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Supplementary Information for

FtsA acts through FtsW to promote cell wall synthesis during cell division in *E. coli*

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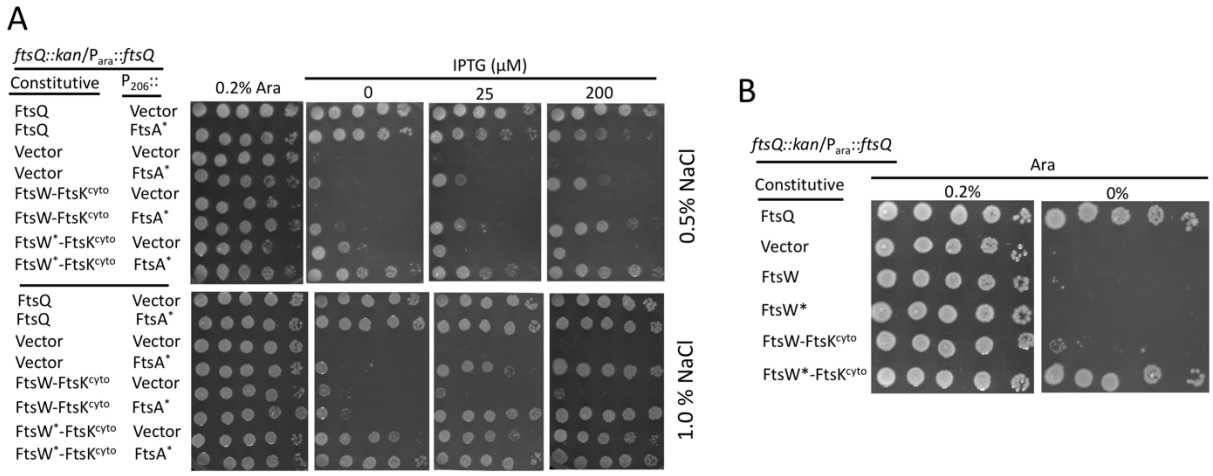

Fig. S1. Testing different conditions for suppression of *ftsQ*. (A) Effect of NaCl concentration on the suppression of the depletion of *ftsQ*. The spot tests were done on LB plates containing 0.5% or 1.0% NaCl in the absence of arabinose (to deplete *ftsQ*) and increasing concentrations of IPTG to induce *ftsA**. Plasmid combinations were introduced into PK3116 (*ftsQ14::kan*/pBAD33-*ftsQ*) to see if they allowed growth in the absence of arabinose. The plasmids included pSEB468 (P_{syn135}::*ftsQ*), pND16 (P_{ftsK}::*ftsW-ftsK*^{cyto}), pND16* (P_{ftsK}::*ftsW*-ftsK*^{cyto}), pSEB306* (P_{206}::*ftsA**) and vector controls. The constructs contained on pGB2 (*ftsQ* and *ftsW-ftsK*^{cyto}) are constitutively expressed whereas *ftsA** was inducible with IPTG. (B) *ftsW** must be fused to *ftsK*^{cyto} to suppress *ftsQ* depletion in the absence of *ftsA**. PK3116 (*ftsQ14::kan*/pBAD33-*ftsQ*) containing the following plasmids that constitutively expressed various genes included: pSEB468 (P_{syn135}::*ftsQ*), pSD257 (P_{syn135} *repA*^{ts}:: *ftsW*), pSD257* (P_{syn135} *repA*^{ts}:: *ftsW*^{M269I}), pND16 (P_{ftsK}::*ftsW-ftsK*^{cyto}), pND16* (P_{ftsK}::*ftsW*-ftsK*^{cyto}) or vector controls were incubated in the absence of arabinose (to deplete *ftsQ*) at 30°C on LB plates with 1% NaCl and increasing concentrations of IPTG.

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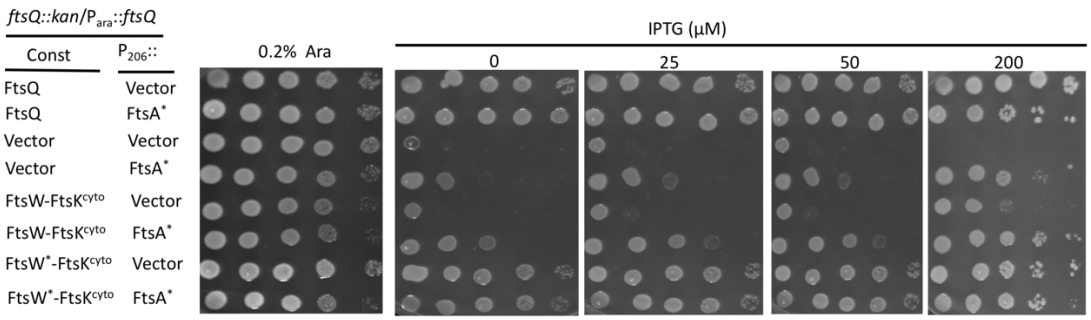

Fig. S2. Suppression of *ftsQ* depletion in MC4100. A) Testing conditions that allow depletion of *ftsQ* in MC4100. Spot tests of JOE417 (MC4100 *ftsQ::kan*/pBAD33-*ftsQ)* containing pND16 (P_{ftsK}::*ftsW-ftsK*^{cyto}) or pSEB468 (P_{syn135}*::ftsQ*) and pSEB306* (P_{206}::*ftsA**) or vector controls were incubated in the absence of arabinose (to deplete *ftsQ*) at 30°C on LB plates with 1% NaCl and increasing concentrations of IPTG. The constructs contained on pGB2 (*ftsQ* and *ftsW-ftsK*^{cyto}) are constitutively expressed whereas *ftsA** was inducible with IPTG (P_{206}::).

