Supporting information for

A role for FtsA in SPOR-independent localization of the essential

***Escherichia coli* cell division protein FtsN**

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SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Immunofluorescence microscopy. Cells of *ftsA12*(Ts) strain WM1115 expressing *gfp* (pWM1088) were fixed using paraformaldehyde/glutaraldehyde fixative and stained as described previously (Levin, 2002). Rabbit anti-His_6-FtsA antibody (Capralogics, Inc.; Hardwick, MA) was affinity purified (Levin, 2002) and applied at a final concentration of 1:500. Goat anti-rabbit Alexa Fluor 488-conjugated antibody (Molecular Probes; Eugene, OR) was applied at a final concentration of 1:200.

Temperature-shift experiments with *ftsQ* TOE1 and *ftsI* AX655 temperature sensitive strains. Cultures of *ftsQ*(Ts) strain WM2193 and *ftsI*(Ts) strain WM2348 expressing *gfp* (pWM1088), *gfp-ftsN*$_{Cyto\text{-}TM}$ (pWM4528), *gfp-ftsN* (pWM1152), or *gfp-ftsZ* (pWM3775), or ^{TT}*mCherry-ftsN*$_{SPOR}$ (pWM4740) were grown at 30˚C without inducer to an OD_{600} of 0.3-0.4 prior to heat shock. Thymine was added to WM2193, which is *thyA*. Cultures were shifted to 42˚C for 30 minutes. DIC and fluorescence images were obtained immediately before and after shifting cultures to the non-permissive temperature.

Spot dilution assays. Spot dilution assays of *ftsN* depletion strain WM4028 harboring pWM2784 (pDSW210-*flag*), pWM4612 (pDSW210-*flag-virB10*$_{Cyto}$*N*$_{TM\text{-}Peri\Delta SPOR}$), pWM4582 (pDSW210-*flag-virB10*$_{Cyto}$*N*$_{TM\text{-}Peri}$), pWM4613 (pDSW210-*flag-ftsN*$_{\Delta SPOR}$), or pWM3157 (pDSW210-*flag-ftsN*) were performed using cultures grown without IPTG to low OD_{600} at 30˚C. Cultures were spot diluted (10^{-3}-10^{-6}) onto LB plates containing 0, 10, or 100 μM IPTG. Plates were incubated at 30˚C and 42˚C.

