Supporting Information

Duman et al. 10.1073/pnas.1313978110

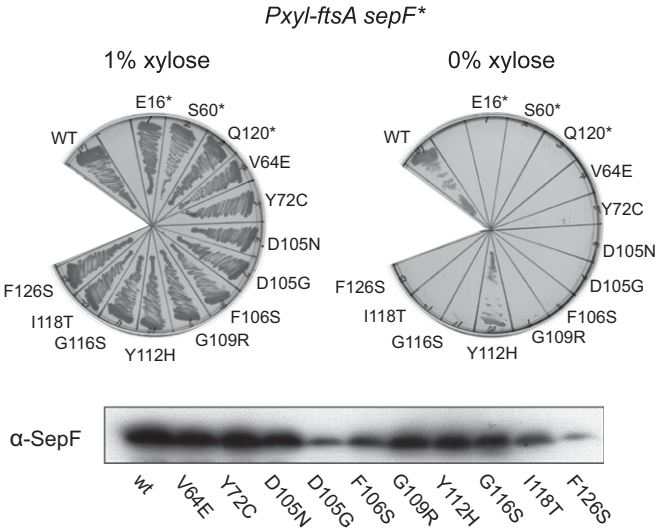

Fig. S1. Growth of *Baccillus subtilis* SepF mutants in the absence of FtsA. Selection of SepF mutant was based on impaired FtsZ interaction in a yeast two-hybrid screen (Fig. 1, main text). Wild-type *sepF* was substituted with mutant *sepF* genes (*sepF**) in a strain containing *ftsA* under control of the xylose-inducible P_{xyl} promoter. If the SepF mutant is inactive, the protein cannot support growth of *B. subtilis* cells in the absence of FtsA (0% xylose). Western blot (*Lower*) shows expression levels of different SepF mutants in *B. subtilis*. We cannot rule out that the mutants D109G and F126S fail to complement wild-type SepF because of low expression levels.