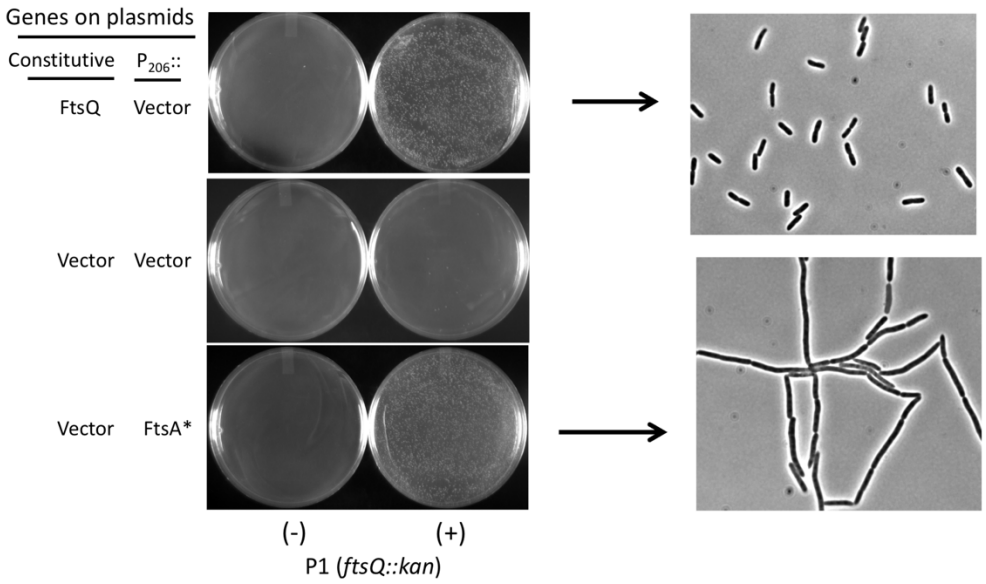

Fig. S3. Deletion of *ftsQ* in the presence of *ftsA**. P1 grown on JOE417 (*ftsQ14::kan/pBAD33-ftsQ*) was used to transduce W3110 to kanamycin resistance. W3110 contained a plasmid constitutively expressing *ftsQ* (pSEB468 [P_{syn135}*::ftsQ*]), a plasmid expressing *ftsA** under an IPTG-inducible promoter (pSEB306* [P_{206}::$ftsA^*$]) or a vector. The experiment is similar to that in Fig. 3. The plates included 200 μM IPTG and were incubated for 2 days at 30°C. The panel on the right contains cells taken from liquid cultures grown under the same conditions. In general, cells observed directly from LB plates displayed less elongated and shorter chaining phenotypes than those from liquid cultures.

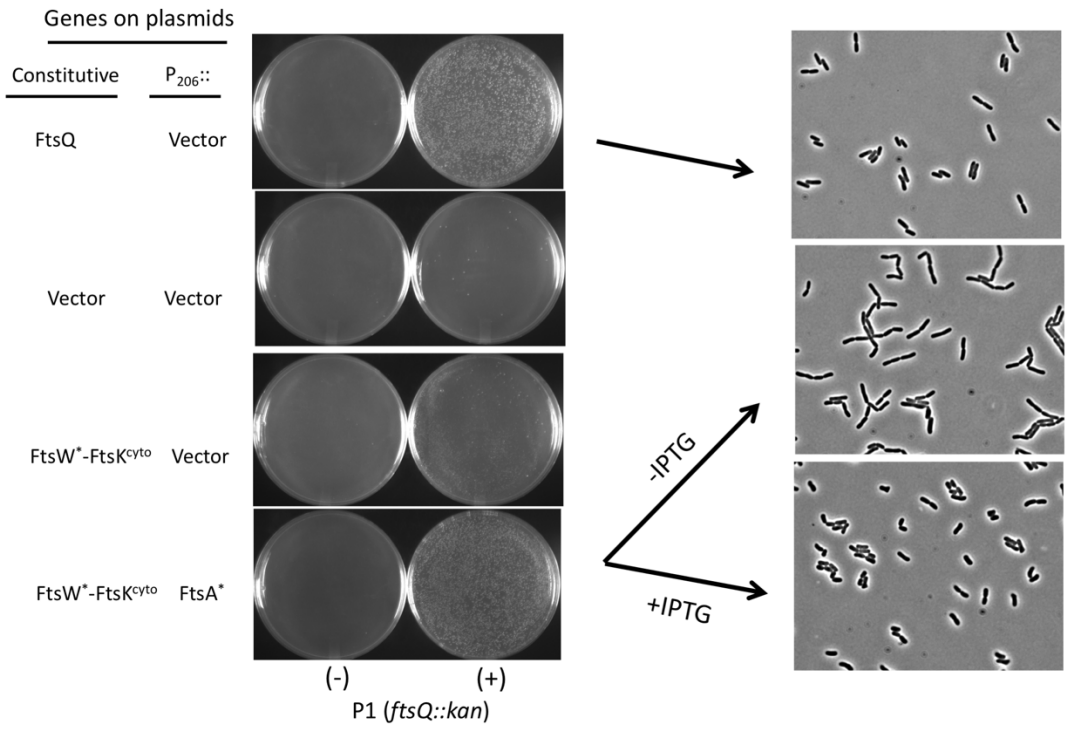

Fig. S4. Deletion of *ftsQ* in the presence of *ftsA** and *ftsW***-ftsK*^{cyto} in MC4100. P1 prepared on JOE417 (*ftsQ14::kan/*pBAD33-*ftsQ)* was used to transduce MC4100 carrying various plasmids to kanamycin resistance. The plasmids were pSEB468 (P_{syn135}*::ftsQ*) or pND16* (P_{ftsK}::*ftsW*-ftsK*^{cyto}) and pSEB306* (P_{206}::*ftsA***)* or a vector. Kanamycin resistant transductants were selected at 30^{o}C on plates containing 200 μM IPTG and appropriate antibiotics. Colonies from the top and bottom rows were re-streaked and grown in liquid culture of the same composition. An exponential culture of a transductant obtained with plasmids containing *ftsW*-ftsK*^{cyto} and *ftsA** was centrifuged, washed and resuspended in LB with or without IPTG. Samples were taken two hours later for photography. Colonies arose on plates expressing just *ftsW*-ftsK*^{cyto} but grew slower and varied in size and were not studied.

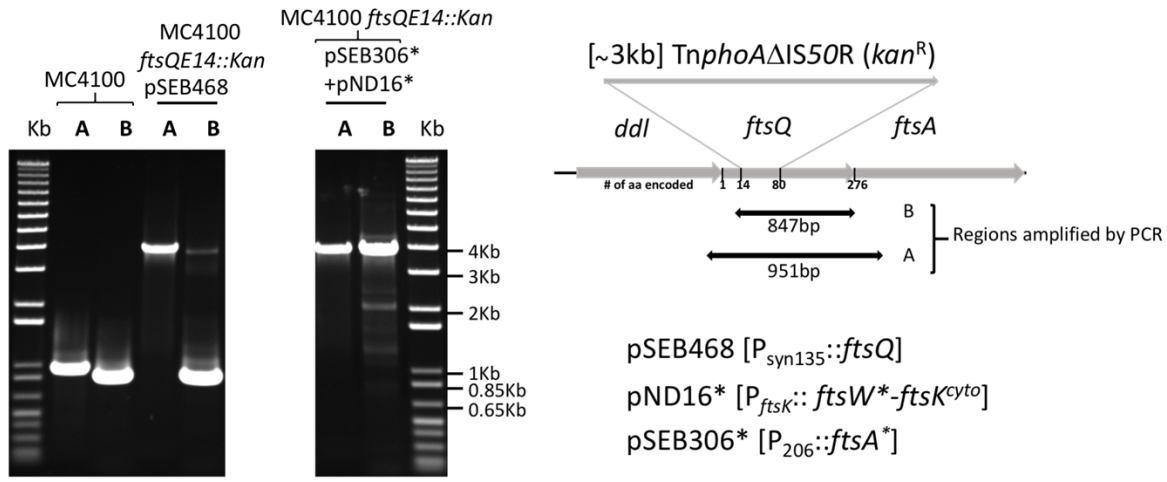

Fig. S5. Confirmation of the deletion of *ftsQ* by PCR. Genomic DNAs were isolated from MC4100 and transductants obtained in Fig. S4 (MC4100 *ftsQ14::kan*/pSEB468 [$P_{syn135:}$*ftsQ*] and MC4100 *ftsQ14::kan*/pND16* [P_{ftsK}::*ftsW*-ftsK*^{cyto}] + pSEB306* [P_{206}::*ftsA**]) and subjected to PCR analysis using two sets of primers as detailed in *Materials and Methods*. One set is internal to *ftsQ* and the other set flanks the *ftsQ* gene. The presence of the *ftsQ14::kan* allele yields a band of ~4 kb.