SUPPLEMENTARY FIGURES

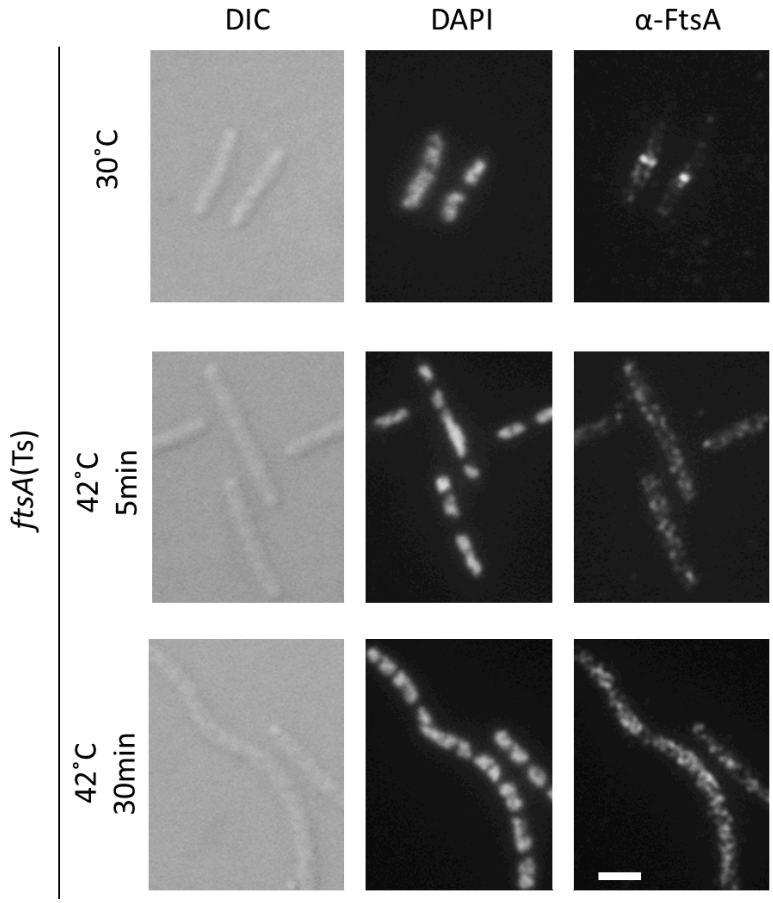

FIG. S1. FtsA12(Ts) delocalizes from division sites within 5 minutes at 42˚C. DIC (left), DAPI (middle), and anti-FtsA (right) images of *ftsA12*(Ts) (WM1115) strain harboring plasmid pWM1088 (pDSW207-*gfp*). Cells were grown to low OD_{600} without IPTG at 30˚C then shifted to 42˚C for 5 and 30 minutes. Cells

were fixed before staining DNA with DAPI and detecting FtsA with anti-FtsA primary and Alexa Fluor 488-conjugated secondary antibodies. Scale bar = 4 µm.