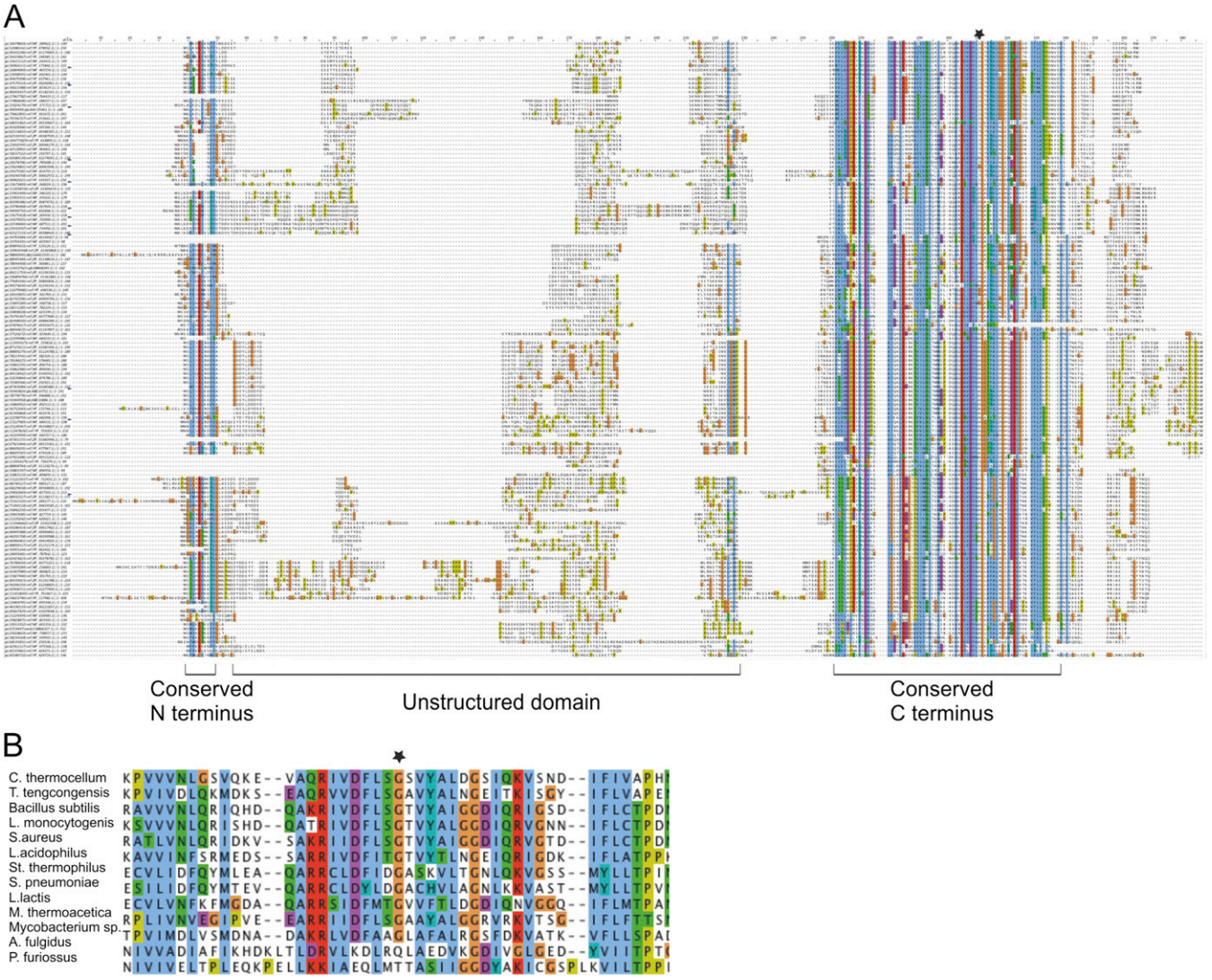

Fig. S2. (*A*) Sequence alignment of SepF-like proteins from 127 different organisms, using Clustal-W. *B. subtilis* SepF sequence is shown at the top. (*B*) Detail of the conserved C-terminal domain sequence containing the conserved glycine (G109) residue responsible for interdimer contacts (marked by a star).

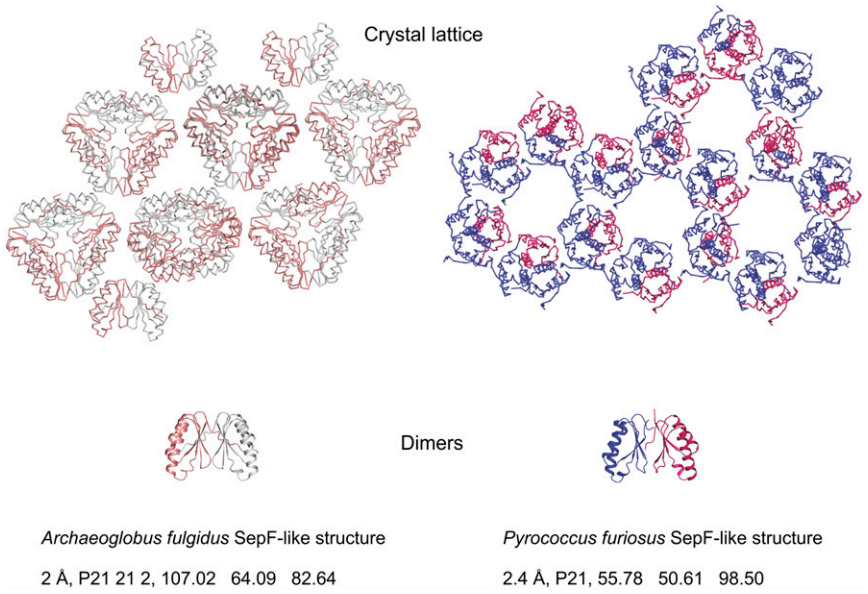

Fig. S3. Crystal lattices and dimer structures of SepF-like proteins from *Archaeoglobus fulgidus* (*Left*) and *Pyrococcus furiosus* (*Right*). Resolution (in angstroms), space group, and unit cell dimensions (in angstroms) of the crystals are shown at the bottom.