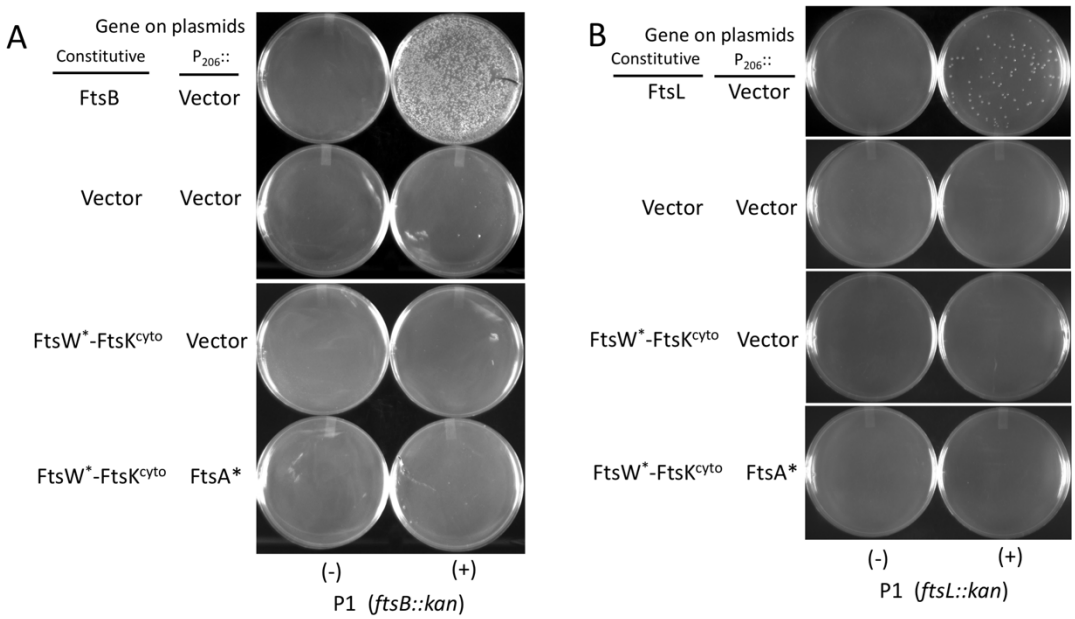

Fig. S6. Combining FtsA* and FtsW*-FtsK^{cyto} cannot bypass *ftsB* or *ftsL*. P1 transduction was used to transduce *ftsB::kan* (SD439) or *ftsL::kan* (SD399) into MC4100 carrying control plasmids expressing *ftsB* (pKTP101 P_{tac}::*ftsB*) or *ftsL* (pKTP100 P_{tac}::*ftsL*) along with a vector or plasmids expressing *ftsW*-ftsK^{cyto}* (pND16*) and a vector or *ftsA** (pSEB306*/P_{206}::*ftsA**). Transductants were selected on plates with 200 μM IPTG for 2 days at 30^{o}C.

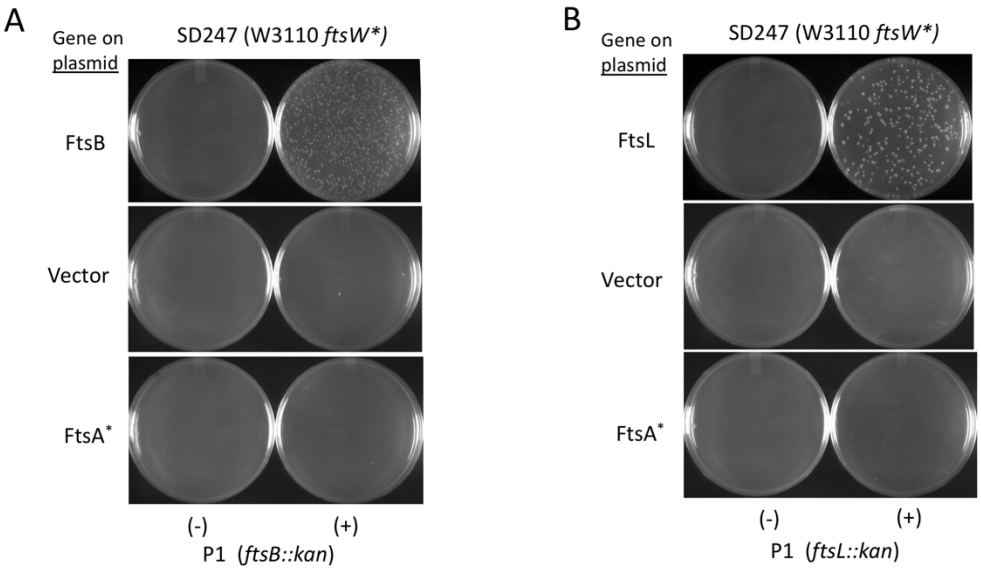

Fig. S7. Combining FtsA* and FtsW* cannot bypass *ftsB* or *ftsL*. P1 phage grown on strains containing *ftsB::kan* (SD439) or *ftsL::kan* (SD399) was used to transduce these alleles into SD247 (*ftsW**) containing plasmids expressing: (A) *ftsB* (pKTP101 P_{tac}::*ftsB*) or (B) *ftsL* (pKTP100 P_{tac}::*ftsL*), a vector or a plasmid expressing *ftsA** (pSEB306* [P_{206}::*ftsA**]). Transductants only emerged with the positive control plasmids. Transductants were selected on plates with 200 μM IPTG for 2 days at 30ºC.

