Supplemental material

Roles of the essential protein FtsA in cell growth and division

in *Streptococcus pneumoniae*

Table S1. Strains, plasmids and constructs used in this study

Strain[a]	Relevant genotype[b,c]	Reference
S. pneumoniae		
Rx1	unencapsulated wild-type	Ravin *et al.*, 1959
Rx1 *ftsA*$^+$//P$_{Zn}$*gfp-ftsA*$^+$	*tet, bgaA'*::P$_{czcD}$*-gfp-ftsA* (Rx1 transformed with PvuI cut pJWV25-*gfp-ftsA*$^+$)	This study
Rx1 Δ*ftsA*//P$_{Zn}$*gfp-ftsA*$^+$	*tet, bgaA'*::P$_{czcD}$*-gfp-ftsA, cm,* Δ*ftsA*::P$_{less}$*-cat* (Rx1 P$_{Zn}$*gfp-ftsA*$^+$ transformed with Δ*ftsA*::P$_{less}$*-cat*)	This study
Rx1 *ftsZ-mCherry*	*ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 transformed with *ftsZ-mCherry*-P$_c$*-erm*)	This study
Rx1 *ftsA*$^+$//P$_{Zn}$*gfp-ftsA*$^+$ *ftsZ-mCherry*	*tet, bgaA'*:: P$_{czcD}$*-gfp-ftsA, ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 P$_{Zn}$*gfp-ftsA*$^+$ transformed with *ftsZ-mCherry*-P$_c$*-erm*)	This study
Rx1 *ftsA*//P$_{Zn}$*gfp-ftsA*$^+$ *ftsZ-mCherry*	*tet, bgaA'*::P$_{czcD}$*-gfp-ftsA, cm,* Δ*ftsA*::P$_{less}$*cat, ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 Δ*ftsA*//P$_{Zn}$*gfp-ftsA*$^+$ transformed with *ftsZ-mCherry*-P$_c$*-erm*)	This study
Rx1 Δ*sepF*	*cm,* Δ*ylmF::cat* (Rx1 transformed with Δ*ylmF::cat*)	Fadda *et al.*, 2003
Rx1 Δ*sepF ftsZ-mCherry*	*cm,* Δ*sepF::cat, ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 Δ*sepF* transformed with *ftsZ-mCherry*-P$_c$*-erm*)	This study
Rx1 Δ*sepF*//P$_{Zn}$*gfp-ftsA*$^+$	*cm,* Δ*sepF::cat; tet, bgaA'*::P$_{czcD}$*-gfp-ftsA*$^+$ (Rx1 Δ*sepF* transformed with PvuI cut pJWV25-*gfp-ftsA*$^+$)	This study
Rx1 Δ*sepF*//P$_{Zn}$*gfp-sepF*$^+$	*cm,* Δ*sepF::cat; tet, bgaA'*::P$_{czcD}$*-gfp-sepF*$^+$ (Rx1 Δ*sepF* transformed with PvuI cut pJWV25-*gfp-sepF*$^+$)	This study
Rx1 Δ*gpsB sup4*	*cm,* Δ*gpsB::cat; sup4* (Rx1 transformed with *gpsB::cat*)	Rued *et al.*, *submitted*
Rx1 Δ*gpsB sup4 ftsZ-mCherry*	*cm,* Δ*gpsB::cat; sup4*; *ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 Δ*gpsB* transformed with *ftsZ-mCherry*-P$_c$*-erm*)	Rued *et al.*, *submitted*
Rx1 Δ*gpsB sup4*//P$_{Zn}$*gfp-ftsA*$^+$	*cm,* Δ*gpsB::cat; sup4*; *tet, bgaA'*::P$_{czcD}$*-gfp-ftsA* (Rx1 Δ*gpsB* transformed with PvuI cut pJWV25-*gfp-ftsA*$^+$)	This study
Rx1 Δ*gpsB sup4*//P$_{Zn}$*gfp-gpsB*$^+$	*cm,* Δ*gpsB::cat; sup4*; *tet, bgaA'*::P$_{czcD}$*-gfp-gpsB* (Rx1 Δ*gpsB* transformed with PvuI cut pJWV25-*gfp-gpsB*$^+$)	This study
Rx1 *sepF*$^+$//P$_{Zn}$*gfp-sepF*$^+$	*tet, bgaA'*::P$_{czcD}$*-gfp-sepF*$^+$ (Rx1 transformed with PvuI cut pJWV25-*gfp-sepF*$^+$)	This study
Rx1 *sepF*$^+$//P$_{Zn}$*gfp-sepF*$^+$ *ftsZ-mCherry*	*tet*, *bgaA'*::P$_{czcD}$*-gfp-sepF, ftsZ::ftsZ-mCherry*-P$_c$*-erm* (Rx1 P$_{Zn}$*gfp-sepF*$^+$ transformed with *ftsZ-mCherry*-P$_c$*-erm*)	This study