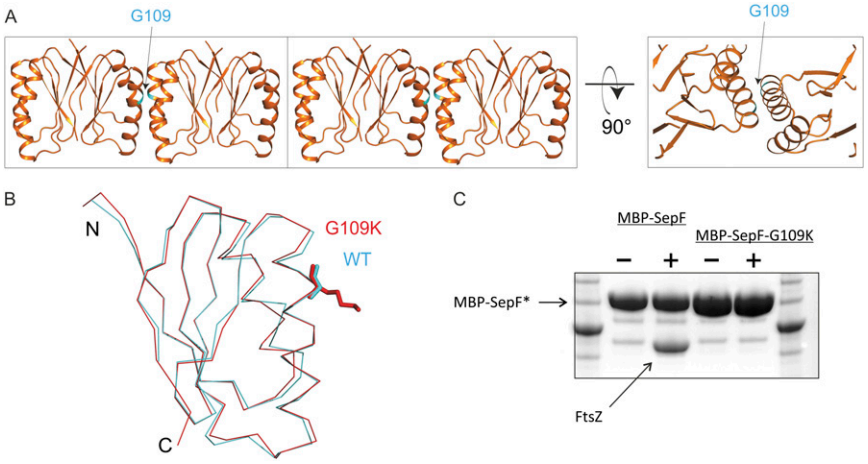

Fig. S4. (*A*) Stereo image of the SepF C-terminal domain dimer showing glycine G109 responsible for the dimer interface (cyan). (*B*) Superposition of the wild-type and G109K mutant in α-carbon traces representation. (*C*) SepF-G109K does not bind FtsZ. Elution fractions of a SepF-FtsZ coelution experiment using MBP-SepF and MBP-SepF-G109K are shown. The MBP-fusion proteins were incubated with (+) or without (–) purified *B. subtilis* FtsZ. Fractions were analyzed by SDS/PAGE and Coomassie staining.

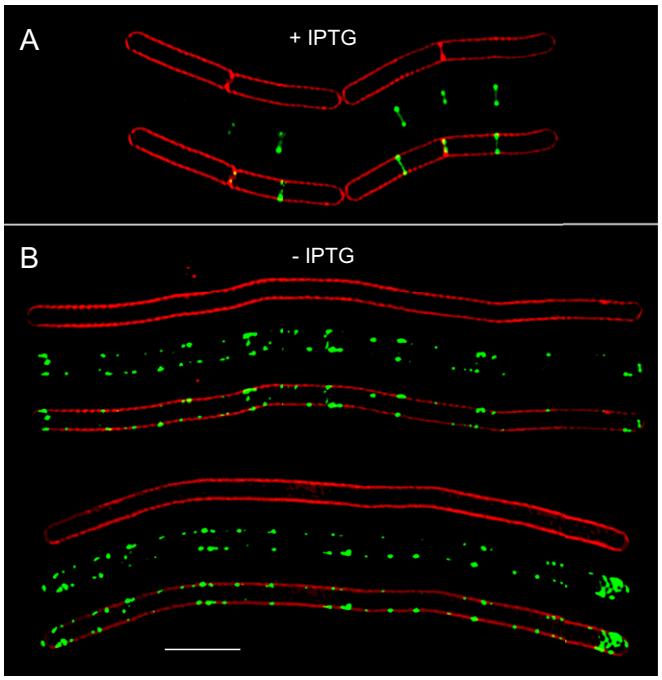

Fig. S5. SepF-GFP localizes close to the cell membrane. (*A* and *B*) Two-dimensional SIM images show SepF-GFP localization in (*A*) the presence (+ITPG) and (*B*) absence (−IPTG) of FtsZ in strain LH21 (*Pxyl-sepF-gfp Pspac-ftsZ*). Membranes were stained with FM-95 (red). SepF-GFP (green) was induced with xylose. (Scale bars, 3 μm.)