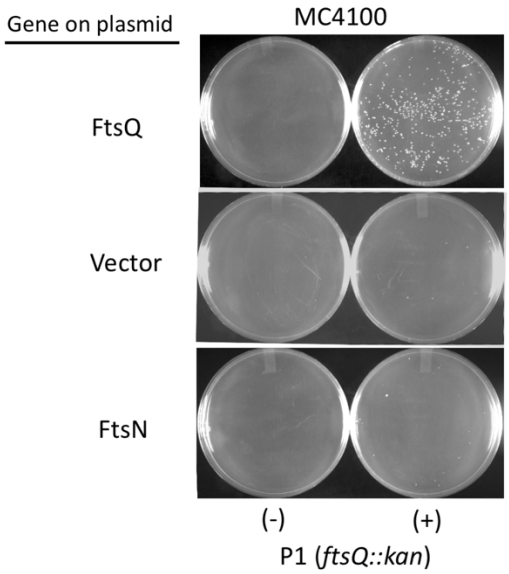

Fig. S8. Overexpression of *ftsN* cannot bypass *ftsQ*. P1 grown on JOE417 (*ftsQ14::kan/pBAD33-ftsQ*) was used to transduce MC4100 to kanamycin resistance. MC4100 contained pSEB468 (P_{syn135}*::ftsQ*), a vector or pKD140 (P_{ftsN}::*ftsN*). Whereas numerous colonies grew on the control plate (pSEB468), only sporadic colonies grew on the plates containing the vector or pKD140. These latter colonies grew poorly when re-streaked and were not studied further.

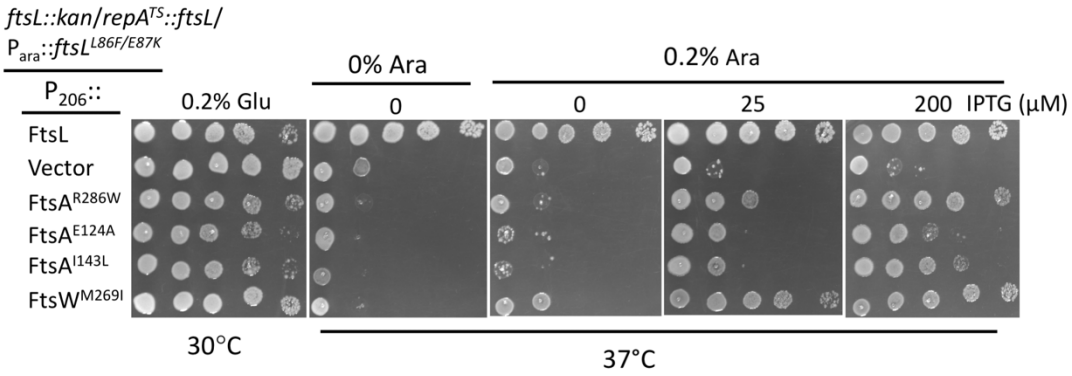

Fig. S9. Effect of *ftsA* alleles on the rescue of *ftsL*$^{L86F/E87K}$. The ability of various *ftsA* alleles to rescue *ftsL*$^{L86F/E87K}$ was examined using strain PK4-1 (*ftsL::kan*/pSC101^{ts} *ftsL*). This strain was transformed with compatible plasmids pSD296-2 (P_{ara}::*ftsL*$^{L86F/E87K}$) and derivatives of pSEB306 (P_{206}::*ftsA*) carrying various alleles of *ftsA*. Controls contained a vector or a plasmid expressing a high level of *ftsL* (pKTP100 [P_{tac}::*ftsL*]). The strains were spot tested on plates at 37°C to deplete *ftsL* and *ftsL*$^{L86F/E87K}$ was induced with arabinose and the *ftsA* alleles were induced with IPTG.

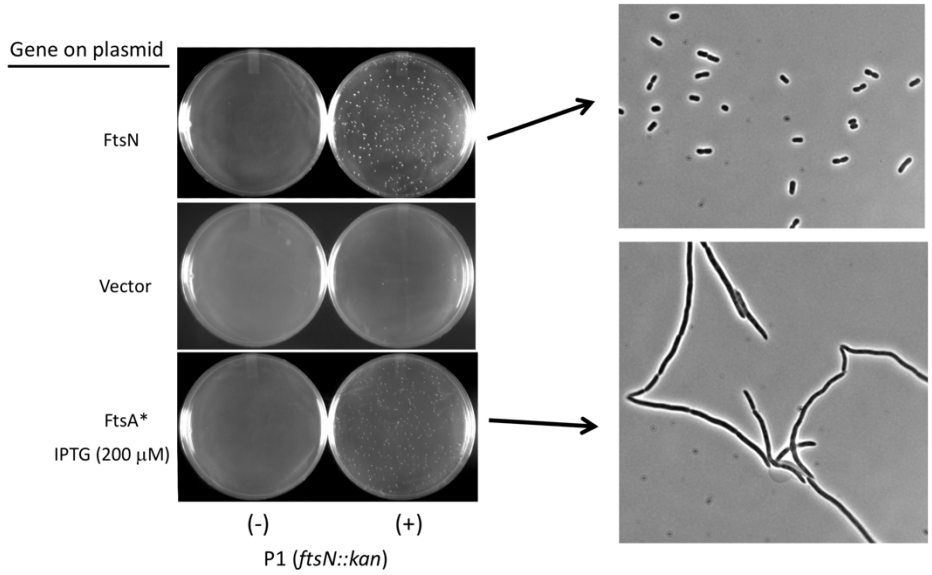

Fig. S10. Deletion of *ftsN* in the presence of *ftsA** leads to slow growing colonies. P1 prepared on CH34 (*ftsN::kan*/pCH201) was used to transduce W3110 to kanamycin resistance containing plasmid pKD140 (P_{ftsN}::*ftsN*) or pSEB306* (P_{206}::*ftsA**). The plates contained 200 μM IPTG and were incubated for 2 days at 30°C. The panel on the right contains cells taken from liquid cultures grown under the same conditions. As in Fig. S3, cells directly taken from LB plates were less elongated and displayed less chaining than those from liquid culture.

