Supplemental Information for:

The Cell Division Protein FtsN: Identification of SPOR Domain Amino Acids Important for Septal Localization, Peptidoglycan-binding, and a Disulfide Bond

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Running title: FtsN SPOR domain

Supplemental Methods

Construction of plasmids

(i) Plasmids for localization of mutant SPOR domains. Most amino acid substitutions were constructed using degenerate primers and megapriming (1). For example, pDSW1230 [P_{204}::^{TT}*gfp-ftsN*^{SPOR} (R247A)] was constructed using P1325, which has a degenerate sequence at the codon for R247. First, P1325 (R247) and P761 were used with pBAD33-*ftsN* (2) as template to amplify a portion of the SPOR domain. The 243 bp product, which carried the desired substitution(s), was isolated using a PCR Cleanup Kit (Qiagen). Second, 10 µl of the 243 bp PCR product were used as a primer along with P760 in a subsequent PCR to produce full length *ftsN* encoding the substitution. The 957 bp product was purified and then used as a template for a third PCR reaction, wherein P1123 and P761 were used to amplify only the mutant SPOR domain. The resulting 270 bp product was cut with BamHI and HindIII, and ligated into the corresponding sites of pDSW962 (3). After transformation, multiple isolates were sequenced to obtain a set of plasmids that encoded ^{TT}*gfp* fusions to SPOR domains with multiple substitutions at codon 247. Similar procedures were used to construct additional mutants using the following degenerate primers: P1326(M249), P1327(Q251), P1328(S254,) P1329(N281), P1330(W283), P1331(R285), P1332(V287), P1333(P290), P1334(R256), P1335(E259), P1336(E262), P1337(T263), P1338(R265), P1339(Q267), P1340(F270), P1341(D274), P1342(K276), P1343(T278), P1344(N280), P1345(K292), P1346(K294), P1347(E295), P1348(N298), P1349(S299), P1350(N302), P1351(R303), P1352(K305), P1353(M306), P1477(R247), and P1497(Q251).

A few mutations were constructed similarly, but with primers that introduced a specific amino acid change (i.e., not degenerate): P1478 (R247K), P1479 (T263S), P1480 (T263D), P1498 (Q251K), P1581 (M249A), P1582 (V287A), P1583 (R256A) and P1584 (K294A).

Amino acid substitutions near the C-terminus of the SPOR domain were obtained by a simpler PCR procedure in which the entire SPOR domain was amplified using as 5' primer P1123 and as 3' primer one of the following primers with a degenerate codon: P1354 (T310), P1355 (N311), P1356 (I313), or P1357 (L315). The resulting 270 bp product was cut with BamHI and HindIII, and then ligated into the same sites of pDSW962 to create the desired ^{TT}*gfp-ftsN*^{SPOR} fusion construct.

To disrupt the putative disulfide bond the C252A and C312A substitutions were introduced by PCR using pDSW992 (4) as template and the following primer pairs: P1790 + P761; P1791 + P1123; or P1790 + P1791. The resulting 270 bp products were cut with HindIII and BamHI, and then ligated into the same sites of pDSW962.

(ii) Plasmids for overproduction of His$_6$-tagged SPOR domains. To overproduce His$_6$-FtsN^{SPOR} with a native C-terminus, primers P1123 and P761 were used to amplify the SPOR domain from template plasmid pDSW992. The resulting 269 bp fragment was cut with BamHI and HindIII, and then ligated into the same sites of pQE80L. This construct resulted in poor overproduction, a problem traced to lack of disulfide bond formation in the cytosol and FtsN's C-terminal AAGG sequence, which appears to be a target for proteolysis. We solved the former problem by expressing FtsN^{SPOR} constructs in *E. coli* Shuffle T7, a strained engineered to promote disulfide bond formation in the cyotosol (5). To reduce proteolysis, two aspartates were added to the C-terminus. Thus, the constructs reported here were cloned by amplifying the coding sequence for FtsN SPOR domains using P1123 as the 5' primer and P1402 as the

3'primer. The templates were the corresponding ^{TT}*gfp-ftsN*^{SPOR} domain plasmids, either wild-type or mutant. The 274 bp product was digested with BamHI and HindIII, and then ligated into corresponding sites of pQE80L. All constructs carry the vector- and primer-derived sequence MRGSHHHHHHGSNNN at the N-terminus, codons 240-319 of *ftsN* and DD at the C-terminus.

Determination of the distance between the sulfur atoms and alpha carbons of C252 and C312. To evaluate the possibility of disulfide bonding between C252 and C312, we have measured the distance of sulfur atoms and alpha carbons of C252 and C312 for 45 lowest energy NMR models of FtsN^{SPOR} from PDB id 1UTA (6) using PyMOL.

Modeling the structure of FtsN^{SPOR} containing a disulfide bond. The structure of FtsN^{SPOR} with a disulfide bond was modeled by choosing the cysteine rotamers in model 1 of 1UTA that brought the two cysteines into disulfide bond forming conformations. The disulfide bond was forced after removing the sulfur hydrogens and creating an SSBOND definition in the PDB header. To remove conformational stress, the model was energy minimized with the NOVA forcefield (7) in YASARA (13.6.16; http://www.yasara.org) by steepest descent minimization and simulated annealing until convergence (less than 0.05 kJ/mol energy improvement per atom during 200 steps) was reached. The starting model with reduced cysteines was also subjected to the same energy minimization process. The overlay shown in Figure S6 compares the energy minimized structures with and without a disulfide bond.