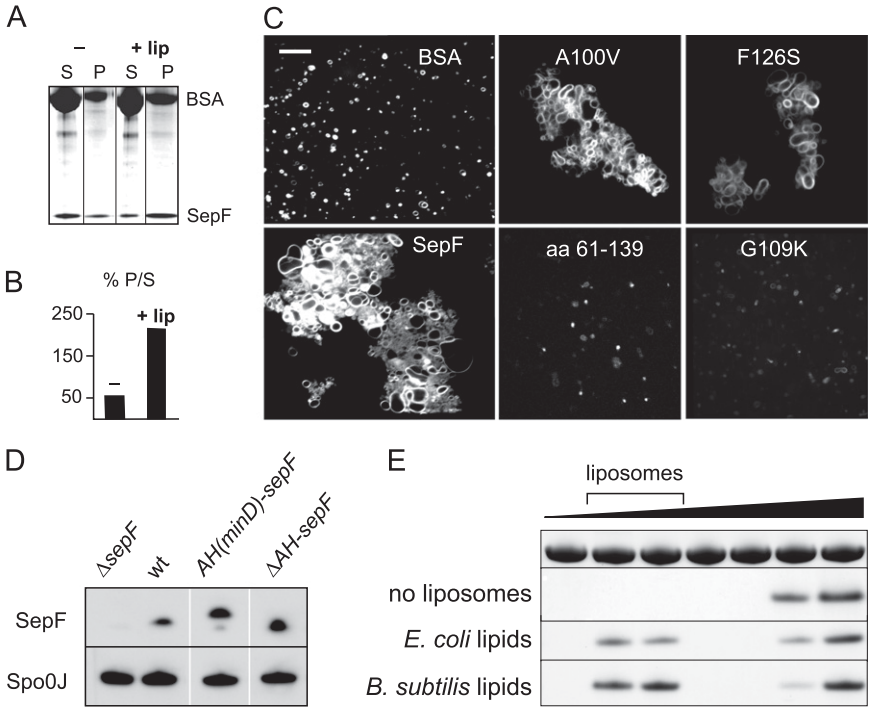

Fig. S6. SepF binds to lipid membranes. (*A*) Pelleting assay with SepF in the absence (–) and presence of liposomes (+ lip). BSA is present to reduce nonspecific interactions. This is a discontinuous image from the same gel. Supernatant (S) and pellet (P) were analyzed using SDS/PAGE. (*B*) Ratios of pelleted SepF (P/S) from *A*. (*C*) SepF causes liposomes to aggregate. The presence of large liposomes suggests that some liposomes fuse due to the presence of SepF. SepF mutants amino acids 61–139, G109K, ΔN13, and FL7D did not induce liposome clustering (ΔN13 and L7D gave the same result as amino acids 61–139 and G109K and are not shown). Liposomes were stained with Bodipy FL C16, and images were taken with a spinning-disk confocal microscope. (Scale bar, 10 μm.) (*D*) Western blot showing that the AH(MinD)-SepF and ΔAH-SepF (lacking first 13 aa) mutants are stable (WT, wild-type strain). Immunodetection of Spo0J was used as a loading control. This is a discontinuous image from the same blot. (*E*) Flotation assay using sucrose density gradient (main text), confirming that SepF also binds to liposomes made from *B. subtilis* lipid extract.

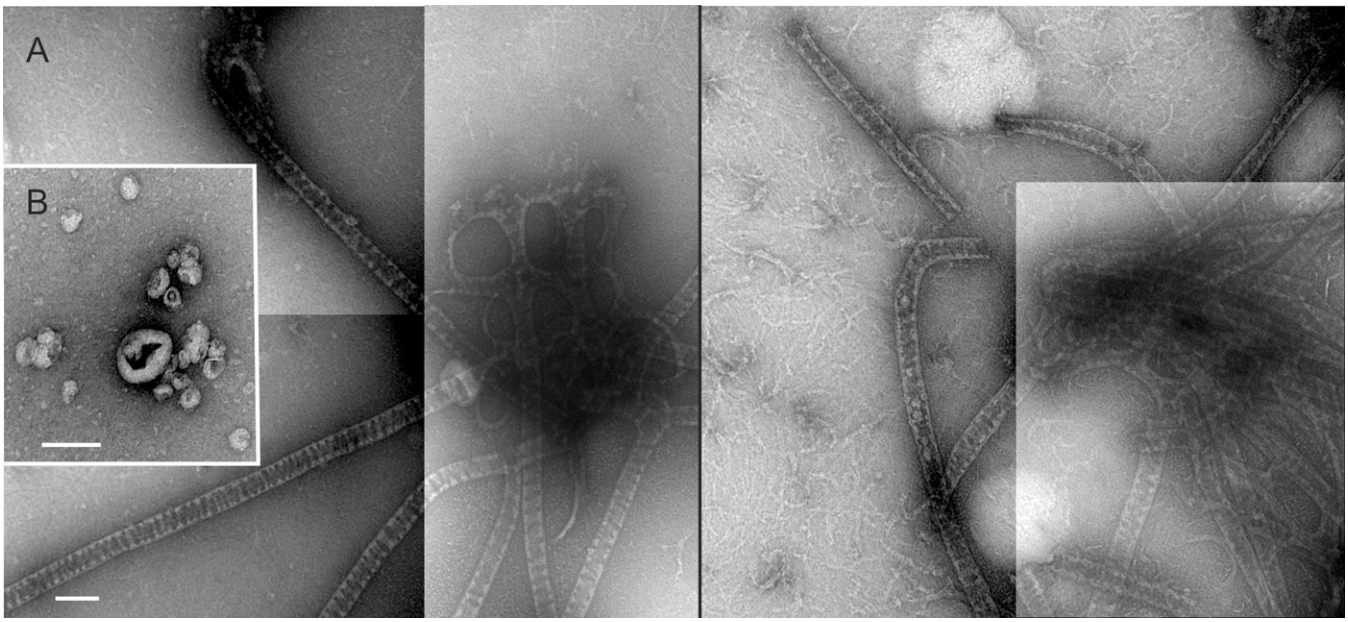

Fig. S7. Liposomes stimulate the formation of FtsZ-SepF tubules. (*A*) EM images of FtsZ-SepF tubules that are formed when purified FtsZ and SepF are mixed with liposomes. Polymerization of FtsZ is initiated by the addition of GTP. (*B*) Liposomes without protein. Liposomes were made from *Escherichia coli* polar lipid extract. (Scale bars, 100 nm.)

