 pXVENftsW2

172 The PCR product obtained using CB15N chromosomal DNA as template and the
173 primers ftsWF2 SacI and ftsWR2 NheI was digested with SacI and NheI and cloned
174 in the SacI/NheI sites of pXVENN-2. This resulted in the fusion of Venus and FtsW
175 by the following linker: PAGALINMHGTLRSRAP.
176
177 pVCHYftsW6
178 The PCR product obtained using CB15N chromosomal DNA as template and the
179 primers ftsWF2 SacI and ftsWR2 NheI in the SacI/NheI sites of pVCHYN-6. This
180 resulted in the fusion of mCherry and FtsW by the following linker:
181 PAGALINMHGTLRSRAP.
182
183 pdipICHY4
184 The PCR product obtained using CB15N chromosomal DNA as template and the
185 primers 3721mchF1 HindIII and 3721mchRI EcoRI in the HindIII/EcoRI sites of
186 pCHYC-4. This resulted in the fusion of DipI and mCherry by the following linker:
187 ENSNVTRHRSAT.
188

189 pVmreBswCHY4

190 The mreBswmCherry construct was obtained essentially as previously
191 described(1).The PCR products obtained with oligonucleotides pairs mreBswF1
192 HindIII, mreBswR1 SphI XhoI and mreBswF2 SphI AscI, mrebr2 EcoRI were
193 digested with SphI and ligated. An aliquot of the ligase reaction was used as a
194 template for a PCR reaction with oligonucleotides mreBswF1 HindIII and mrebr2
195 EcoRI. The PCR product was purified, digested with HindIII and EcoRI and cloned

196 in pTZ19R. The resultant plasmid was digested with XhoI and AscI and ligated with
197 the PCR product digested with the same enzymes that was obtained with primers
198 mCherry F2 XhoI and mCherry R2 AscI and pCHYC-4 as template. The resulting
199 plasmid was used as template in a PCR reaction with the oligonucleotides mreBF1
200 NdeI and mrebr2 EcoRI, the PCR product was digested with NdeI and EcoRI and
201 ligated with the 3.9 kb fragment obtained from a NdeI, EcoRI double digestion of
202 pVCHYN-4.

203 pADdipI and pBDdipI

204 The PCR product obtained using CB15N chromosomal DNA as template and the
205 primers 3721F6 NdeI and 3721R6 EcoRI was digested with EcoRI and NdeI and
206 cloned in the NdeI/EcoRI sites of pBGKT7 and pGADT7.
207

208 pADftsI and pBDftsI

209 The PCR product obtained using CB15N chromosomal DNA as template and the
210 primers ftsIF1 NdeI and ftsIR1 EcoRI was digested with EcoRI and NdeI and cloned
211 in the NdeI/EcoRI sites of pBGKT7 and pGADT7.
212

213 pADftsN and pBDftsN

214 The PCR product obtained using CB15N chromosomal DNA as template and the
215 primers ftsNF1 NdeI and ftsNR1 EcoRI was digested with EcoRI and NdeI and
216 cloned in the NdeI/EcoRI sites of pBGKT7 and pGADT7.

217

218 pADftsB and pBDftsB

219 The PCR product obtained using CB15N chromosomal DNA as template and the
220 primers ftsBF2 NdeI and ftsBR1 EcoRI was digested with EcoRI and NdeI and
221 cloned in the NdeI/EcoRI sites of pBGKT7 and pGADT7.

222 pADftsL and pBDftsL

223 The PCR product obtained using CB15N chromosomal DNA as template and the
224 primers ftsLF2 NdeI and ftsLR1 EcoRI was digested with EcoRI and NdeI and cloned
225 in the NdeI/EcoRI sites of pBGKT7 and pGADT7.

226 pADftsQ and pBDftsQ

227 The PCR product obtained using CB15N chromosomal DNA as template and the
228 primers ftsQF2 NdeI and ftsQR1 EcoRI was digested with EcoRI and NdeI and
229 cloned in the NdeI/EcoRI sites of pBGKT7 and pGADT7.