Supplemental Results

Overproduction of FtsN^{SPOR} for purification purposes. We constructed a plasmid that directed expression of FtsN^{SPOR} with an N-terminal His_6-tag under control of a T7 RNA polymerase-dependent promoter. The plasmid was transformed into BL21(DE3), but overproduction of His_6-FtsN^{SPOR} was barely detected by SDS-PAGE (Figure S2, compare lanes 2 and 3). We tried to improve overproduction by changing the growth temperature, changing the amount of IPTG, letting the induction proceed for longer before harvest, and switching to a host with reduced RNase activity [BL21 Star^{TM} (DE3) from Invitrogen]. None of these measures were of much help.

These difficulties were perplexing because neither Ursinus et al. nor Yang et al. had reported difficulty overproducing their FtsN^{SPOR} constructs (6, 8). Looking more closely at what they had done, we realized their constructs contained a C-terminal His_6-tag, whereas ours had an N-terminal His_6-tag, so we were trying to overproduce the SPOR domain with its native C-terminus. The final 4 amino acids of FtsN are AAGG, which bears some resemblance to one of the recognition signals for the ClpXP protease system (9). We therefore added two aspartates to the C-terminus of our construct and discovered this greatly improved overproduction in BL21 (DE3) (Figure S2, compare lanes 3 and 5). At this point we cloned our mutants of FtsN^{SPOR} into the overproduction vector but included the two C-terminal aspartates. We also constructed a ^{TT}GFP-FtsN^{SPOR} derivative with two aspartates to determine whether modifying the C-terminus affected septal localization. It did not (Figure 3A; Table S3). We later realized that FtsN^{SPOR} has a disulfide bond, so we tested an overproduction host engineered to form proper disulfide bonds in the cytoplasm (*E. coli* SHuffle T7 from New England Biolabs). Switching from BL21(DE3) to SHuffle T7 greatly improved overproduction even for an FtsN^{SPOR} construct with a native C-terminus (Figure S3, compare lanes 3 and 7), but adding C-terminal aspartates

increased protein production even further (Figure S3, compare lanes 7 and 9). Therefore, the $FtsN^{SPOR}$ proteins used in the PG binding assays were overproduced in SHuffle T7 and have a His_6-tag at the N-terminus plus two aspartates at the C-terminus.

Distance between the sulfur atoms and alpha carbons of C252 and C312. To evaluate the possibility of disulfide bonding between C252 and C312, we have measured the distance of sulfur atoms and alpha carbons of C252 and C312 for 45 lowest energy structures of 1UTA PDB structures using PyMOL The distance for two sulfur atoms was 5.3 ± 0.9 Å, and the minimum distance was 3.3 Å (Table S5). This indicates that the disulfide bond is not observed in the 1UTA PDB structure. On the other hand, the distance for two alpha carbon atoms of C252 and C312 converged on 3.6 ± 0.3 Å, suggesting they are close enough for disulfide bond formation.

References

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Table S1: Strains used in this study

Strain	Genotype or relevant features	Source or reference
BW25113	Δ(*araD-araB*)*567*, *lacZ4787*(Δ)::*rrnB-3*, *rph-1*, Δ*(rhaD-rhaB)568*, *hsdR514*	(10)
CH34/pMG20	MG1655 *lacIZYA*<>*frt ftsA*(E124A) *ftsN*<>*kan*. The plasmid is a pACYC derivative carrying P_{BAD}::*torA*ss-*bfp*-T7.tag-*ftsN*(71-105)	(11)
EC251	Our isolate of MG1655 (wild-type)	Lab collection
ER1821	*glnV44 e14*$^{-}$*(McrA*$^{-}$*) rfbD1 relA1 endA1 spoT1 thi-1* Δ*(mcrC-mrr)114::IS10*	New England Biolabs
JP120	ER1821 *dsbA*::*kan1*	(12)
JW3832	BW25113 Δ*dsbA*::*kan*	(10)
JCB570	MC4100 *phoR zig*::Tn*10*	(12)
JCB571	JCB570 *dsbA*::*kan1*	(12)
JCB502	Bardwell lab MG1655 isolate	J. Bardwell
JCB572	JCB572 *dsbA*::*kan1*	(12)
Shuffle T7	*fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal* λ*att::pNEB3-r1-cDsbC* (SpecR, *lacIq*) Δ*trxB sulA11 R(mcr-73::miniTn10*--TetS*)2 [dcm] R(zgb-210::Tn10* --TetS*) endA1* Δ*gor* Δ*(mcrC-mrr)114::IS10*	New England Biolabs