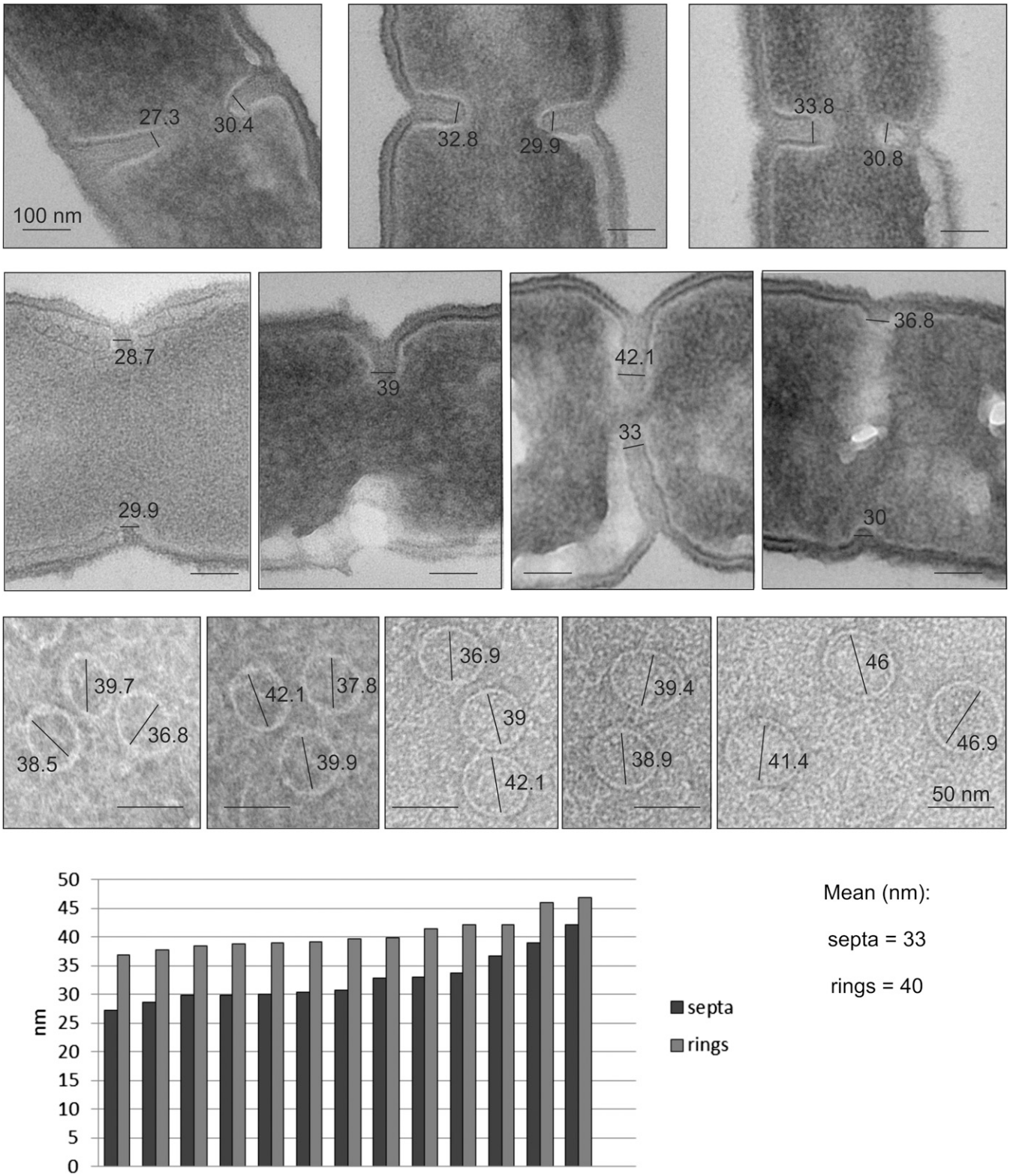

Fig. S8. Transmission EM images of dividing *B. subtilis* cells used for septum width measurements and SepF rings used to measure the inner diameter of the rings. The measured values are ordered in the bar diagram.