230

231 **Strain construction**

232 SP2 strain was obtained by independently electroporating plasmids pNPTΔdipIΩ
233 and pXdipI5 into CB15N cells, transformants that integrated the plasmids in the
234 chromosome were selected by plating in PYE plates containing kanamycin or
235 tetracycline respectively. The *xylR*::pXdipI5 allele was then transduced into the
236 pNPTΔdipIΩ transformant strain. Excision of the plasmid and replacement of the *dipI*
237 allele by the Ω^{Spc} cassette was selected by plating in PYE plates containing 3%
238 sucrose, 0.2% xylose, spectinomycin and tetracycline followed by replica plating in
239 PYE-Kan and PYE-Tc/Spc plates both containing 0.2% xylose.
240
241 SP3 strain was obtained by independently electroporating plasmids pNPTΔdipIΩ
242 and pXdipItm5 into CB15N cells, transformants that integrated the plasmids in the
243 chromosome were selected by plating in PYE plates containing kanamycin or
244 tetracycline respectively. The *xylR*::pXdipItm5 allele was then transduced into the
245 pNPTΔdipIΩ transformant strain. Excision of the plasmid and replacement of the *dipI*
246 allele by the Ω^{Spc} cassette was selected by plating in PYE plates containing 3%
247 sucrose, 0.2% xylose, spectinomycin and tetracycline followed by replica plating in
248 PYE-Kan and PYE-Tc/Spc plates both containing 0.2% xylose.
249
250 SP4 strain was obtained by transducing the *vanR*::pMT383 into SP3 strain
251
252 SP5 strain was obtained by independently electroporating plasmids pNPTΔftsI and
253 pXftsI5 into CB15N cells, transformants that integrated the plasmids in the
254 chromosome were selected by plating in PYE plates containing kanamycin or
255 tetracycline respectively. The *xylR*::pXftsI5 allele was then transduced into the
256 pNPTΔftsI transformant strain. Excision of the plasmid and replacement of the *dipI*
257 allele by the Ω^{Spc} cassette was selected by plating in PYE plates containing 3%
258 sucrose, 0.2% xylose and tetracycline followed by replica plating in PYE-Kan and
259 PYE-Tc plates both containing 0.2% xylose.
260
261 SP6 strain was obtained by transducing the *vanR*::pVdipMCHYC2 allele into SP3
262 strain
263
264 SP7 strain was obtained by transducing the *vanR*::pVmreBswCHY4 allele into SP3
265 strain
266
267 SP8 strain was obtained by transducing the *murG*::pmurGCHY4 allele into SP3
268 strain
269
270 SP9 strain was obtained by transducing the *vanR*::pVmCHYftsI4 allele into SP3
271 strain
272
273 SP10 strain was obtained by transducing the *vanR*::pVmCHYftsW6 allele into SP3
274 strain
275
276 SP12 strain was obtained by independently electroporating plasmids pNPTΔftsN
277 and pXftsN5 into CB15N cells, transformants that integrated the plasmids in the

chromosome were selected by plating in PYE plates containing kanamycin or tetracycline respectively. The *xylR*::pXftsN5 allele was then transduced into the pNPTΔftsN transformant strain. Excision of the plasmid and replacement of *ftsN* allele by the Δ*ftsN* allele was selected by plating in PYE plates containing 3% sucrose, 0.2% xylose and tetracycline followed by replica plating in PYE-Kan and PYE-Tc plates both containing 0.2% xylose.

SP13 strain was obtained by independently electroporating plasmids pNPTΔftsI and pXftsI5 into CB15N cells, transformants that integrated the plasmids in the chromosome were selected by plating in PYE plates containing kanamycin or tetracycline respectively. The *xylR*::pXftsI5 allele was then transduced into the pNPTΔftsI transformant strain. Excision of the plasmid and replacement of *ftsI* by the Δ *ftsI* allele was selected by plating in PYE plates containing 3% sucrose, 0.2% xylose and tetracycline followed by replica plating in PYE-Kan and PYE-Tc plates both containing 0.2% xylose.

SP14 strain was obtained by independently electroporating plasmids pNPTΔftsL and pXftsL5 into CB15N cells, transformants that integrated the plasmids in the chromosome were selected by plating in PYE plates containing kanamycin or tetracycline respectively. The *xylR*::pXftsL5 allele was then transduced into the pNPTΔftsI transformant strain. Excision of the plasmid and replacement of *ftsL* by the Δ *ftsL* allele was selected by plating in PYE plates containing 3% sucrose, 0.2% xylose and tetracycline followed by replica plating in PYE-Kan and PYE-Tc plates both containing 0.2% xylose

SP15 strain was obtained by electroporating plasmid pdipICHY4 into CB15N cells.