Rx1 *gpsB*$^{+}$//P_{Zn}*gfp-gpsB*$^{+}$	*tet, bgaA'::*P_{czcD}*-gfp-gpsB* (Rx1 transformed with PvuI cut pJWV25-*gfp-gpsB*$^{+}$)	This study
Rx1 *gpsB*$^{+}$//P_{Zn}*gfp-gpsB*$^{+}$ *ftsZ-mCherry*	*tet*, *bgaA'::*P_{czcD}*-gfp-gpsB, ftsZ::ftsZ-mCherry*-P_c-*erm* (Rx1 P_{Zn}*gfp-gpsB*$^{+}$ transformed with *ftsZ-mCherry*-P_c-*erm*)	This study
IU1824	D39 Δ*cps, str*, *rpsL1*	Lanie *et al*., 2007
IU1945	D39 Δ*cps*	Lanie *et al*., 2007
IU6962	D39 Δ*cps, kan*, *ftsZ*-Myc-P_c-*kan*	Land *et al*., 2013
IU7612	D39 Δ*cps, rpsL1*, *kan*, Δ*sepF*::P_c-[*kan-rpsL*$^{+}$] (IU1824 transformed with amplicon Δ*sepF*:: P_c-[*kan-rpsL*$^{+}$] from K734)	This study
IU7614	D39 Δ*cps, rpsL1*, *kan*, *ftsZ*$^{+}$-P_c-[*kan-rpsL*$^{+}$]	Tsui *et al.*, 2016
IU7667	D39 Δ*cps, str*, *rpsL1*, *ftsZ*-Myc (IU7614 transformed with fusion amplicon *ftsZ*-Myc)	This study
IU8039	D39 Δ*cps, str*, *rpsL1*, Δ*sepF* markerless (IU7612 transformed with fusion amplicon Δ*sepF* markerless)	This study
IU8499	D39 Δ*cps, rpsL1*, *kan*, *ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF*::P_c-[*kan-rpsL*$^{+}$] (IU1824 was transformed with fusion amplicon *ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF*::P_c-[*kan-rpsL*$^{+}$])	This study
IU8503	D39 Δ*cps, str*, *rpsL1*, *ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF* markerless (IU7612 transformed with fusion amplicon *ftsZ*-Myc Δ*sepF* markerless)	This study
IU9767	D39 Δ*cps, rpsL1*, *kan*, P_c-[*kan-rpsL*$^{+}$]-*ftsA*$^{+}$ (IU1824 transformed with P_c-[*kan-rpsL*$^{+}$]-*ftsA*$^{+}$ fusion amplicon)	This study
IU9969	D39 Δ*cps, str*, *rpsL1*, FLAG-*ftsA* (IU9767 transformed with FLAG-*ftsA* fusion amplicon)	This study
IU10236	D39 Δ*cps, rpsL1*, *kan*, FLAG-*ftsA-ftsZ*-P_c-[*kan-rpsL*$^{+}$] (IU9969 transformed with *ftsZ*-P_c-[*kan-rpsL*$^{+}$] amplicon from IU7614)	This study
IU10304	D39 Δ*cps, str*, *rpsL1*, FLAG-*ftsA ftsZ*-Myc (IU10236 was transformed with *fts*Z-Myc amplicon from IU7667)	This study
IU10576	D39 Δ*cps, rpsL1*, *kan*, FLAG-*ftsA ftsZ*-Myc Δ*sepF*::P_c-[*kan-rpsL*$^{+}$] (IU10304 transformed with *ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF*::P_c-[*kan-rpsL*$^{+}$] amplicon from IU8499)	This study
IU10632	*str*, *rpsL1*, FLAG-*ftsA ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF* (IU10576 transformed with *ftsZ*-Myc-*ylmE*$^{+}$-Δ*sepF* amplicon from IU8499)	This study
IU12307	D39 Δ*cps, tet*, *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^{+}$ (IU1945 transformed with fusion *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^{+}$ fusion amplicon)	This study