Table S1. Crystallographic data

Statistics	*Archaeoglobus fulgidus*	*Pyrococcus furiosus*	*Bacillus subtilis*	*B. subtilis*
Protein	II05M, SeMet	SeMet	BsSepF-C NATI	BsSepF-C G109K
UniProt IDs	NP_069616	YP_006493105	SEPF_BACSU	SEPF_BACSU
Data collection				
Beamline	ESRF ID23eh1	ESRF ID23eh1	ESRF ID29	ESRF ID23eh1
Wavelengths, Å	0.9793; 0.9798	0.9793; 0.9798	0.9762	0.9000
Method crystal	SeMet MAD	SeMet MAD	MR with *Pf*	MR with BsSepF-C
Space group	$P2_12_12_1$	$P6_522$	$P3_221$	$C222_1$
Cell, Å	107.0, 64.1, 82.6	59.3, 59.3, 177.5	40.6, 40.6, 170.3	42.0, 80.1, 51.8
Scaling				
Resolution, Å	2.0	2.5	1.9	2.2
Completeness, %*	99.9 (99.9)	99.9 (99.9)	99.9 (99.9)	100 (100)
Multiplicity*	14.0 (14.5)	26.2 (25.8)	9.6 (8.6)	6.8 (7.0)
$(I)/\sigma(I)$*	28.7 (21.6)	31.1 (14.6)	14.5 (4.5)	8.8 (4.5)
R_{merge}*	0.072 (0.110)	0.077 (0.230)	0.092 (0.415)	0.14 (0.40)
R_{pim}*	0.029 (0.043)	0.020 (0.063)	0.032 (0.148)	0.062 (0.164)
Refinement				
R/R_{free}†	0.188 (0.244)	0.219 (0.289)	0.184 (0.246)	0.194 (0.245)
Model	3 dimers: 37–121; 372 H_2O	2 monomers packing as dimer: 49–130; 46–129; 29 H_2O	1 dimer: 61–139; 61–140; 59 H_2O	1 monomer: 61–142; 30 H_2O
Bond length rmsd, Å	0.025	0.008	0.024	0.007
Bond angle rmsd, °	2.055	0.973	1.815	0.947
Most favored, %‡	97.3	91.6	93.6	100
Disallowed, %‡	0.0	0.0	0.0	0.0
PDB ID	3ZIE	3ZIG	3ZIH	3ZII

ESRF, European Synchrotron Radiation Facility; MAD, multi-wavelength anomalous dispersion; PDB, Protein Data Bank.
*Values in parentheses refer to the highest recorded resolution shell.
†Five percent of reflections were randomly selected before refinement.
‡Percentage of residues in the Ramachandran plot (PROCHECK).