SP16 strain was obtained by transducing the *dipI*::pdipICHY4 allele into CJW3186 strain

SP17 strain was obtained by transducing the *dipI*::pdipICHY4 allele into SP14 strain

SP18 strain was obtained by transducing the *dipI*::pdipICHY4 allele into SP13 strain

SP19 strain was obtained by transducing the *dipI*::pdipICHY4 allele into SP12 strain

SP20 strain was obtained by electroporating plasmid pVmCHYftsN6 into CB15N cells.

SP21 strain was obtained by transducing the *xylR*::pXVENftsB2 allele into SP15 strain.

SP22 strain was obtained by transducing the *xylR*::pXVENftsN2 allele into SP15 strain.

322 SP23 strain was obtained by transducing the *xylR*::pXVENftsW2 allele into SP15
323 strain.
324 SP24 strain was obtained by electroporating plasmid pXdipICHY5 into CB15N cells.

325 SP25 strain was obtained by electroporating plasmid pVsp2dipICHY4 into CB15N
326 cells.

327 SP26 strain was obtained by transducing the *xylR*::pXVENftsI2 allele into SP15
328 strain.
329

330 SP27 strain was obtained by transducing the *vanR*::pVftsLCHY4 allele into SP3
331 strain.

332 SP28 strain was obtained by transducing the *vanR*::pVftsQCHY4 allele into SP3
333 strain.

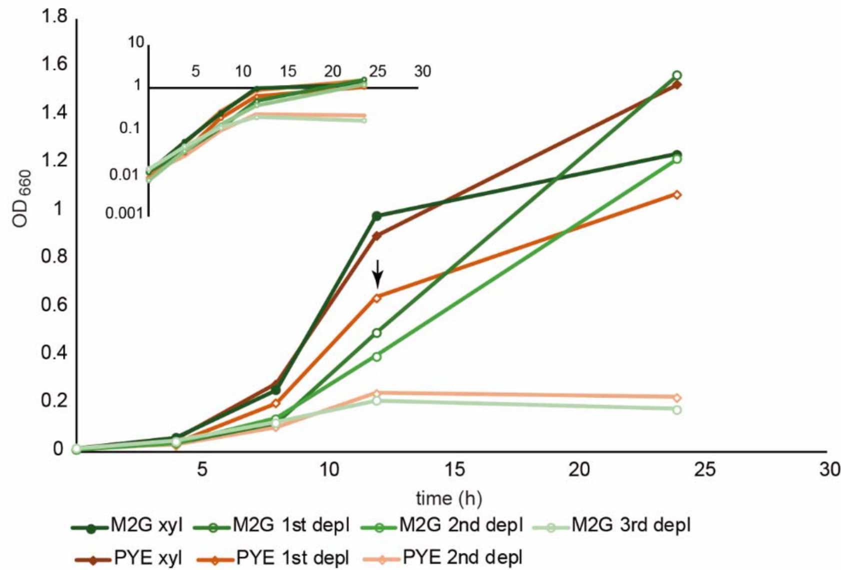
334 SP29 strain was obtained by transducing the *ftsZ*::pPJM1 allele into SP15 strain.

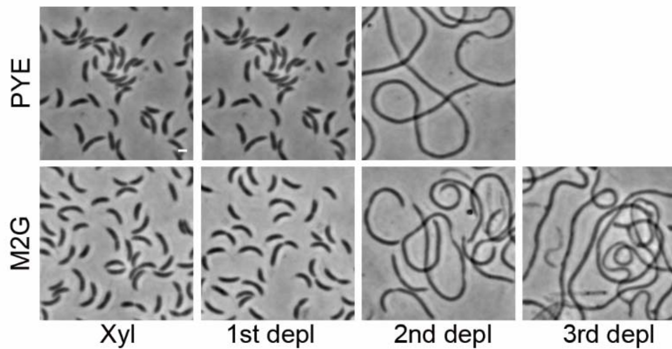
335

336 References

337 1. **Bendezu FO, Hale CA, Bernhardt TG, de Boer PA.** 2009. RodZ (YfgA) is
338 required for proper assembly of the MreB actin cytoskeleton and cell shape
339 in *E. coli*. EMBO J. **28:**193-204.