IU12310	D39 Δ*cps, str*, *rpsL1*, *tet*, *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^+$ (IU1824 transformed with *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^+$ fusion amplicon)	This study
IU12323	D39 Δ*cps, spec*, Δ*ftsA*::*aad9*, *tet*, *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^+$ (IU12307 transformed with Δ*ftsA*::*aad9* fusion amplicon)	This study
IU12326	D39 Δ*cps, str*, *rpsL1*, Δ*sepF*, *tet*, *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^+$ (IU8039 transformed with *bgaA*'::*tet*-P_{Zn}-RBS_{ftsA}-*ftsA*$^+$ fusion amplicon)	This study
K734	D39 Δ*cps, kan*, Δ*sepF(spd_1477)*::P_c-[*kan-rpsL*$^+$] (IU1945 transformed with Δ*sepF*::P_c-[*kan-rpsL*$^+$] fusion amplicon)	This study
Escherichia coli		
DH5α	F$^-$, 80*dlacZ* M15 *(lacZYA-argF)* U169 *recA1 endA1hsdR17(rk-, mk+) phoAsupE44 -thi-1 gyrA96 relA1*	Hanahan, 1983
Plasmid		
pR326	*cm, source of cat cassette*	Fadda *et al.*, 2003
p*ply::cat*	*cm, ply::cat*	Fadda *et al.*, 2003
pJWV25	*amp*, *tetM*, *bgaA*, *-gfp*$^+$	Eberhardt *et al.*, 2009
pJWV25-*gfp-ftsA*$^+$	*amp*, *tetM*, *bgaA*, *-gfp-ftsA*$^+$	Beilharz *et al.*, 2012
pJWV25-*gfp-sepF*$^+$	*amp, tetM, bgaA, -gfp-sepF*$^+$	This study
pJWV25-*gfp-gpsB*$^+$	*amp, tetM, bgaA, -gfp-gpsB*$^+$	This study
Construct		
ftsZ-mCherry-P_c-*erm*	*erm*	Sham *et al.*, 2011
P_c-[*kan-rpsL*$^+$]	*kan*	Tsui *et al.*, 2011

[a]P_{Zn} stands for $P_{czcD,}$ Zn^{2+} inducible promoter.

[b]Strains were constructed as described in Materials and Methods.

[c]Antibiotic resistance markers: *cm,* chloramphenicol; *erm*, erythromycin; *kan,* kanamycin*; aad9/spec,* spectinomycin; *str*, *streptomycin*; *tet*, tetracycline; *amp*, ampicillin.