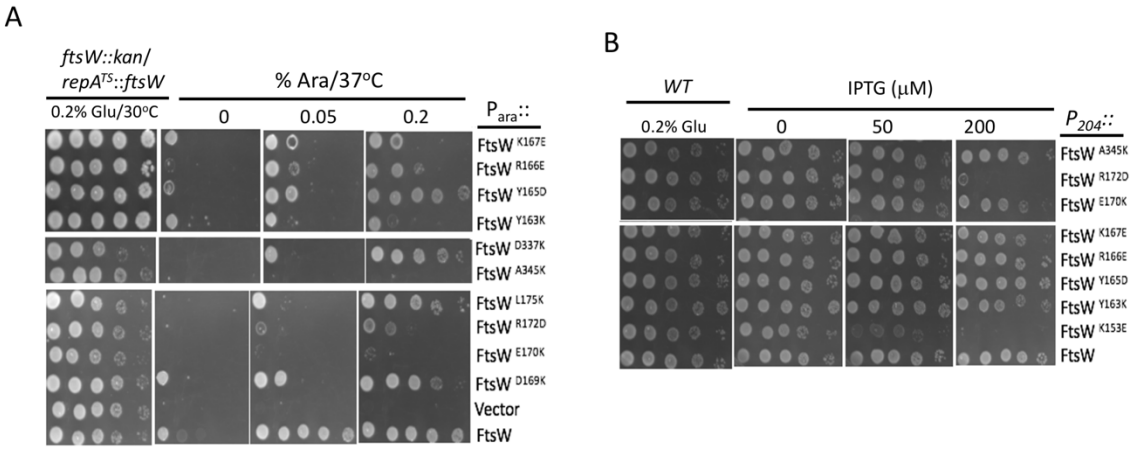

Fig. S11. Characterization of FtsW mutants. A) Complementation test. Spot tests were conducted to determine the ability of *ftsW* mutants to complement a FtsW depletion strain. To do this, derivatives of pDSW406 (P_{ara}::*ftsW*) carrying various *ftsW* mutations were introduced into SD295 (*ftsW::kan*/pSD256 [*repA*^{ts} P_{syn135}::*ftsW*]) at 30°C. Colonies from the transformation were picked into 300 µl LB, serial diluted 10-fold, and 3 µl from each dilution was spotted on LB plates with increasing concentrations of arabinose. B) Dominant negative test of FtsW mutants. To test if the *ftsW* mutations were dominant negative, they were introduced into pSEB429 (P_{204}::*ftsW*) (to achieve higher expression) and the resulting plasmids were transformed into W3110 *recA*. The *ftsW*^{Y165D} mutation was included as a control as it complements a depletion strain and was not expected to be dominant negative. Colonies from the transformation were spotted on plates with increasing IPTG. One additional mutation (*ftsW*^{K153E}) that altered a charged residue in TM4 was also included as a control. It was expected to be dominant negative (the corresponding residue in RodA is nonmutable (17)) and tests showed that it was.

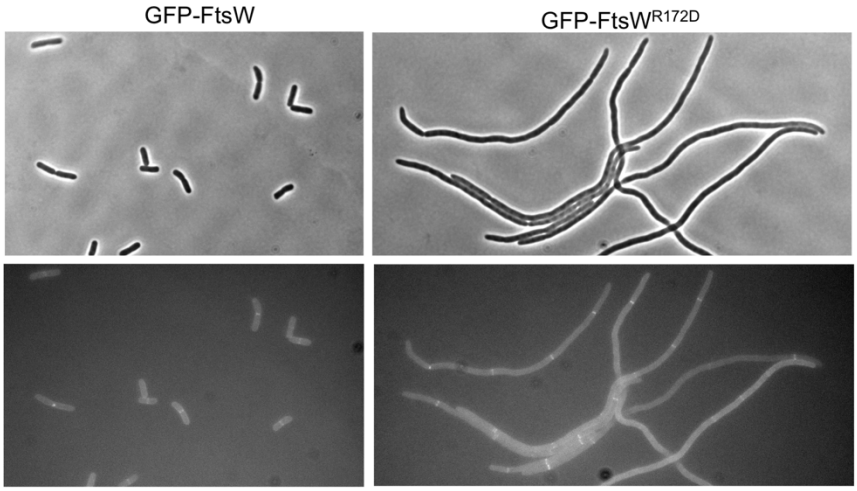

Fig. S12. GFP-FtsW^{R172D} does not have a defect in localization. EC912 (*ftsW::kan*/pDSW406 [P_{ara}::*ftsW*]) containing pDSW311 (P_{206}::*gfp-ftsW*) or pDSW311-1 (P_{206}::*gfp-ftsW^{R172D}*) was grown to exponential phase in the presence of 0.2% arabinose at 30^{o}C, centrifuged and resuspended in media without arabinose. After 2 hours IPTG was added at 1 mM and samples taken for photography 2 hour later.

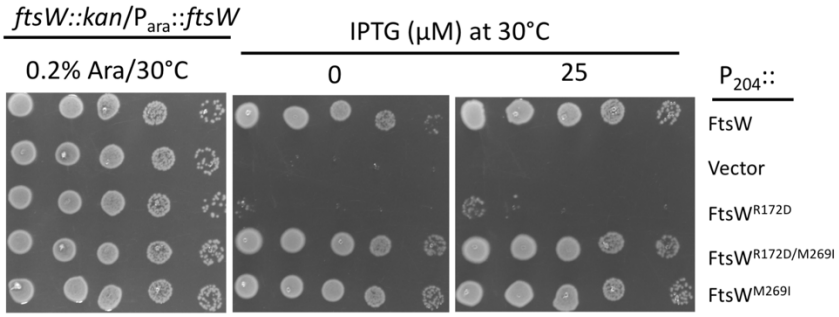

Fig. S13. *ftsW** is an intragenic suppressor of *ftsW*^{R172D}. To test if the *ftsW** activation mutation could rescue *ftsW*^{R172D}, EC912 (*ftsW::kan*/pDSW406 (P_{ara}::*ftsW*) was transformed with derivatives of pSEB429 (P_{204}::*ftsW*) carrying various alleles of *ftsW*. The transformants were tested on plates without arabinose (to deplete WT *ftsW*) and containing increasing amounts of IPTG. Note, basal expression of *ftsW* from pSEB429 (P_{204}::*ftsW*) is sufficient to complement an *ftsW* depleteion strain.

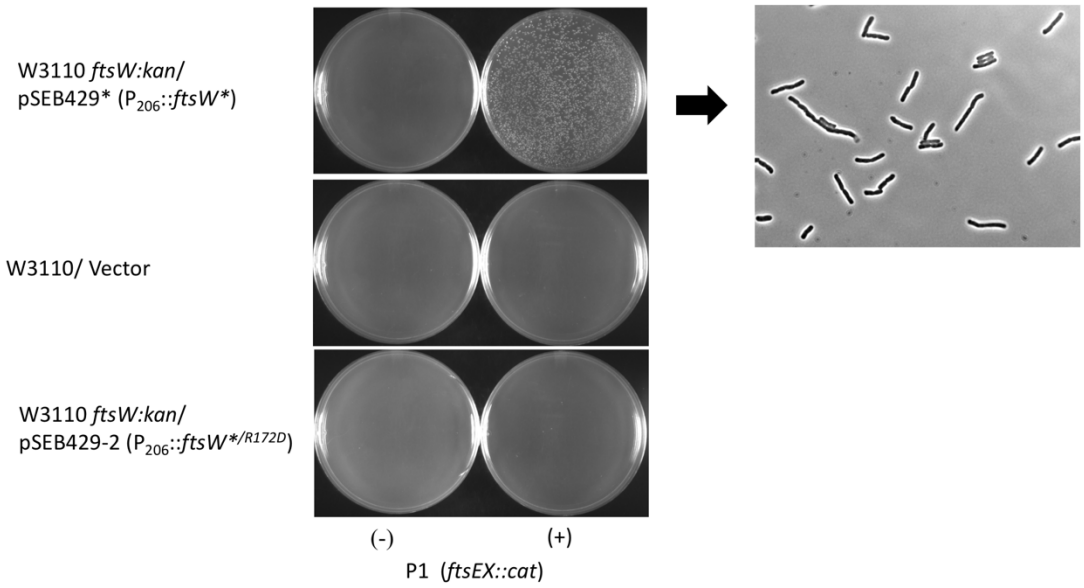

Fig. S14. *ftsEX::cat* can be transduced into *ftsW** but not *ftsW*$^{R172D/}$*. P1 grown on SD205 (*ftsEX::cat*) was used to transduce various derivatives of W3110 to chloramphenical resistance. The stains included W3100 *ftsW::kan*/pSEB429-1 (P_{206}::*ftsW**), W3110 (pDSW208) and W3110 *ftsW::kan*/pSEB429-3 (P_{206}::*ftsW**$^{/R279E}$).