340





341

342 Fig.S1 Growth curve of DipI depletion strain. Strain SP3 was grown ON in PYE and
343 or M2G medium supplemented with 0.3% xylose. Cultures with or without xylose
344 were inoculated with 1/100 for PYE or 1/50 for M2G of the culture volume to obtain
345 ON cultures that have been growing without xylose for 12 or 24 h. The culture
346 containing xylose was used to inoculate cultures with or without xylose and the
347 cultures without xylose were used to inoculate cultures without xylose. Cells
348 growing in M2G with xylose were washed once with fresh media before inoculating
349 cultures without xylose. Samples were taken from these cultures and the OD660
350 was measured every four hours. The same graph with logarithmic scale is shown
351 in the insert. Growth rate of the cultures with xylose and the first depletion are
352 similar, the higher terminal OD of the culture with xylose is probably due to the
353 additional carbon source. The majority of the increment in OD of the 2nd and 3rd
354 depletion cultures of PYE and M2G respectively, is due to the filamentation of the
355 cells. The arrow indicates the point at which the depletion culture is used to
356 inoculate the next depletion culture. At the 12 h timepoint, pictures of the cells were
357 taken and are shown at the bottom of the figure. White bar indicates 1μm.

358

359

360

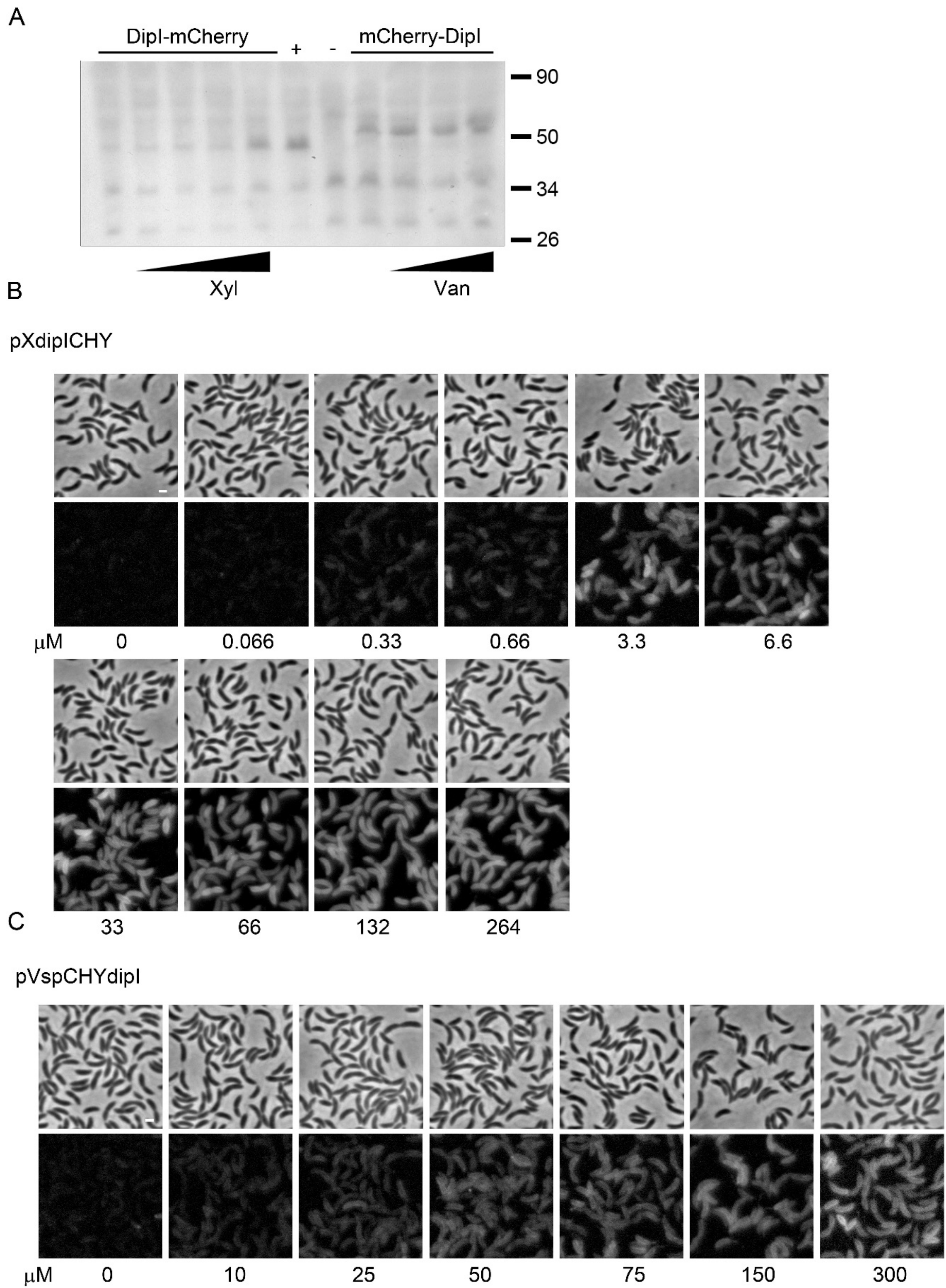
361 Table S1. Generation time and average cell length of WT and SP15 strains