Table S2: Primers used in this study

Primer	Codon[b]	Sequence[a]
P455		CACAGAATTCATTAAAGAGGAG
P760		CGGGATCCGGTGGTCTGTACTTCATTACG
P761		CCCAAGCTTTCAACCCCCGGCGGCGAGCCG
P1123		GCCGGATCCAACAACAACGCGGAGAAAAAAGACGAA
P1325	R247	AAAGACGAACGC**GMN**TGGATGGTGCAG
P1326	M249	GAACGCCGCTGG**GHG**GTGCAGTGCGGT
P1327	Q251	CGCTGGATGGTG**GMN**TGCGGTTCGTTC
P1328	S254	GTGCAGTGCGGT**RMR**TTCAGAGGCGCG
P1329	N281	ATCACCACCAAC**GMN**GGCTGGAATCGT
P1330	W283	ACCAACAATGGC**KMY**AATCGTGTGGTC
P1331	R285	AATGGCTGGAAT**RMR**GTGGTCATTGGC
P1332	V287	TGGAATCGTGTG**ADY**ATTGGCCCGGTG
P1333	P290	GTGGTCATTGGC**GHG**GTGAAAGGCAAA
P1334	R256	TGCGGTTCGTTC**GHN**GGCGCGGAACAG
P1335	E259	TTCAGAGGCGCG**GHY**CAGGCAGAGACG
P1336	E262	GCGGAACAGGCA**GHY**ACGGTACGTGCT
P1337	T263	GAACAGGCAGAG**KCN**GTACGTGCTCAG
P1338	R265	GCAGAGACGGTA**GHN**GCTCAGCTGGCG
P1339	Q267	ACGGTACGTGCT**RMN**CTGGCGTTCGAA
P1340	F270	GCTCAGCTGGCG **KMY**GAAGGCTTTGAC
P1341	D274	TTCGAAGGCTTT **RMR**TCGAAAATCACC
P1342	K276	GGCTTTGACTCG **RMY**ATCACCACCAAC
P1343	T278	GACTCGAAAATC**KCC**ACCAACAATGGC
P1344	N280	AAAATCACCACC**GMN**AATGGCTGGAAT
P1345	K292	ATTGGCCCGGTG**RMY**GGCAAAGAGAAC
P1346	K294	CCGGTGAAAGGC**RMY**GAGAACGCAGAC
P1347	E295	GTGAAAGGCAAA**GHY**AACGCAGACAGC
P1348	N298	AAAGAGAACGCA**RMR**AGCACCCTCAAT
P1349	S299	GAGAACGCAGAC**RCN**ACCCTCAATCGG

P1350	N302	GACAGCACCCTC**GMN**CGGTTGAAGATG
P1351	R303	AGCACCCTCAAT**GHN**TTGAAGATGGCG
P1352	K305	CTCAATCGGTTG**RMY**ATGGCGGGTCAT
P1353	M306	AATCGGTTGAAG**GHG**GCGGGTCATACA
P1354	T310	CCCAAGCTTCAACCCCCGGCGGCGAGCCGAATGCAGTT**N DC**ATGACCCGCCAT
P1355	N311	CCCAAGCTTCAACCCCCGGCGGCGAGCCGAATGCA**NDC**T GTATGACCCGC
P1343	N281	ATCACCACCAAC**GMN**GGCTGGAATCGT
P1356	I313	CCCAAGCTTCAACCCCCGGCGGCGAGCCG**NDC**GCAGTTT GTATG
P1357	L315	CCCAAGCTTCAACCCCCGGCGGC**NDC**CCGAATGCAGTT
P1402	Adds DD to C-terminus	CGTAAGCTTAATCGTCACCCCCGGCGGCGAGCCGAATGCA G
P1477	R247	AAAGACGAACGC**RCA**TGGATGGTGCAG
P1478	R247K	AAAGACGAACGC**AAG**TGGATGGTGCAG
P1479	T263S	GAACAGGCAGAG**AGC**GTACGTGCTCAG
P1480	T263D	GAACAGGCAGAG**GAC**GTACGTGCTCAG
P1497	Q251	CGCTGGATGGTG**RCA**TGCGGTTCGTTC
P1498	Q251K	CGCTGGATGGTG**AAG**TGCGGTTCGTTC
P1581	M249A	GAACGCCGCTGG**GCG**GTGCAGTGCGGT
P1582	V287A	TGGAATCGTGTG**GCG**ATTGGCCCGGTG
P1583	R256A	TGCGGTTCGTTC**GCA**GGCGCGGAACAG
P1584	K294A	CCGGTGAAAGGC**GCA**GAGAACGCAGAC
P1790	C252A	GCCGGATCCAACAACAACGCGGAGAAAAAAGACGAACGC CGCTGGATGGTGCAG**GCC**GGTTCGTTCAGAGGC
P1791	C312A	CCCAAGCTTTCAACCCCCGGCGGCGAGCCGAAT**GGC**GTTT GTATGACCCGC

[a]DNA sequence is given in the 5' to 3' direction for all primers; [b]Codon targeted for mutagenesis

Base pairs underlined are restriction enzyme sites and base pairs that are bolded are the mutagenized codon

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Table S3. Localization of ^{TT}GFP-FtsN^{SPOR} in wild-type background.

Amino Acid Substitution	n*	% cells with ring (s)	Amino Acid Substitution	n*	% cells with ring (s)
WT	30	70±9	W283D	4	9±6
WT^{DD}	2	73±14	W283A	3	23±3
R247A	2	69±5	R285K	3	44±5
R247E	2	78±7	R285A	4	22±2
R247T	2	70±1	R285E	4	11±1
R247K	2	75±1	V287I	2	67±15
M249V	3	58±15	V287S	3	60±20
M249E	2	44±4	V287A	2	53±7
M249A	2	58±1	P290V	4	20±5
Q251E	4	11±8	P290E	2	40±1
Q251T	3	9±2	P290A	3	25±6
Q251A	3	18±4	K292D	2	57±11
Q251K	2	17±6	K292A	2	54±16
S254K	3	5±8	K292N	3	58±12
S254E	3	13±8	K294N	2	63±10
S254A	2	82±6	K294D	2	61±22
R256D	2	24±9	K294T	2	60±7
R256V	2	31±1	K294A	2	48±5
R256E	2	46±13	E295V	2	59±2
R256A	2	28±3	E295A	2	68±1
E259A	2	49±9	E295D	2	56±2
E259T	2	71±16	D298E	3	66±8
E262A	4	51±15	D298K	2	61±0
E262V	2	33±7	D298A	3	54±9