Table S2. List of primers used in this study

Primer	Sequence (5' to 3')	Template[a]	Product amplicon
For Construction of the Rx1 Δ*ftsA*//P_{Zn}*gfp-ftsA*$^+$ depletion mutant			
AKOF1_6297	TGCCACTGTCAGAAAGCCTA	Rx1	flanking region left upstream of *ftsA*
AKOR2_P_{less}-*cat*	AGTTCATTTGATATGCCTCCTAATCCAAGT TTCGCACTTCGTAT		
AKOF3_P_{less}-*cat*	ATACGAAGTGCGAAACTTGGATTAGGAGG CATATCAAATGAACT	pR326	P_{less}*cat*
AKOR4_P_{less}-*cat*	CAATACCACCAGGGAGGTCCAATATTATAA AAGCCAGTCATTAG		
AKOF5_P_{less}-*cat*	CTAATGACTGGCTTTTATAATATTGGACCT CCCTGGTGGTATTG	Rx1	flanking region right downstream of *ftsA*
AKOR6_8470	ACCTCCACCGACACCAATTAC		
Verification of integration of Δ*ftsA::*P_{less}*cat* at the *ftsA* native locus			
ADF1	AACCAAGCCGCCCAAATTTG	Rx1Δ*ftsA*//P_{Zn} *gfp-ftsA*[c]	upstream of *ftsA,*
ADR6	GCTTTACGACCAACCTCAGG		downstream of *ftsA*
Cloning of *gfp* fusions in the pJWV25 plasmid			
*sepF*_SpeIF[b]	GCGCACTAGTTCTTTAAAAGATAGATTCGA TAGATTTATAG	Rx1	*sepF*$^+$
*sepF*_NotIR[b]	ATAAGAATGCGGCCGCTTATTATCGTACTC TATTTCGCTTCATATCAAAACC		
*gpsB*_SpeF[b]	GGACTAGTATGGCAAGTATTATTTTTTCAG		*gpsB*$^+$
*gpsB*_NotIR[b]	ATTAGCGGCCGCTTAAAAATCTGAGTTATC TAAAA		
Verification of integration of *gfp-fusions* at the ectopic *bgaA* region			
*bga*_check_F	CCACTCGCAACAATCACTTGG	Rx1 P_{Zn}*gfp* fusion	*bgaA* region external
*bga*_PF_ext_661	GCTCATCATGTTGATTGTCA		
PR1_4392	TGTCAATTTGATAGCGGGAAC		pJWV25 internal
PF1_4372	GTTGGGAAGTGGAATGCAGTA		
*bga*_PR_ext_7834	AGGACAAGAGTTTTTCTTTGG		*bgaA r*egion external
*bga*_check_R	GGGATTGGTACTTATGGCCAATAACC		
For construction of IU7667 (*ftsZ*-Myc markerless)			
TT165	AGTGGTGCCGATATGGTCTTCATCACTGC T	IU6962[c]	3' *ftsZ*-Myc
TT587	GTATTTTCTTTTACATTCATTTACTTAAAGA TCTTCTTCAGAAATAAGTTTTTGTTCACG		
TT588	ACTTATTTCTGAAGAAGATCTTTAAGTAAAT GAATGTAAAAGAAAATACAGAACTTGTTT	D39	downstream of *ftsZ*
TT166	TCATTGGGAGAGCCGGTTCCTGTGAAGAA T		
For construction of IU8039 (Δ*sepF* markerless)			
P1477	ACTACCGTGAGACAGTGAAACCAGCTCAT TC	D39	upstream of *sepF*$^+$ 60 bp of *5' sepF*
AJP22	CACCCTGTTGATCTTCATCTGGTGAATCCT CATCCTCCGTAAAATAATCTAT		