362

Strain	G M2G	G PYE	cell length M2G	cell length PYE
WT	139 ±8	101 ±6	2.6 ±0.8	2.02 ±0.4
SP15	140 ±11	121 ± 9	2.7 ±0.6	3.18 ±0.97

363

364 Generation time (G) and average cell length of CB15N (WT) and SP15 (CB15N
365 *dipI*::pdipICHY-4) in M2G minimal media and PYE rich medium. Generation
366 time is in minutes and cell length in μm. Generation times are the average of
367 three independent experiments and cell lenghts are the representative
368 averages of at least 500 cells from three independent measurements.
369

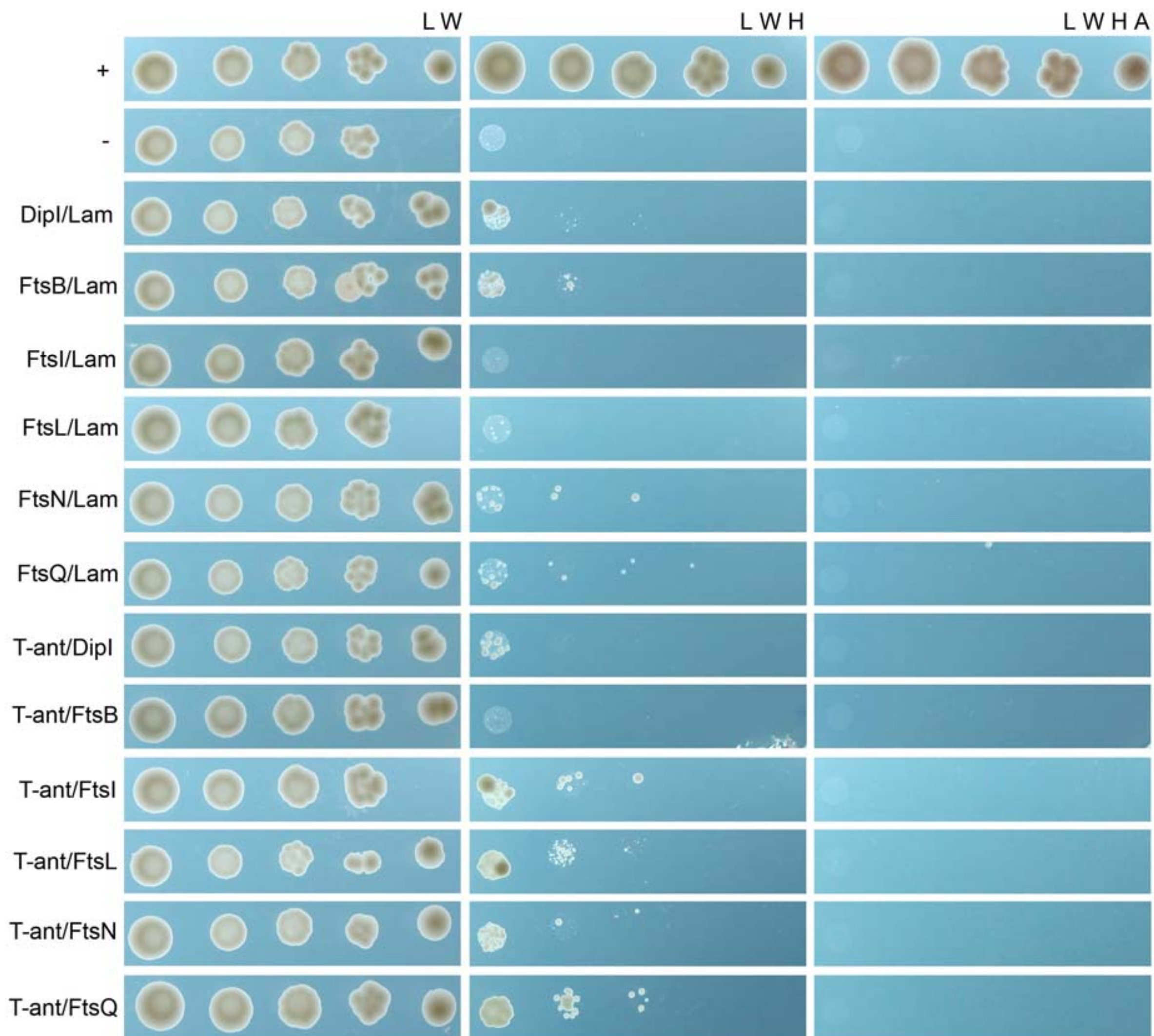


370

371 Fig. S2 Localization of the inducible N-terminal and C-terminal fusions of DipI with
372 mCherry. (A) Western blot of xylose inducible mCherry-DipI and vanillic acid
373 inducible DipI-mCherry. Total cell extracts obtained from cultures of SP24 and SP25

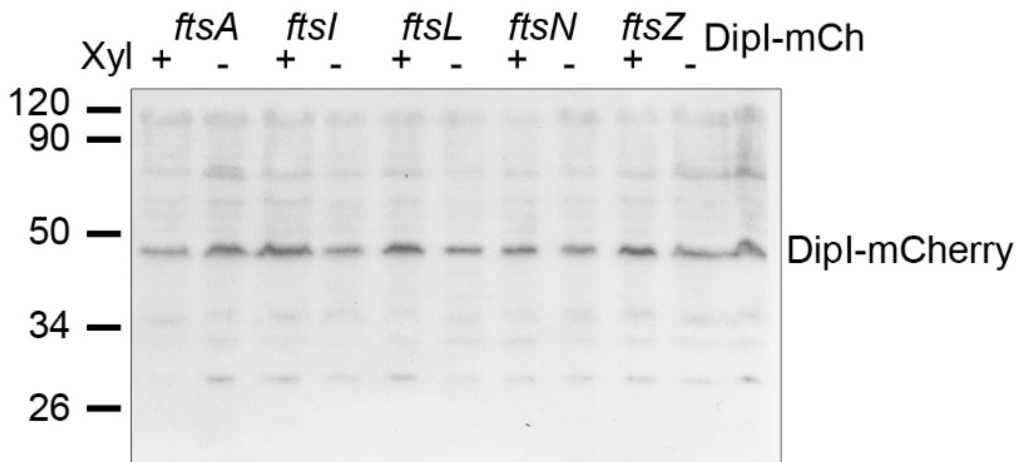
374 strains induced with increasing concentrations of xylose or vanillic acid respectively
375 (Xylose: 0.066, 0.33, 0.66 and 3.3μM; Vanillic acid: 10, 25 and 50μM) were used to
376 immuno-detect the inducible N-terminal and C-terminal mCherry fusions of DipI. (B)
377 Localization of xylose inducible DipI-mCherry at increasing inducer concentrations.
378 Cultures of strain SP24 were induced with the indicated concentrations of xylose for
379 3 h before acquiring phase contrast and fluorescent images. (C) Localization of
380 vanillic acid inducible mCherry-DipI at increasing inducer concentrations. Cultures
381 of strain SP25 were induced with the indicated concentrations of vanillic acid for 3 h
382 before acquiring phase contrast and fluorescent images. + (positive control, total
383 extract of strain SP15); - (negative control, total cell extract of CB15N). Expected
384 molecular weight of fusion proteins in kDa: DipI-mCherry, 44.2; mCherry-DipI 53.6.
385 Whit bars indicate 1 μm.