Amino Acid Substitution	n*	% cells with ring (s)	Amino Acid Substitution	n*	% cells with ring (s)
T263A	2	68±8	S299A	2	66±2
T263S	2	82±4	S299T	2	66±5
T263D	4	29±8	N302E	2	73±2
R265V	3	48±5	N302D	2	73±4
R265A	2	62±16	N302A	3	64±4
R265E	2	26±2	R303V	2	52±12
Q267A	2	49±2	R303A	2	57±9
Q267E	2	54±8	K305D	2	59±8
F270S	3	54±17	K305A	2	62±9
F270A	2	59±1	M306A	2	52±4
F270Y	2	39±6	M306E	2	73±1
F270D	4	33±11	M306V	2	59±8
D274A	2	41±5	T310A	2	65±4
D274E	2	73±2	T310D	2	63±4
D274T	2	36±5	T310E	3	66±15
K276A	2	59±2	N311A	3	59±13
K276D	2	49±9	N311V	3	57±18
T278S	2	55±1	N311E	2	57±6
T278A	2	61±6	I313A	5	25±9
N280A	2	60±0	I313D	4	2±1
N280E	2	56±12	I313V	3	56±9
N281E	2	65±13	L315A	4	37±5
N281A	2	64±0	L315V	2	52±10

*n = number of independent experiments. Approximately 200 cells scored for most experiments.

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Table S4. Localization of ^{TT}GFP-FtsN^{SPOR} in a FtsN^{SPOR} null background (CH34/pMG20).

Amino Acid Substitution	n*	% cells with ring (s)	Amino Acid Substitution	n*	% cells with ring (s)
WT	7	60±5	T278A	2	42±6
R247E	2	62±12	N281E	2	52±3
Q251E	3	9±1	W283D	2	4±1
E259A	3	46±0	K294N	2	25±4
T263S	2	63±8	D298E	2	50±7
T263D	2	17±0	N302D	2	64±5
F270A	2	36±6	M306E	3	68±6
D274E	2	66±1			

*n = number of independent experiments. Approximately 200 cells scored for most experiments.

Table S5. Estimated distance between sulfur and α-carbons of C252 and C312 in 45 lowest energy structures of $FtsN^{SPOR}$.

Structure # in PDB (1UTA)	S-S Distance (Å)	Cα-Cα Distance (Å)	Structure # in PDB (1UTA)	S-S Distance (Å)	Cα-Cα Distance (Å)
1	6.2	3.5	26	6.3	4.0
2	3.6	3.8	27	5.7	3.7
3	5.0	3.3	28	6.3	3.6
4	7.0	4.0	29	4.5	3.5
5	5.0	3.2	30	5.0	3.2
6	4.9	3.2	31	4.9	3.3
7	6.0	3.8	32	6.4	3.5
8	3.4	3.6	33	5.0	3.7
9	5.0	5.2	34	3.5	3.7
10	4.7	3.4	35	6.3	3.2
11	3.3	3.6	36	6.5	4.0
12	6.5	3.7	37	6.0	3.8
13	4.3	3.8	38	6.2	3.2
14	6.5	3.4	39	4.8	3.2
15	4.9	3.3	40	5.8	3.8
16	4.9	3.4	41	6.5	3.5
17	4.9	3.2	42	5.0	3.6
18	5.0	3.3	43	5.6	3.4
19	4.7	3.3	44	4.9	3.4
20	5.0	3.5	45	5.6	3.8
21	5.8	3.6			
22	5.7	3.8			
23	4.6	3.6			
24	4.8	3.3			
25	5.6	3.3			

	S-S Distance (Å)	Cα-Cα Distance (Å)
Average	5.3	3.6
Standard deviation	0.9	0.3
Median	5.0	3.5
Minimum	3.3	3.2
Maximum	7.0	5.2

Table S6. SPOR domain sequences with two cysteines. These domains were retrieved from the Pfam seed alignment of 136 SPOR domain on June 14, 2013. Cysteines are in red.