AJP23	TTTACGGAGGATGAGGATTCACCAGATGAAGATCAACAGGGTGAGTT	D39	3’ 60 bp of *sepF*$^+$ downstream of *sepF*
P1478	GTTCCTCCAGCGAAACAGGTATACGACCAA		
For construction of IU8499 (*ftsZ*-Myc-*ylmE*$^+$-Δ*sepF*::P$_c$-[*kan-rpsL*$^+$])			
P1477	ACTACCGTGAGACAGTGAAACCAGCTCATTC	IU7667[c]	5’ of *ftsZ*-Myc-*ylmE*$^+$-and 5’ 60 bp of *sepF*
P1479	CATTATCCATTAAAAATCAAACGGATCCTATGAATCCTCATCCTCCGTAAAATAATCTAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-[*kan-rpsL*$^+$] cassette	P$_c$-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1480	CAAAAGCATAAGGAAAGGGGCCCCCAGATGAAGATCAACAGGGTGAGTT	D39	3’ 60 bp of *sepF*$^+$ downstream
P1478	GTTCCTCCAGCGAAACAGGTATACGACCAA		
For construction of IU8503 (*ftsZ*-Myc-*ylmE*$^+$-Δ*sepF* markerless)			
P1477	ACTACCGTGAGACAGTGAAACCAGCTCATTC	IU7667[c]	3’ of *ftsZ*-Myc-*ylmE*$^+$-and 5’ 60 bp of *sepF*
AJP22	CACCCTGTTGATCTTCATCTGGTGAATCCTCATCCTCCGTAAAATAATCTAT		
AJP23	TTTACGGAGGATGAGGATTCACCAGATGAAGATCAACAGGGTGAGTT	D39	3’ 60 bp of *sepF*$^+$ downstream
P1478	GTTCCTCCAGCGAAACAGGTATACGACCAA		
For construction of IU9767 (Pc-[*kan-rpsL+]-ftsA+*)			
TT780	CGCATTACCAAGGAGCAAATAGAGCTTCTTTGGCAGG	D39	3’ *spd_1481* + 70 bp intergenic region
TT751	ATTATCCATTAAAAATCAAACGGATCCTATCTATTCAGAAATTCTTATTTTATAAGCTGC		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P$_c$-[*kan-rpsL*$^+$] cassette	P$_c$-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
TT781	CAAAAGCATAAGGAAAGGGGCCCGCAGAAAAAATGATTGCAAAGGAAGC	D39	30 bp 3’ *spd_1481,* intergenic (281 bp) and 5’ *ftsA*
TT753	GCCTTCCGCTAATTTGCGAGAGGTTTTCAA		
For construction of IU9969 (FLAG-*ftsA*)			
TT780	CGCATTACCAAGGAGCAAATAGAGCTTCTTTGGCAGG	D39	Upstream of *ftsA* and FLAG
TT767	TCTCTAGCTTTATCATCATCATCTTTATAATCCATTACATCGCTTCCTCTCTATCTTCCA		
TT768	ATGGATTATAAAGATGATGATGATAAAGCTAGAGAAGGCTTTTTTACAGGTCTAGATATT	D39	FLAG-*ftsA*
TT753	GCCTTCCGCTAATTTGCGAGAGGTTTTCAA		
For construction of IU12307, IU12310 and IU12326 (*bgaA*’::*tet*-P$_{Zn}$-RBS$_{ftsA}$-*ftsA*$^+$)			
TT657	CGCCCCAAGTTCATCACCAATGACATCAAC	pJWV25	*bgaA*’::*tet*-P$_{Zn}$
AJP32	ACATCGCTTCCTCTCTATCTTCCTTGTTATAATAGATTTATGAACACCTTGTTCATTATC		