386



387

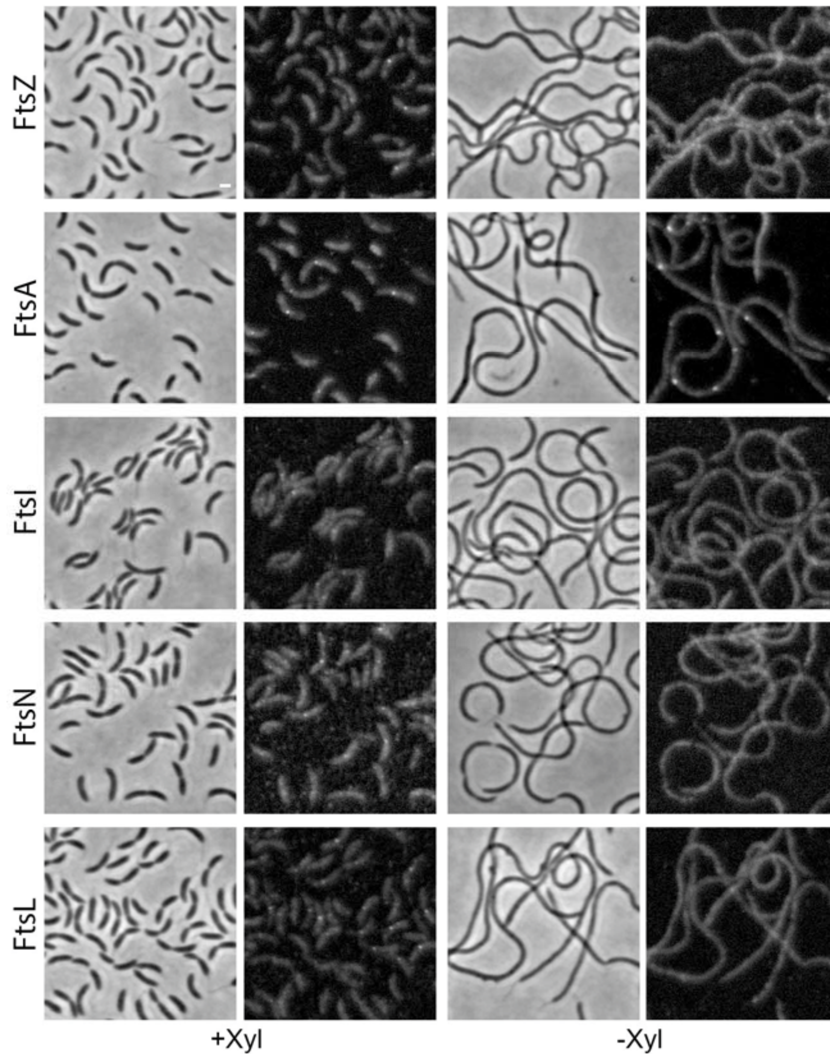
388 Fig. S3 Negative control interactions of division proteins with unrelated proteins.
389 The interaction of DipI and the periplasmic domain of different cell division proteins
390 with unrelated proteins was tested in a yeast two-hybrid assay. The mature DipI
391 protein and the periplasmic domains of FtsQ, L, B, I and N were fused to the
392 activator and DNA binding domains (AD and DBD respectively) of the Gal4 protein
393 and their ability to interact with the human lamin C or the SV40 large T-antigen
394 (expressed from the control plasmids pGBKT7-Lam and pGADT7-T respectively)
395 was determined by the restauration of histidine prototrophy if the interaction is
396 weak and of adenine if it is strong. Serial dilutions of the yeast strain carrying the
397 plasmids being tested were spotted in agar plates lacking leucine and triptophan
398 (growth control), leu, trp and histidine (weak interaction) and trp, leu, his and
399 adenine (strong interaction). The missing amino acid or nucleotide base are
400 indicated at the top of each column (A stands for adenine). Protein fusions are
401 indicated in the following order DBD fusion/AD fusion. B, FtsB; L, FtsL; Q, FtsQ; I,
402 FtsI; N, FtsN, T-ant; SV40 large T-antigen, Lam; human lamin C.



403

404 Fig. S4 Stability of DipI-mCherry in cells depleted of different cell division proteins.

405 The stability of DipI-mCherry in cells depleted of the division proteins FtsA, FtsI,
406 FtsL, FtsN and FtsZ was tested by western blot using mouse α-mCherry
407 antibodies. The gene under control of the inducible promoter is indicated at the top
408 of the figure. A total cell extract of strain SP15 that expresses DipI-mCherry was
409 used as positive control.



410

411 Fig. S5 The localization of DipI-mCherry was determined in cells depleted of
412 different cell division proteins (indicated at the left of each row). Cells were grown
413 in M2G and depletion was carried out as described in Material and Methods.
414 Strains used from top to bottom are: SP29, SP16, SP18, SP19, SP17 Bar
415 indicates 1 μm.

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