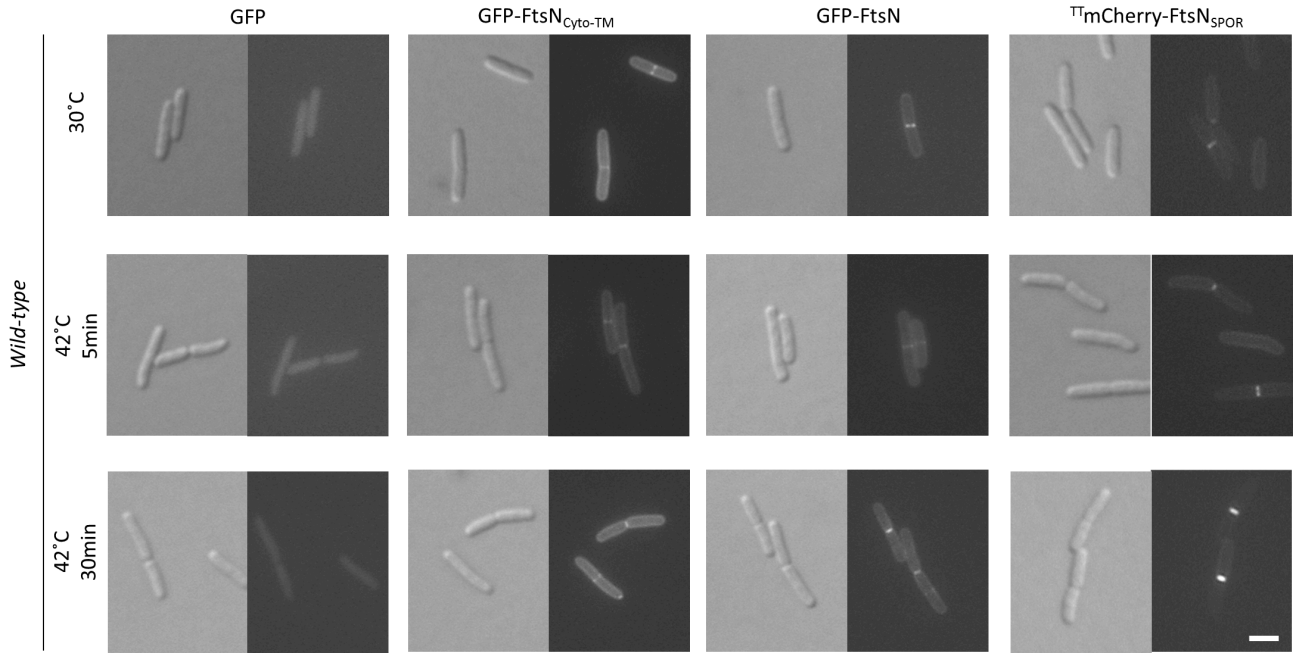

FIG. S2. Localization of GFP- and ^{TT}mCherry-tagged constructs is not disrupted at 42˚C in wild-type cells. DIC (left) and fluorescence (right) images of wild-type (WM1074) strains harboring plasmids pWM1088 (pDSW207-*gfp*), pWM4528 (pDSW207-*gfp-ftsN$_{Cyto-TM}$*), pWM1152 (pDSW207-*gfp-ftsN*), or pWM4740 (pKG116-*^{TT}mCherry-ftsN$_{SPOR}$*). Cells were grown to low OD_{600} without IPTG (pDSW207 strains) or with 1 µM sodium salicylate (pKG116 strain) at 30˚C then shifted to 42˚C for 5 and 30 minutes. Scale bar = 4 µm.