AJP48	AACAAGGTGTTCATAAATCTATTATAACAA GGAAGATAGAGAGGAAGCGATGTAATGG	D39	RBS_{ftsA}-*ftsA*$^+$ (24 bp upstream of *ftsA* + *ftsA*)
AJP49	CAACTGGTTTATGAGAAAGTAAGTTCTTTT ATTCGTCAAACATGCTTCCGATC		
AJP50	CGGAAGCATGTTTGACGAATAAAAGAACTT ACTTTCTCATAAACCAGTTGC	D39	3' fragment containing *bgaA'*
CS121	GCTTTCTTGAGGCAATTCACTTGGTGC		
For construction of IU12323 (Δ*ftsA*::*aad9*)			
AJP43	CTTTATGGTTGGTACTGGGTCAATC	D39	5' flanking region upstream of *ftsA*
AJP44	CGTATGTATTCAAATATATCCTCCTCACTA CATCGCTTCCTCTCTATCTTCCAAG		
AJP45	GGAAGATAGAGAGGAAGCGATGTAGTGAG GAGGATATATTTGAATACATACGAAC	IU1753[d]	*aad9* ORF replaces *ftsA* reading frame with extra 9 bp for primer design
AJP46	CTTGTTGGAAATCCGCCATATGTCGTTCTT ATAATTTTTTTAATCTGTTATTTAA		
AJP47	ACAGATTAAAAAAATTATAAGAACGACATA TGGCGGATTTCCAACAAGCTT	D39	90 bp of 3' *ftsA* and downstream of *ftsA*
TT462	CAGATGTTCACTCCTTGACCTGCTGCCTG G		
For construction of K734 (Δ*sepF*::P_c-[*kan-rpsL*$^+$])			
P1477	ACTACCGTGAGACAGTGAAACCAGCTCAT TC	D39	upstream of *sepF* + 60 bp of *sepF*
P1479	CATTATCCATTAAAAATCAAACGGATCCTA TGAATCCTCATCCTCCGTAAAATAATCTAT		
Kan rpsL forward	TAGGATCCGTTTGATTTTTAATGGATAATG	P_c-[*kan-rpsL*$^+$] cassette	P_c-[*kan-rpsL*$^+$]
Kan rpsL reverse	GGGCCCCTTTCCTTATGCTTTTG		
P1480	CAAAAGCATAAGGAAAGGGGCCCCCAGAT GAAGATCAACAGGGTGAGTT	D39	3' 60 bp of *sepF*$^+$ downstream
P1478	GTTCCTCCAGCGAAACAGGTATACGACCAA		

[a]Genomic DNA of indicated *S. pneumoniae* strains was used as templates for PCR reactions except for P_{less}-*cat* and P_c-[*kan-rpsL*$^+$] (Janus) cassette.

[b]The underlined text in the primer sequence column indicates restriction sites.

[c]Genotype of strain described in Table S1.

[d]Genotype of IU1753 is R6 Δ*mreD*<>*aad9* (Land & Winkler, 2011).