Identifier/residues	Sequence of SPOR domain
>Q5E2H5_VIBF1/123-196	SEIPYIMQ**C**GAYKNRSQAEERKMNIAFQGITSTVRHAEGSSWYKVVLGPY KFKRDAEKDRHKLQRAKIEP**C**AIW
>FTSN_ECOLI/244-315	DERRWMVQ**C**GSFRGAEQAETVRAQLAFEGFDSKITTNNGWNRVVIGPVKG KENADSTLNRLKMAGHTN**C**IRL
>Q7MQG0_VIBVY/72-144	KEDFFWIQ**C**GILNQPMPLADAKPLYKQITTDVWMKPENKTYR**C**LIGPYQS FAQASKDLKQVKKLGDYREAFIR
>Y896_HAEIN/133-203	DSKKFGLQ**C**GAFKNRAQAENLQGRLQMTGLNAQIQTNGEWNRVRVASFDT RELAVQAQSRAKTVTD**C**VVIG
>Q68XU0_RICTY/134-215	SHTSYKVQLGSVKSEAEAMEEGAKIKKKFPKILQNVVITTKKVKYDDGKF YYLILAGEYSSLNQAQAV**C**KKLAHNQQS**C**VLK
>Q6N5W7_RHOPA/457-539	TSGGYVVQVSSQRNEADAKASYRSLQSKFPSVLGSQPPLIKRVDLGSKGT YYRAMVGPFSSAEQAQQV**C**GNLKSAGGQ**C**VVQR
>Q6G315_BARHE/758-839	NSENYYVQLASQPTHALAKDSLKNMKSKFGFLIGTRPLNIQSAVIPGKGT YYRVRVQAQNRNEAINL**C**ENIKNSGGS**C**FITR
>Q57DM9_BRUAB/909-990	GAGGYFIQIASQPSAELAQKSYANMAQKYASVIGGHSVDIKRADIQGKGT YYRVRVQAGSKEDALAL**C**SRLKSAGGS**C**FVTQ
>Q8YIE5_BRUME/5-87	KPKPWGIQLAGNFRRSVAINQWNRLRKQFASVLAGHNPVISRIRTPIGRR GIYAVRIGADSRKEADGI**C**SSLHAVGGA**C**IVSR
>Q6N538_RHOPA/285-368	ADRPWGVQLAAGFNRNRALASYARAMSRLSTVIGERDPTLLSGVFRSRGT RPFYQVRIGAETRGEADDL**C**KQIRRAGQA**C**LVLR
>Q57D64_BRUAB/329-409	GRSNWRIQLAATPSRAGASELQEKYAPVVSRIVPGAKGEISPSPKGRKVY RVRFSGVRDSAAASKA**C**AQLKRQQIA**C**LAIQ
>Q7CXC2_AGRT5/348-428	ERSRWEVQIAATDSEAAARSLLANARSNIGSYTGIAPYTEAVQSGSATLY RARFTGFEDQSSAVSA**C**KELKAQSYA**C**VVMT
>Q57CW7_BRUAB/388-469	AASGWAIQIGSLPSEGQARDMLAKASATAGRTLRSASPYTETFNKGSATF YRARFVGFTSKQAAWDA**C**ASLKRNNFG**C**YAVA
>Q6N533_RHOPA/503-584	AHSGWIIQVGALESEPEARKRLEAAREQASGLLGKADPFTETVTTKGDRK LYRARFAGLERDEAEAV**C**RKLKRSDIS**C**FTIK
>Q5FT21_GLUOX/335-415	SYGPWAVQVGAFGSIGQAKFAATMARQAAFTSLQSARIEVHPTPSHGTTV WRARLTGISRVGAAQA**C**STLSGQGMA**C**MAVP
>Q5FR24_GLUOX/369-448	ATGNWAIQVGAFANAKQASIATSAAHSKGGVVVASARSQVESVKGGRSHL YRARLTGLTHENAVAA**C**RRLSHGSP**C**VVVP
>Q9A6S6_CAUCR/186-266	STGPASVQIGALSSPALADKAWAEAVRLAPGLAAGKGKKVETVDKNGTTL YRTSVTGFATREAAKAF**C**EAIAASGKS**C**FVK
>Q5FQ53_GLUOX/291-370	SGGTHEVQLGALDSEAAARKEWDSLRHQAPALFAGHTPLFEKTTRGDHTF VRLRIGGFADLKSARAY**C**VKLHAQSVA**C**TP
>Q5NP92_ZYMMO/297-374	PAGAGVIQLGAFGSEAKANEVWSHLTQRYSWIKPLPHQIISVKIGEKTFY RLRATAGSQANSF**C**SQLQAAGET**C**AHIG
>Q5LQI7_SILPO/325-406	PVGTRLAQLGAYDSPEIARAEWDRLNGRFGEYLDGKQRVIQEASSGGRTF YRLRAAGFGDLAEARHF**C**SVLVAERAD**C**IPVT
>Q9A6D3_CAUCR/358-434	PKGEWGVQVGAFRSKSLANEQLKLVRGRITKLVSDAEGAVEGAGGMFRAQ FQGMTNEAAREA**C**SALKAKRMP**C**IVLK
>Q6N6F9_RHOPA/319-400	PRTDFGVDLGAANTVEGLRGLWRKLSKTQKALKGLQPIIMIKENGNATQL RLIAGPIADAAAAAKV**C**AALGSADRA**C**EASVY

Figure S1. Western blot using anti-GFP antibody to document expression levels of select ^{TT}GFP-FtsN^{SPOR} mutant proteins. The R285 proteins are also shown in Figure 3A, but the blot below used independent cultures. Substitutions at P290 greatly impaired localization, but as shown by this blot, the proteins were unstable, so we did not classify P290 as a residue important for septal localization. The R285A and T263D substitutions impaired localization, but not sufficiently to make our cut-off for classifying R285 and T263 as important for localization. The blot below shows the corresponding mutant ^{TT}GFP-FtsN^{SPOR} constructs are produced at normal levels.