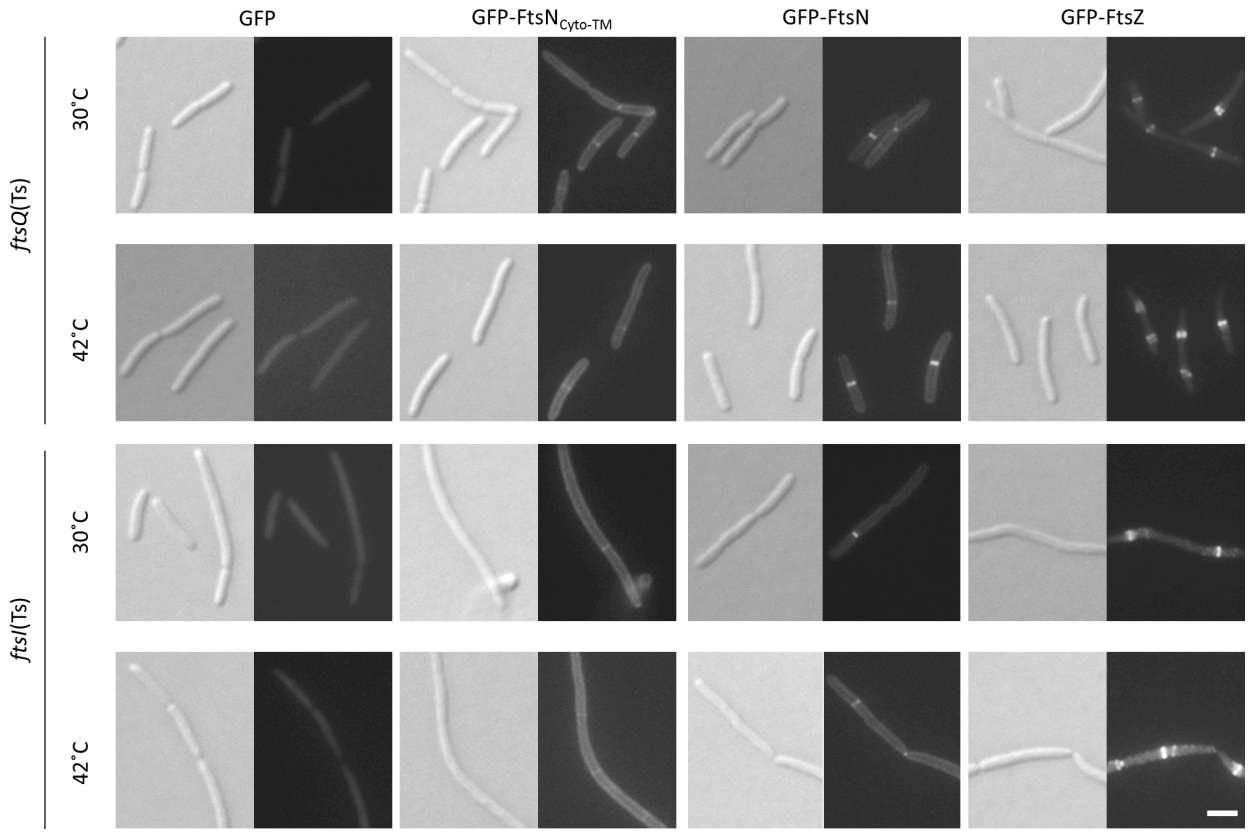

FIG. S3. Localization of GFP-FtsN$_{Cyto-TM}$ in *ftsQ*(Ts) and *ftsI*(Ts) strains. DIC (left) and fluorescence (right) images of *ftsQ*(Ts) and *ftsI*(Ts) strains harboring plasmids pWM1088 (pDSW207-*gfp*), pWM4528 (pDSW207-*gfp-ftsN$_{Cyto-TM}$*), pWM1152 (pDSW207-*gfp-ftsN*), or pWM3775 (pDSW207-*gfp-ftsZ*). Cells were grown to low OD_{600} without IPTG at 30˚C then shifted to 42˚C for 30 minutes. Scale bar = 4 µm.