Table S1. *E. coli* strains used in this study

Strain	**Genotype**	**Source or reference**
BL155/pBL194	TB28 *ftsB::kan* / pSC101 (*repA*^{ts} P_{syn135}::*gfp-ftsB*)	(13)
BL156/pBL195	TB28 *ftsL::kan* / pSC101 (*repA*^{ts} P_{syn135}::*gfp-ftsL)*	(13)
BTH101	F-, *cya-99 , araD139, galE15, galK16, rpsL1 (Str*^{r}*) , hsdR2, mcrA1, mcrB1*	(8)
CH34	TB28 *ftsN::kan/*pCH201	(2)
EC912	W3110 *ftsW*::*kan*/pDSW406	(4)
JOE417	MC4100 *JOE309 (MC4100 ara+) ftsQE14::kan/*pBAD33-*ftsQ*	(4)
PK3116	W3110 *ftsQE14::kan/*pBAD33-*ftsQ*	P1(JOE417) x W3110 (pBAD33- *ftsQ*); select Kan^{R} with 0.2% Ara
PK4-1	W3110 *ftsL::kan/*pKTP108	(9)
PK7	W3110 *ftsN::kan*/ pSEB306* P_{206}::*ftsA**)	P1 (BL156) x SD247/ pSEB306*; Select Kan^{R} in the presence of 200 μM IPTG
JS238	MC1061 *malPp*::*lacI*^{Q} *srlC*::Tn10 *recA1*	(11)
MC4100	F^{-} *araD139*Δ(*argF-lac*)*U169 rspL150 relA1 flbB5301 fruA25 deoC1 ptsF25*	(11)
PB143/pCX41	JK268 *ftsZ*^{0} *recA::*Tn*10* pCX41 (cat *repA*^{ts}) *ftsZ*$^{+}$)	(14)
SD205	W3110 *ftsEX::cat*	(15)
SD247	W3110 *ftsW*^{M269I}	(15)
SD264	W3110 *leu*::*Tn10 ftsN::kan*/pBL154	(15)
SD292	W3110 *ftsW::kan*/pSD257 (*aadA repA*^{ts} P_{syn135}*:: ftsW)*	P1 from EC912 x W3110/ pSD257; select Kan^{R} at 30^{o}C
SD295	SD292 *recA::Tn10*	P1 (PB143) X SD292; select Tet^{R}

SD368	W3110 *ftsB::kan*/pSD255 (pSC101 *repA*^{ts} P_{syn35}::*ftsB*)	W3110/ pSC101^{ts} *ftsB* P1(BL155/pBL194) X W3110/pSD255; select for Kan^{R} on LB plates at 30 C and test for TS growth
SD399	W3110 *ftsL::kan*/pSD256 (pSC101 *repA*^{ts} *ftsL*)	P1 (BL156/pBL19 X W3110/pSD256 and select for Kan^{R} on LB plates at 30^{o}C and test for TS growth
SD439	W3110 *ftsL::kan*/pSD296	Transform SD292with pSD296 and passage at 37^{o}C on arabinose
TB28	MG1655, *lacIZYA<>frt*	(2)
W3110	Wild type strain	Laboratory collection
W3110 recA	W3110 *recA56 Tn*10	P1(PB143) X W3110, select Tet^{R} and screen UV sensitivity
WM2355	W3110 *ftsN::kan*/pSC101 *repA*^{ts} *ftsN*	(16)

Table S2. Plasmids used in this study

Plasmid	Genotype	Origin	Source or reference
pBAD33-ftsQ	*cat* P_{ara}*::ftsQ*	pACYC184	(1)
pBL154	*aadA* P_{syn135}*:: ftsN*	pSC101	(2)
pCH201	*bla lacI*^{q} P_{lac}*:: gfp-ftsN*	ColE1	(2)
pDSW208	*bla lacI*^{q} P_{204}*::gfp*	ColE1	(3)
pDSW209	*bla lacI*^{q} P_{206}*::gfp*	ColE1	(3)
pDSW210	*bla lacI*^{q} P_{206}*::gfp*	ColE1	(3)
pDSW311	*bla lacI*^{q} P_{206}*::gfp-ftsW*	ColE1	(4)
pDSW311-1	*bla lacI*^{q} P_{206}*::gfp-ftsW*^{R172D}	ColE1	This work
pDSW406	*cat* P_{ara}*::ftsW*	ColE1	(4)
pJF118EH	*bla lacI*^{q} P_{tac}*::vector*	ColE1	(5)
pKD123	*cat repA*^{ts} P_{ftsN}*::ftsN*	pSC101	(6)
pKD140	*bla* P_{ftsN}*::ftsN*	ColE1	(6)
pKD146	*bla malG*$^{1–33}$*–ftsN*$^{46–319}$	ColE1	(7)
pKT25	*kan Plac-T25-MCS*	p15A	(8)
pKT25-ftsW	*kan Plac-T25-ftsW*	p15A	(9)
pKT25-ftsW*	*kan Plac-T25-ftsW**	p15A	(9)
pKTP100	*bla lacI*^{q} P_{tac}*::ftsL*	ColE1	(9)
pKTP100*	*bla lacI*^{q} P_{tac}*::ftsL*$^{L86F/E87K}$	ColE1	(9)
pKTP101	*bla lacI*^{q} P_{tac}*::ftsB*	ColE1	(9)
pKTP107	*cat* P_{ara}*::ftsL*$^{\Delta cyto}$	pACYC184	(9)
pKTP108	*aadA repA*^{TS} P_{syn135}*:: ftsL*	pSC101	(9)
pND16	*aadA* P_{ftsK}*:: ftsW-ftsK*$^{179-1329(cyto)}$	pSC101	(10)
pND16*	*aadA* P_{ftsK}*::ftsW*-ftsK*$^{179-1329}$	pSC101	This work
pSD255	*aadA repA*^{ts} P_{syn135}*:: ftsB*	pSC101	This work
pSD256	*aadA repA*^{ts} P_{syn135}*::ftsL*	pSC101	(8)
pSD257	*aadA repA*^{ts} P_{syn135}*:: ftsW*	pSC101	This work
pSD257*	*aadA repA*^{ts} P_{syn135}*:: ftsW**	pSC101	This work
pSD295	*cat pBAD33* P_{ara}*::ftsB*	pACYC184	(9)
pSD295-54	*cat pBAD33* P_{ara}*::ftsB*$^{1\text{-}54}$	pACYC184	This work
pSD296	*cat* pBAD33 P_{ara}*::ftsL*	pACYC184	(9)
pSD296-2	*cat* pBAD33 P_{ara}*::ftsL*$^{L86F/E87K}$	pACYC184	(9)
pSEB306	*bla lacI*^{q} P_{206}*::ftsA*	ColE1	(11)
pSEB306*	*bla lacI*^{q} P_{206}*::ftsA**	ColE1	(11)
pSEB417	*bla lacI*^{q} P_{204}*::ftsN*	ColE1	(11)
pSEB428	*bla lacI*^{q} P_{204}*::ftsEX*	ColE1	(11)
pSEB429	*bla lacI*^{q} P_{204}*::ftsW*	ColE1	(11)
pSEB429*	*bla lacI*^{q} P_{204}*::ftsW**	ColE1	This work