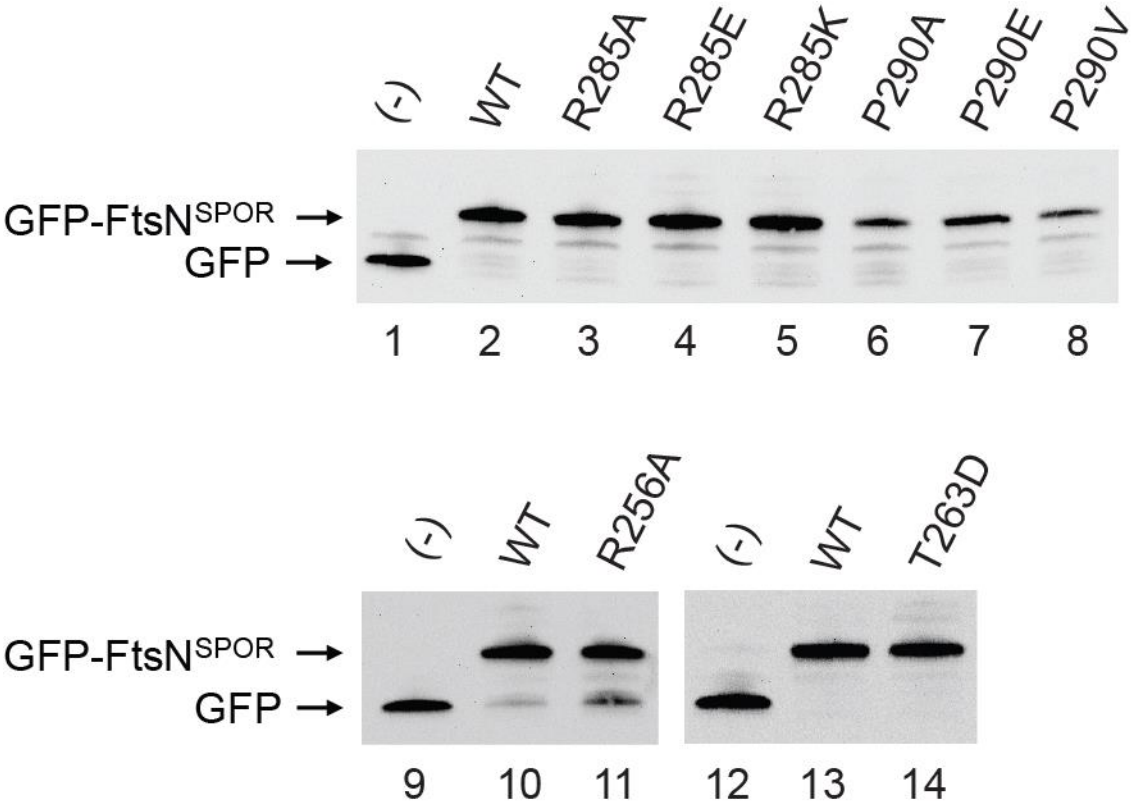

Figure S2. Overproduction of His_6-$FtsN^{SPOR}$ proteins. Cells grown in LB at 37 °C to OD_{600} ~0.5 were induced overnight at 20 °C with 1 mM IPTG and then samples were harvested for SDS-PAGE (Mini Protean TGX precast gels: Any kD; from BioRad, Hercules, CA). The plasmids used were pDSW 1333 (Lane 2, 3, 6, and 7) or pDSW1314 (Lane 4, 5, 8, and 9).