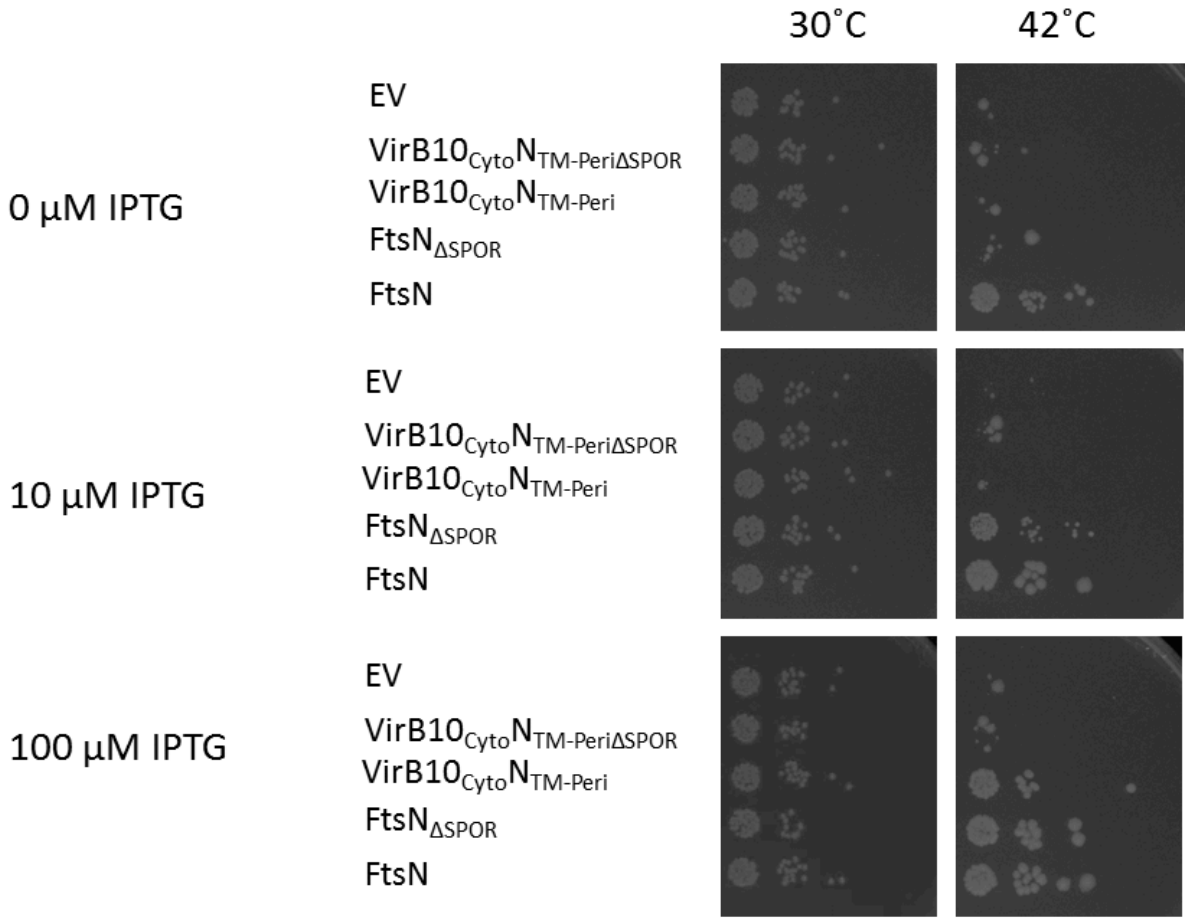

FIG. S4. Substitution and/or deletion of the cytoplasmic and SPOR domains of FtsN reduce viability in an *ftsN* depletion strain. Spot dilution assays of *ftsN* depletion strain WM4028 harboring pWM2784 (pDSW210-*flag*), pWM4612 (pDSW210-*flag-virB10*$_{Cyto}$*N*$_{TM\text{-}Peri\Delta SPOR}$), pWM4582 (pDSW210-*flag-virB10*$_{Cyto}$*N*$_{TM\text{-}Peri}$), pWM4613 (pDSW210-*flag-ftsN*$_{\Delta SPOR}$), or pWM3157 (pDSW210-*flag-ftsN*). Cultures were grown without IPTG to low OD_{600} at 30˚C. Cultures were diluted to 10^{-3}-10^{-6} concentrations and spotted onto LB plates containing 0, 10, or 100 μM IPTG and incubated at 30˚C and 42˚C.

TABLE S1. Oligonucleotide primers used in this study.

Primer #	Sequence (5’ – 3’)
111	ACGAAGCTTTCAACCCCCGGCGGCGAG
112	CGAGAATTCAACAACAACGCACAACGAGATTATG
1115	GCTCTAGAGCACAACGAGATTATGTACGCCGC
1116	GCCTGCAGTCAACCCCCGGCGGCGAGCCGAATGC
1803	GTCTTTCGGGGTCTCAGAAACTGCCTGCGGTTTCTCCCGC
1804	GCGGGAGAAACCGCAGGCAGTTTCTGAGACCCCGAAAGAC
1809	GGTCTAGAAATAACGATAGTCAGCAAG
2003	ACGAAGCTTTCACGTAATGAAGTACAG
2047	ACGAAGCTTTCAACCTAGCCAAATGAGG
2048	CGAGAATTCAACAACAACAATAACGATAGTCAGC
2049	GCCTGCAGTCATTCTCCGCCGTCGGTTTTGGCGC
2051	AATCTAGAATGATCAAGGCGACGGACAG
2052	TTCTGCAGCTCTCCGATTTGTGCCTGTC
2054	CGACTGCGGCGCAAGCGGCGATGGTGAGCAAGGGCGAGG
2055	CCTCGCCCTTGCTCACCATCGCCGCTTGCGCCGCAGTCG
2056	GCGGAATTCATGGCGAACAATAACGATC
2057	CGCGGATCCCTTGTACAGCTCGTCCATGC
2058	GCGGGATCCGAGAAAAAAGACGAACGC
2059	CGCTCTAGATCAACCCCCGGCGGCGAGC
2060	ACGAAGCTTTCATTCTCCGCCGTCGGTTTTGGCGC
2063	GCGATGCATATGGCGAACAATAACGATC

TABLE S2. Supplemental strains.

Strain or plasmid	Genotype or description	Source or reference
WM2193	*ftsQ1*(Ts) strain TOE1, K12 parent strain AB2497	Begg et al. (1980)
WM2348	*ftsI*(Ts) strain AX655, K12 parent strain AB1157	Allen et al. (1974)

REFERENCES

Allen, J. S., C. C. Filip, R. A. Gustafson, R. G. Allen & J. R. Walker (1974) Regulation of bacterial cell division: genetic and phenotypic analysis of temperature-sensitive, multinucleate, filament-forming mutants of *Escherichia coli*. *J Bacteriol* 117: 978-986.

Begg, K. J., G. F. Hatfull & W. D. Donachie (1980) Identification of new genes in a cell envelope-cell division gene cluster of *Escherichia coli:* cell division gene *ftsQ*. *J Bacteriol* 144: 435-437.

Levin, P. A. (2002) Light microscopy techniques for bacterial cell biology. In: Methods in Microbiology Sansonetti, P. and Zychlinsky, A. (eds). London: Academic Press Ltd., pp. 112-132.