pSEB429-2	*bla lacI*^{q} P_{204}*::ftsW*/R172D*	ColE1	This work
pSEB453	*bla* P_{ftsN}*::malG*$^{1–33}$*–ftsN*$^{46–319}$	ColE1	This work
pSEB468	*aadA* P_{syn135}*:: ftsQ*	pSC101	This work
pUT18C	*bla Plac-T18-MCS*	ColE1	(8)
pUT18-ftsA	*bla Plac-T18-ftsA*	ColE1	(12)
pUT18-ftsA^{R300E}	*bla Plac-T18-ftsA*^{R300E}	ColE1	(12)
pUT18-ftsA$^{R300E/*}$	*bla Plac-T18-ftsA*$^{R300E/*}$	ColE1	(12)
pUT18C-ftsL	*bla Plac-T18-ftsL*	ColE1	(9)

Table S3. Rescue of FtsW mutants by FtsL** and FtsA*.

FtsW mutant	Complementation	Dominant negative[2]	Rescued by FtsL**	Rescued by FtsA*
Y163K	-	+	+	-
V165D	+	-	NA[1]	NA
R166E	-	-	NA	NA
K167E	-	+	+	+
D169K	+	NA	NA	NA
E170K	-	++	+	+
R172D	-	+++	+	-
L175K	+	NA	NA	NA
D337K	+	NA	NA	NA
A345K	-	-	NA	NA

[1]Not applicable;
[2] +++, inhibition of colony formation; ++, slight ihibition of colony formation with filamentous cells; +, filamentous cells; -, no filamentous cells

Table S4. List of the primers used in this study

Primer Name[a]	**Sequence**
ftsA-E124A-F	5’-CGTGTGCGCGATGCGCATCGTGTGCTGCATGTGA-3’
ftsA-E124A-R	5’-TCACATGCAGCACACGATGCGCATCGCGCACACG-3’
ftsA-I143L-F	5’- CTATCAGGAAGGGCTCAAGAATCCGGTAGGACT-3’
ftsA-I143L-R	5’- AGTCCTACCGGATTCTTGAGCCCTTCCTGATAG-3’
ftsA-R286W-F	5’-GTCGTCCGCCATGGAGTCTGCAACGTCAGACAC-3’
ftsA-R286W-R	5’- GTGTCTGACGTTGCAGACTCCATGGCGGACGAC-3’
*ftsB-Hin*dIII-F	5’- TACTAAGCTTGGGCTAATTTGTAC-3’
*ftsB-EcoR*I-R	5’- TCGGGAATTCCCAGGACTTATGGCAATG-3’
*ftsB-Xba*I-F	5’-TCCTCTAGAGCCGTTTTTCAGGGGGCAGGATGGGTAAACTAACGC TGCTGTTGC-3’
*ftsB-Hind*III-R	5’- CAGCCAAGCTTTTATCGATTGTTTTGCCCCG-3’
ftsB-AE55,56 Stop-F	5’- CGATCAACTTTTTTAGTAAATTGACGATCTCAATGGC-3’
ftsB-AE55,56 Stop-R	5’-GCCATTGAGATCGTCAATTTACTAAAAAAGTTGATCG-3’
ftsB-YR85,86 Stop-F	5’- AGGCCGGGCGAAACTTTTTAGTGACTGGTGCCTGA-3’
ftsB-YR85,86 Stop-R	5’- TCAGGCACCAGTCACTAAAAAGTTTCGCCCGGCCT-3’
*ftsL-Hin*dIII-F	5’-TACTAAGCTTCGTATTGTGAAACG-3’
*ftsL-EcoR*I-R	5’-CGGGAATTCAGAGAACGCATGTC-3’
ftsL-L86F/E87K-F	5’-GCAACCTGATCTTTAAAGAGAATGCGCTCGGCGACCAT-3’
ftsL-L86F/E87K-R	5’-ATGGTCGCCGAGCGCATTCTCTTTAAAGATCAGGTTGC-3’
ftsL-E88K/G92D-F	5’-CTGATCCTTGAAAAGAATGCGCTCGACGACCATAG-3’
ftsL-E88K/G92D-R	5’-CTATGGTCGTCGAGCGCATTCTTTTCAAGGATCAG-3’
ftsL-IL85,86 stop-F	5’-GGCGCAACCTGTAGTAGGAAGAGAATGCGCTCGGCG-3’
ftsL-IL85,86 stop-R	5’- CGCCGAGCGCATTCTCTTCCTACTACAGGTTGCGCC-3’
ftsL-AL90,91 stop-F	5’- TCCTTGAAGAGAATTAGTAGGGCGACCATAGCC-3’
ftsL-AL90,91 stop-R	5’- GGCTATGGTCGCCCTACTAATTCTCTTCAAGGA-3’
*ftsQ-Xba*I-F	5’- TACCTCTAGAATTTAAGAAGGAGATATACATATGTCGCAGGCTGCTCT GAACACGCG-3’
*ftsQ-Hind*III-R	5’-TACTAAGCTTTATCATTGTTGTTCTGCCT-3’
ftsQ-(-55)	5’-GCAGGTATGAGCTTCTCGCAGTTGGTAGTACGAATTCTG-3’
ftsQ-(+65)	5’- TAAAGCGGCAACCTTCGCGGTACCAATCTCCAGTCCTAC-3’
ftsQ-L6E FW	5’-CTAATATGTCGCAGGCTGCTGAGAACACGCGAAACAGC-3
ftsQ-HindIII	5’-CGTAAGCTTTATCATTGTTGTTCTGCCTGTGC-3’
*ftsW-Hin*dIII-F	5’-TACTAAGCTTCGGTCGTGACGGCG-3’
*ftsW-EcoR*I-R	5’- CGGGAATTCCACGCAGACCAGAGATAC-3’
ftsW-Y163K-F	5’- TTTTGCTATATCGCCAACAAACTGGTGCGTAAAGG-3’
ftsW-Y163K-R	5’- CTTTACGCACCAGTTTGTTGGCGATATAGCAAAA-3’
ftsW-V165D-F	5’- TATATCGCCAACTATCTGGACCGTAAAGGCGACG-3’
ftsW-V165D-R	5’-CGTCGCCTTTACGGTCCAGATAGTTGGCGAT-3’
ftsW-R166E-F	5’-CAACTATCTGGTGGAAAAAGGCGACGAAGTACG-3’
ftsW-R166E-R	5’- GTACTTCGTCGCCTTTTTCCACCAGATAGTTG-3’
ftsW-K167E-F	5’- CAACTATCTGGTGCGTGAAGGCGACGAAGTACG-3’
ftsW-K167E-R	5’-CGTACTTCGTCGCCTTCACGCACCAGATAGTTG-3’