References Table S1 and Table S2

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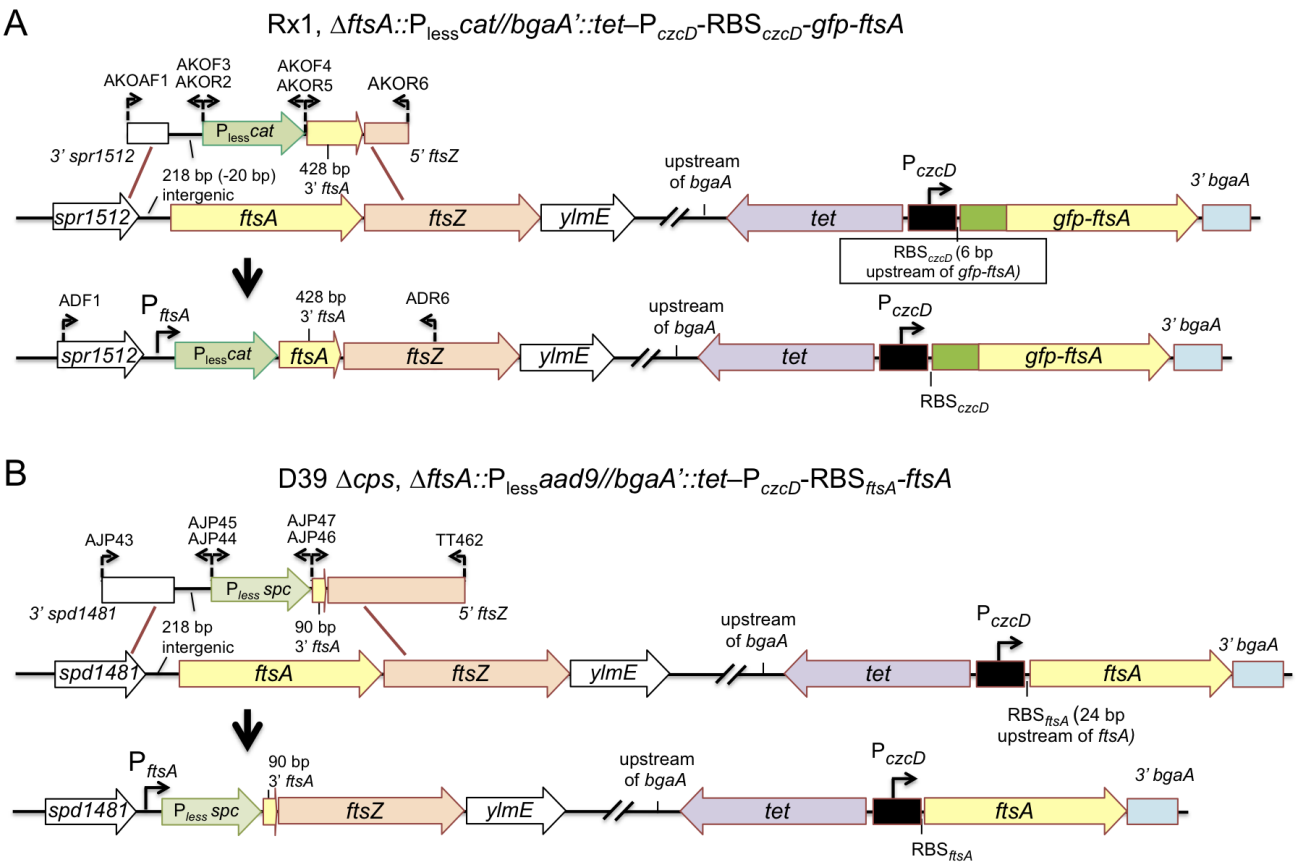

Fig. S1. Construction of (A) Rx1 Δ*ftsA*::P$_{less}$*cat//bgaA'*::*tet*–P$_{czcD}$-RBS$_{czcD}$-*gfp-ftsA* and (B) D39 Δ*cps* Δ*ftsA*::P$_{less}$*aad9//bgaA'*::*tet*–P$_{czcD}$-RBS$_{ftsA}$-*ftsA*. Amplicons containing promoterless *cat* or *spc* marker replacement of 5' *ftsA* and flanking regions were transformed into the merodiploid Rx1 strain containing P$_{czcD}$-RBS$_{czcD}$-*gfp-ftsA* or D39 Δ*cps* strain containing *tet*–P$_{czcD}$-RBS$_{ftsA}$-*ftsA* in the ectopic *bgaA* site. Primers used for construction of the Δ*ftsA* alleles are shown in the first line. P$_{czcD}$ is a Zn-dependent promoter described in *Materials and Methods* . P$_{ftsA}$ is the native promoter for the *ftsA-ftsZ-ylmE-sepF-ylmG-ylmH-divIVA* operon, although the exact location of this promoter has not been determined.