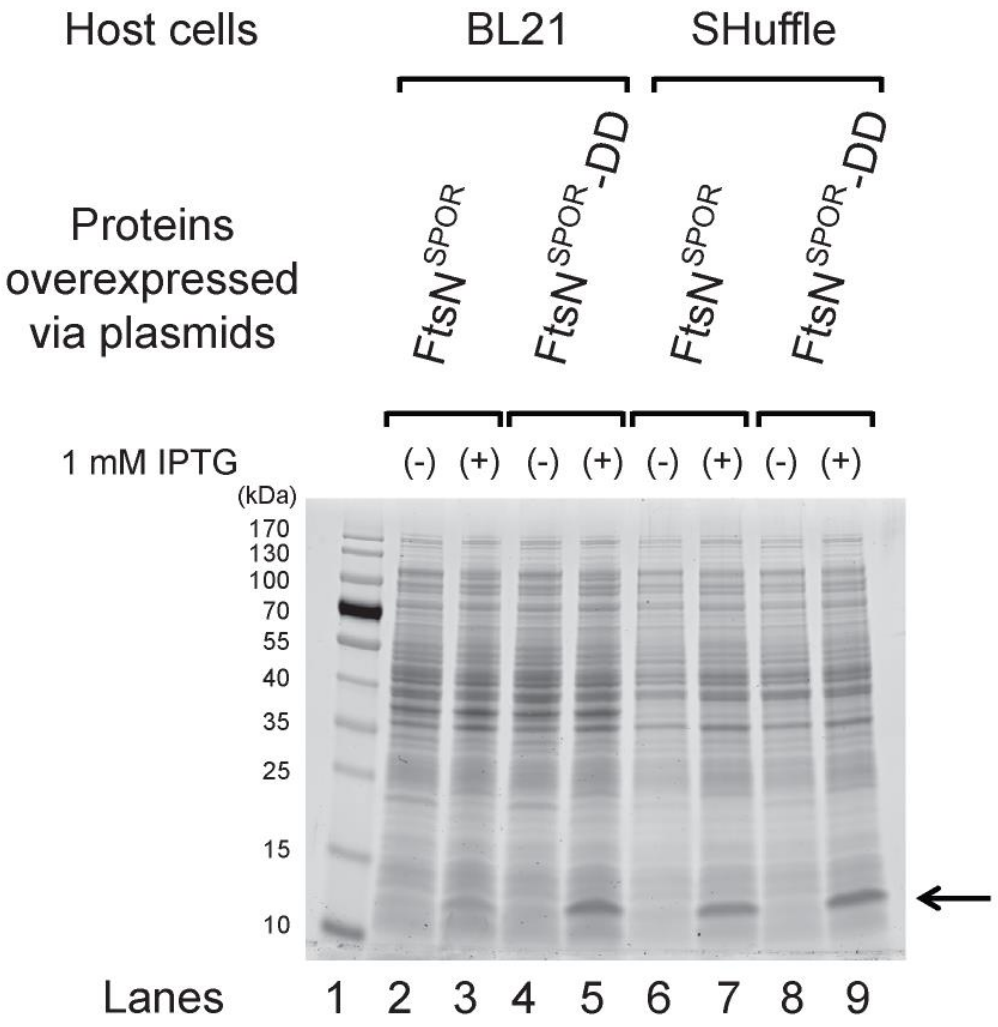

Figure S3. FtsN contains a disulfide bond. Whole cell extracts in Laemmli sample buffer containing (lanes 2, 4) or lacking (lanes 3, 5) 5% ß-mercaptoethanol and analyzed by Western blot with anti-FtsN (top panel) or anti-FtsQ (bottom panel). Samples were loaded twice to facilitate visualization of any mobility differences. For the experiment shown here, samples were loaded on an Any kD™ Mini-PROTEAN® TGX™ gel (Bio-Rad) and run for 100 min. at 120 V, whereas the experiment shown in Figure 4B used a 10% polyacrylamide gel that was run for 60 min. at 120V.

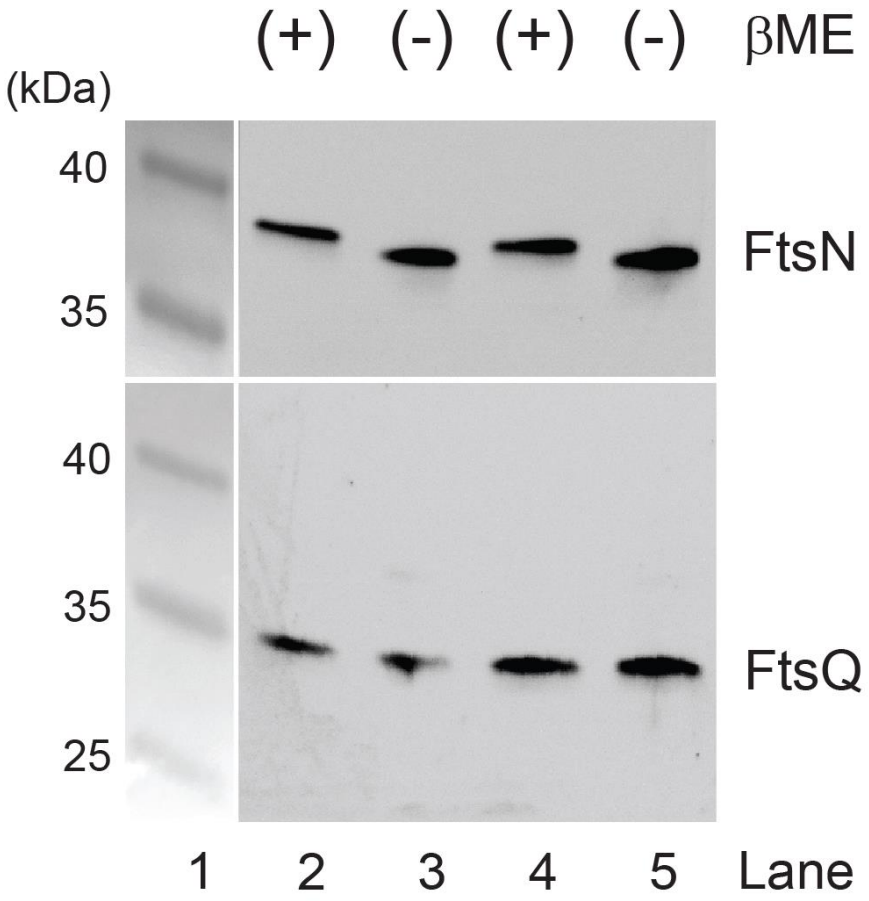

Figure S4. Reduced abundance of FtsN in *dsbA* mutants grown in LB. The indicated strains were grown at 30°C in LB to OD_{600} ~0.5, then whole cell extracts were analyzed by Western blotting using anti-FtsN or anti-DamX as the primary antibody.