ftsW-D169K-F	5’-TCTGGTGCGTAAAGGCAAAGAAGTACGTAATAACC-3’
ftsW-D169K-R	5’-GGTTATTACGTACTTCTTTGCCTTTACGCACCAGA-3’
ftsW-E170K-F	5’- TGGTGCGTAAAGGCGACAAAGTACGTAATAACCT-3’
ftsW-E170K-R	5’- GCGCAGGTTATTACGTACTTTGTCGCCTTTACGC-3’
ftsW-R172D-F	5’- AGGCGACGAAGTAGATAATAACCTGCGCGGCTTC-3’
ftsW-R172D-R	5’- AGGAAGCCGCGCAGGTTATTATCTACTTCGT-3’
ftsW-L175K-F	5’-GACGAAGTACGTAATAACAAGCGCGGCTTCCTG-3’
ftsW-L175K-R	5’- CAGGAAGCCGCGCTTGTTATTACGTACTTCGTC-3’
ftsW-D337K-F	5’- GCATTAGAAATTAAACACCGTTTTTCCGGTTTTC-3’
ftsW-A345K-F	5’- TTTTCCGGTTTTCTCAAATGTTCTATTGGCATC-3’
ftsW-A345K-R	5’- GATGCCAATAGAACATTTGAGAAAACCGGAAAA-3’
ftsW-M269I-F	5’-CGCAATCGCTGATCGCGTTTGGTCGCGGCGAACTT-3’

[a]Primers used for mutagenesis come in pairs (F-forward, R-reverse) and are named according to the amino acid substitution or the position at which the stop codons were introduced (two stop codons in tandem).

SI References

1. Goehring NW, Petrovska I, Boyd D, & Beckwith J (2007) Mutants, suppressors, and wrinkled colonies: mutant alleles of the cell division gene ftsQ point to functional domains in FtsQ and a role for domain 1C of FtsA in divisome assembly. *J Bacteriol* 189(2):633-645.
2. Gerding MA*, et al.* (2009) Self-enhanced accumulation of FtsN at Division Sites and Roles for Other Proteins with a SPOR domain (DamX, DedD, and RlpA) in Escherichia coli cell constriction. *J Bacteriol* 191(24):7383-7401.
3. Weiss DS, Chen JC, Ghigo JM, Boyd D, & Beckwith J (1999) Localization of FtsI (PBP3) to the septal ring requires its membrane anchor, the Z ring, FtsA, FtsQ, and FtsL. *J Bacteriol* 181(2):508-520.
4. Mercer KL & Weiss DS (2002) The Escherichia coli cell division protein FtsW is required to recruit its cognate transpeptidase, FtsI (PBP3), to the division site. *J Bacteriol* 184(4):904-912.
5. Furste JP*, et al.* (1986) Molecular cloning of the plasmid RP4 primase region in a multi-host-range tacP expression vector. *Gene* 48(1):119-131.
6. Dai K, Xu Y, & Lutkenhaus J (1993) Cloning and characterization of ftsN, an essential cell division gene in Escherichia coli isolated as a multicopy suppressor of ftsA12(Ts). *J Bacteriol* 175(12):3790-3797.
7. Dai K, Xu Y, & Lutkenhaus J (1996) Topological characterization of the essential Escherichia coli cell division protein FtsN. *J Bacteriol* 178(5):1328-1334.
8. Karimova G, Ullmann A, & Ladant D (2001) Protein-protein interaction between Bacillus stearothermophilus tyrosyl-tRNA synthetase subdomains revealed by a bacterial two-hybrid system. *J Mol Microbiol Biotechnol* 3(1):73-82.
9. Park KT, Du S, & Lutkenhaus J (2020) Essential Role for FtsL in Activation of Septal Peptidoglycan Synthesis. *mBio* 11(6).
10. Dubarry N, Possoz C, & Barre FX (2010) Multiple regions along the Escherichia coli FtsK protein are implicated in cell division. *Mol Microbiol* 78(5):1088-1100.
11. Pichoff S, Du S, & Lutkenhaus J (2015) The bypass of ZipA by overexpression of FtsN requires a previously unknown conserved FtsN motif essential for FtsA-FtsN interaction supporting a model in which FtsA monomers recruit late cell division proteins to the Z ring. *Mol Microbiol* 95(6):971-987.
12. Busiek KK, Eraso JM, Wang Y, & Margolin W (2012) The early divisome protein FtsA interacts directly through its 1c subdomain with the cytoplasmic domain of the late divisome protein FtsN. *J Bacteriol* 194(8):1989-2000.
13. Liu B, Persons L, Lee L, & de Boer PA (2015) Roles for both FtsA and the FtsBLQ subcomplex in FtsN-stimulated cell constriction in Escherichia coli. *Mol Microbiol* 95(6):945-970.
14. Raskin DM & de Boer PA (1999) Rapid pole-to-pole oscillation of a protein required for directing division to the middle of Escherichia coli. *Proc Natl Acad Sci U S A* 96(9):4971-4976.

15. Du S, Pichoff S, & Lutkenhaus J (2016) FtsEX acts on FtsA to regulate divisome assembly and activity. *Proc Natl Acad Sci U S A* 113(34):E5052-5061.
16. Bernard CS, Sadasivam M, Shiomi D, & Margolin W (2007) An altered FtsA can compensate for the loss of essential cell division protein FtsN in Escherichia coli. *Mol Microbiol* 64(5):1289-1305.
17. Meeske AJ*, et al.* (2016) SEDS proteins are a widespread family of bacterial cell wall polymerases. *Nature* 537(7622):634-638.