Table S2. Strains and plasmids

Name	Relevant genotype	Source
Strain		
168	*trpC2*	Laboratory stock
LH21	*amy::Pxyl-sepF-gfp Spc, Pspac-ftsZ ble*	Same as strain 4182 in ref. 1
YK012	CRK6000, *ezrA::spec*	(2)
YK204	CRK6000, *ΔsepF::spec*	(3)
YK206	CRK6000, *ΔftsA:::erm-Pspac-ftsZ (ΔftsA:::erm)*	(3)
MD120	*ezrA::spec*	S. Ishikawa
MD136	*ΔftsA:::erm-Pspac-ftsZ (ΔftsA:::erm)*	S. Ishikawa
MD172	*amyE::Pxyl-ftsA (Pxyl-ftsA)*	S. Ishikawa
MD175	*ΔftsA:::erm* Pxyl-*ftsA*	S. Ishikawa
MD194	*ΔsepF::spec*	S. Ishikawa
MD197	*ftsA::erm* Pxyl-*ftsA ΔsepF::spec*	S. Ishikawa
SD261	*sepF-E16*-cat*	This study
SD264	*sepF-K60*-cat*	This study
SD269	*sepF-R120*-cat*	This study
SD270	*sepF-V64E-cat*	This study
SD271	*sepF-Y72C-cat*	This study
SD272	*sepF-D105N-cat*	This study
SD273	*sepF-D105G-cat*	This study
SD274	*sepF-F106S-cat*	This study
SD275	*sepF-G109R-cat*	This study
SD276	*sepF-Y112H-cat*	This study
SD277	*sepF-G116S-cat*	This study
SD278	*sepF-I118T-cat*	This study
SD279	*sepF-F126S-cat*	This study
SD283	*sepF-wild-cat*	This study
SD331	*ΔftsA:::erm Pxyl-ftsA sepF-E16*-cat*	This study
SD332	*ΔftsA::erm Pxyl-ftsA sepF-K60*-cat*	This study
SD333	*ΔftsA:::erm Pxyl-ftsA sepF-R120*-cat*	This study
SD334	*ΔftsA:::erm Pxyl-ftsA sepF-V64E-cat*	This study
SD335	*ΔftsA:::erm Pxyl-ftsA sepF-Y72C-cat*	This study
SD336	*ΔftsA:::erm Pxyl-ftsA sepF-D105N-cat*	This study
SD337	*ΔftsA:::erm Pxyl-ftsA sepF-D105G-cat*	This study
SD338	*ΔftsA:::erm Pxyl-ftsA sepF-F106S-cat*	This study
SD339	*ΔftsA:::erm Pxyl-ftsA sepF-G109R-cat*	This study
SD340	*ΔftsA:::erm Pxyl-ftsA sepF-Y112H-cat*	This study
SD341	*ΔftsA:::erm Pxyl-ftsA sepF-G116S-cat*	This study
SD342	*ΔftsA:::erm Pxyl-ftsA sepF-I118T-cat*	This study
SD343	*ΔftsA:::erm Pxyl-ftsA sepF-F126S-cat*	This study
SD347	*ΔftsA:::erm Pxyl-ftsA sepF-wild-cat*	This study
HS206	*amyE::spec Pxyl-sepF1-39* (SepF1-13)-*gfp*	This study
HS207	*amyE::spec Pxyl-sepF1-75* (SepF1-25)-*gfp*	This study
HS208	*amyE::spec Pxyl-sepF1-75* (SepF1-25)-*junLZ-gfp*	This study
HS223	*amyE::spec Pxyl-sepF1-39* (SepF1-13, L7D)-*gfp*	This study
NC21	*sepF::ery amy::spec Pxyl-sepF* (G109K)-*gfp*	This study
NC40	sepF::neo	This study
HS230	*aprE::spc Pxyl-SepF*$_{\Delta AH}$ (SepFΔ2–13)	This study
HS232	*aprE::spc Pxyl-AH*$_{MinD}$*-sepF* (MinD$_{248–268}$-SepF$_{14–151}$)	This study
HS242	*sepF::neo ΔftsA:::erm aprE::spc Pxyl-AH*$_{MinD}$*-sepF* (MinD$_{248–268aa}$-SepF$_{14–151aa}$)	This study
Plasmids		
pMAL-C2	*Ptac, bla, ColE1, malE, lacZα, lacI*^{q}	New England Biolabs
pNC12	*Ptac-malE-sepF*	This work
pMBP-SepF A100V	*Ptac-malE-sepF* (A100V)	(4)
pMBP-SepF F126S	*Ptac-malE-sepF* (F126S)	(4)
pNC13	*Ptac-malE-sepF* (G109K)	This work
pHJS106	*Ptac-malE-sepF*ΔN13 (SepF14-151)	This work
pHJS107	*Ptac-malE-sepF* (SepF L7D)	This work
pNC14	*bla spec amyE3′ Pxyl-sepF* (G109K)-*gfp amyE5′*	This work
pSG1154	*bla spec amyE3′ Pxyl-gfp amyE5′*	(5)
pTS37	*cat gfp-junLZ-EcMTS256–270*	(6)
pHJS108	*bla spec amyE3′ Pxyl-sepF1-39-gfp amyE5′*	This work
pHJS109	*bla spec amyE3′ Pxyl-sepF1-75-gfp amyE5′*	This work
pHJS110	*bla spec amyE3′ Pxyl-sepF1-75-junLZ-gfp amyE5′*	This work
pHJS111	*bla spec amyE3′ Pxyl-sepF1-39 (L7D)-gfp amyE5′*	This work

1. Hamoen LW, Meile JC, de Jong W, Noirot P, Errington J (2006) SepF, a novel FtsZ-interacting protein required for a late step in cell division. *Mol Microbiol* 59(3):989–999.
2. Kawai Y, Ogasawara N (2006) *Bacillus subtilis* EzrA and FtsL synergistically regulate FtsZ ring dynamics during cell division. *Microbiology* 152(Pt 4):1129–1141.
3. Ishikawa S, Kawai Y, Hiramatsu K, Kuwano M, Ogasawara N (2006) A new FtsZ-interacting protein, YlmF, complements the activity of FtsA during progression of cell division in *Bacillus subtilis*. *Mol Microbiol* 60(6):1364–1380.
4. Gündoğdu ME, et al. (2011) Large ring polymers align FtsZ polymers for normal septum formation. *EMBO J* 30(3):617–626.
5. Lewis PJ, Marston AL (1999) GFP vectors for controlled expression and dual labelling of protein fusions in *Bacillus subtilis*. *Gene* 227(1):101–110.
6. Szeto TH, Rowland SL, Habrukowich CL, King GF (2003) The MinD membrane targeting sequence is a transplantable lipid-binding helix. *J Biol Chem* 278(41):40050–40056.