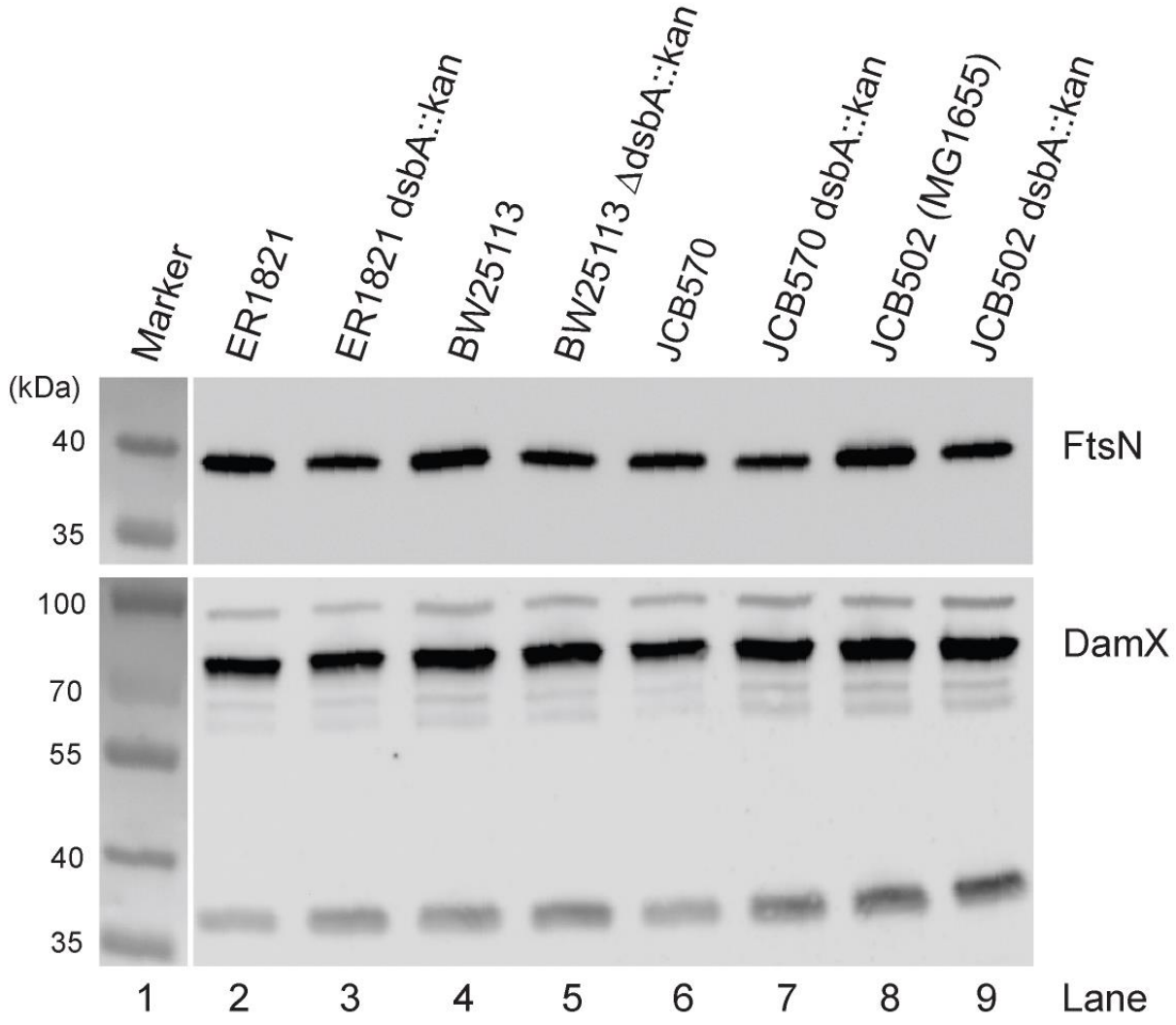

Figure S5. Efficient localization of ^{TT}GFP-DamX^{SPOR} does not require DsbA. The indicated strains were grown to midlog phase, then immobilized on an agarose pad and photographed under fluorescence microscopy.

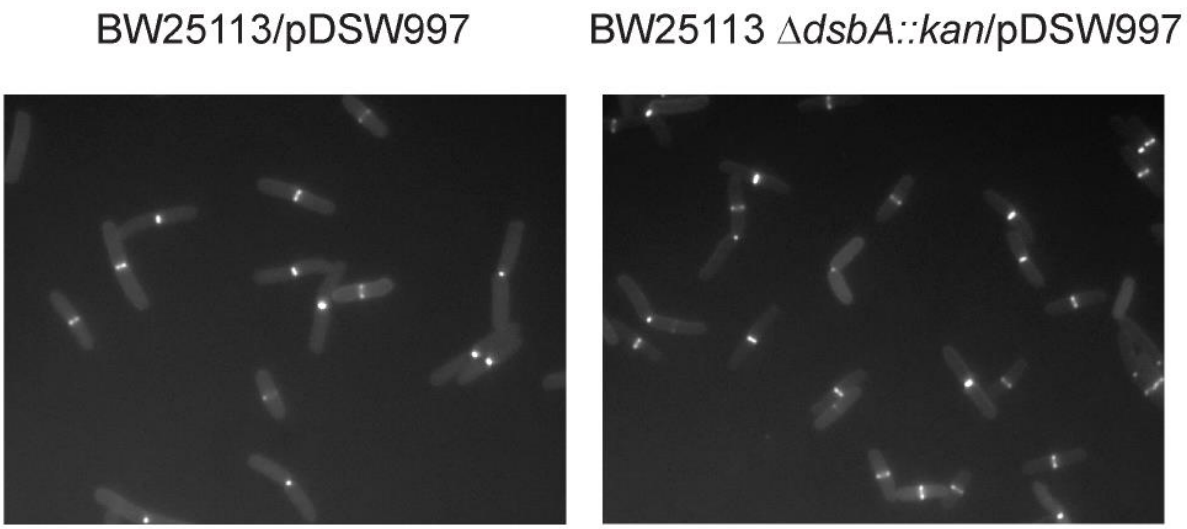

Figure S6. Modeling the effect of the disulfide bond on the structure of FtsN^{SPOR} reveals only minor changes. A. Overlays of modeled structures for the reduced (red) and disulfide-bonded forms of FtsN^{SPOR} viewed from the side (left) or the bottom (right). The disulfide bond is shown in orange. For clarity, only the backbone is shown. B. Comparison of disulfide-bonded FtsN^{SPOR} to DamX^{SPOR} and CwlC^{SPOR}. Note that the ß-sheet in the modeled FtsN^{SPOR} is slightly more curved than it was in Figure 1 of the main text, but the sheet is still flat in comparison to the other SPOR domains. In the modeled FtsN^{SPOR} structure, two cysteine residues (including the disulfide bond) are in orange. Other features are colored as in Figure 1.