Table S3. Oligonucleotides

Name	Information	Sequence
inc22	ΔN(14–151) F	GTTGCCCGGGGAAGATGAAGAATAC
inc6	ΔN(14–151) R	GACTCTAGATTACCACCTCTGATGTTC
HS492	L7D F	GAGTATGAAAAATAAAGACAAAAACTTTTTCTCAATG
HS493	L7D R	CATTGAGAAAAAGTTTTTGTCTTTATTTTTCATACTC
inc40	G109K F	GACTTTTTAAGCAACACCGTTTATG
inc41	G109K R	CATAAACGGTGTTGCTTAAAAAGTC
sepFgwR		agaaagctgggtcTTACCACCTCTGATGTTCGT
sepF-ΔN1-gwF		aaaaagcaggctcgCCGGCTTACAACGGGAATAAAC
sepF-ΔN2-gwF		aaaaagcaggctcgAAAGTGGTGTTGAGTGAGCC
sepF-ΔN3-gwF		aaaaagcaggctcgCCTGACAACGTAGATGTATCAGG
Adapter-attB1		Ggggacaagtttgtacaaaaaagcaggctcg
Adapter-attB2		Ggggaccactttgtacaagaaagctgggtc
pDONR-F		TCGCGTTAACGCTAGCATGGATCTC
pDONR-R		GTAACATCAGAGATTTTGAGACAC
sepF-mutF-f		ACAGGAATTGATGAAAGGGAATCCG
sepF-mutF-r2		CTTCCATTGAGAAAAAGTTTTTCAG
sepF-mutIn-f2		CTGAAAAACTTTTTCTCAATGGAAG
pGBT9-mutIn-r		gctcttctggtggagtctatccGGCTGCAGGTCGACGGAATC
rPCR-CmF2		ggatagactccaccagaagagcATCATCGGCAATAGTTACCC
rPCR-CmR(-ter)		ccaggatgtagtatccttccgTTATAAAAGCCAGTCATTAGGCC
ylmG-rPCR-f		CGGAAGGATACTACATCCTGG-AGAGGTGGTAAAGCGAGATG
sepF-mutB-r		TCCCGTAACAGCAGAGAGGATGACC
HS09	pSG1154 R	CATCCTAGGAATCTCCTTTCTAG
HS479	pSG1154 F	AGCGGCTCAGGATCCATGAGTAAAGGAGAAGAACTTTTCAC
HS478	SepF F	GAGATTCCTAGGATGAGTATGAAAAATAAACTGAAAAACTTTTTC
HS480	N13 R	GGATCCTGAGCCGCTTCCTGACATTGAGAAAAAGTTTTTCAGTTTATTTTTC
HS481	N25 R	GGATCCTGAGCCGCTTCCTGATGTCTCAATATATTCATATTCGTATTC
HS484	JunLZ F	GCGCGGGATCCGGTGGTCGTATCGCTCGTCTG
HS485	JunLZ R	CGCGCGGATCCTGAGCCGCTTCCGTTCATAACTTTCTGTTTCAGCTG
HS495	Pxyl F	AATTCGTCTCCCGGCCTTCAAAGCCTGTCGGAATTG
HS497	Pxyl(Δ2–13) R	AATTCGTCTCTCCATCCTAGGAATCTCCTTTCTAGATG
HS498	Pxyl(AH_{MinD}) R	AATTCGTCTCACCATCCTAGGAATCTCCTTTCTAGATG
HS501	SepF(Δ2–13) F	AATTCGTCTCTATGGAAGATGAAGAATACGAATATGAATATATTG
HS503	SepF(AH_{MinD}) F	AATTCGTCTCTATGGTGCTTGAAGAGCAAAACAAAGGAA TGATGGCTAAGATTAAGTCATTTTTCGGAGTAAGATCTGA AGATGAAGAATACGAATATG
HS500	SepF R	AATTCGTCTCCCCAATTACCACCTCTGATGTTCG

Tag sequence for adapter and recombinant PCR is shown in lowercase.