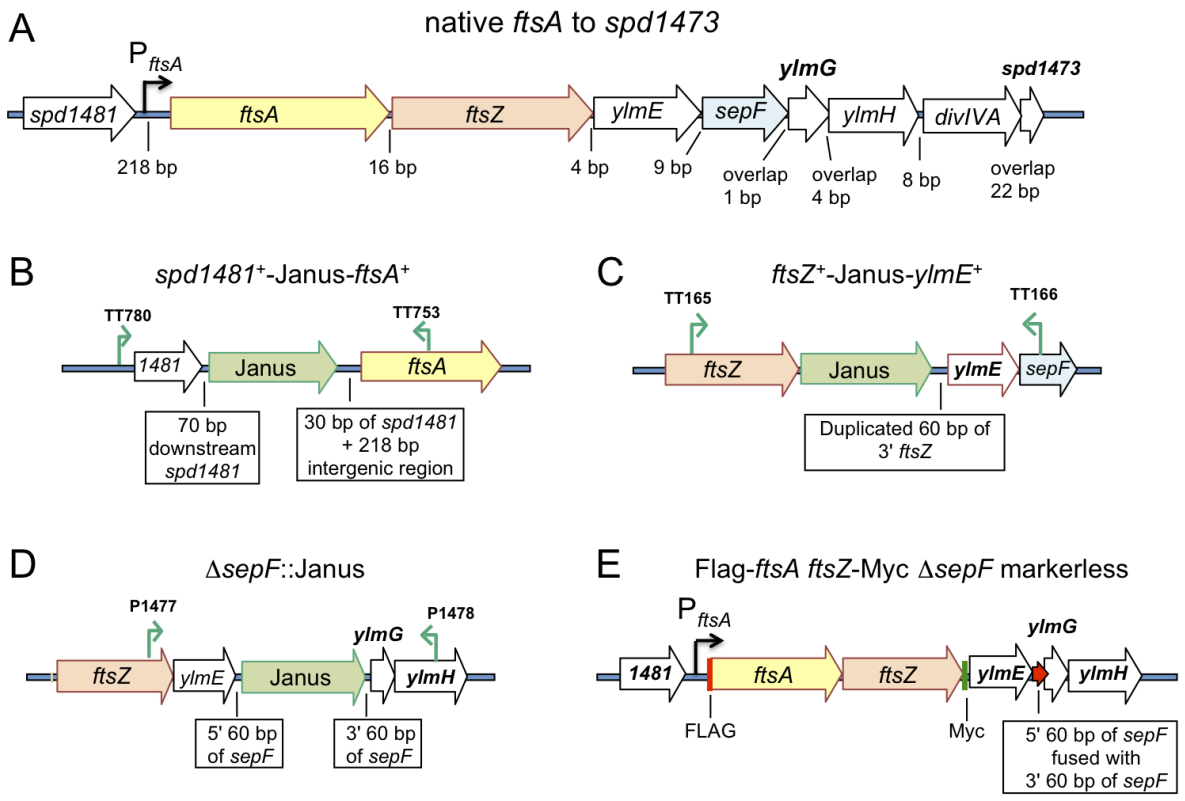

Fig. S2. Construction of D39 FLAG-*ftsA ftsZ*-Myc Δ*sepF* markerless strain. (A) The *ftsA-ftsZ-ylmE-sepF-ylmG-ylmH-divIVA-spd1473* operon in *S. pneumoniae* D39 strain as predicted by the DOOR 2.0 operon database (http://csbl.bmb.uga.edu/DOOR/). The exact location of the promoter for the operon (P_{ftsA}) has not been determined. (B-D) Intermediate constructs *spd1481*$^{+}$-Janus-*ftsA*$^{+}$: (B), *ftsZ*$^{+}$-Janus-*ylmE*$^{+}$ (C), and Δ*sepF*::Janus (D) containing the Janus cassette (P_c-[*kan-rpsL*$^{+}$]) to create markerless FLAG-*ftsA, ftsZ*-Myc or Δ*sepF* markerless alleles in native chromosomal loci (see Supporting Information Table S1). Outside primers (green arrows) are labeled on each figure and fusion primers used for the generation of amplicons are listed in Supporting Information Table S2. (E) Final FLAG-*ftsA ftsZ*-Myc Δ*sepF* markerless construct. All native intergenic sequences are retained in this construct.

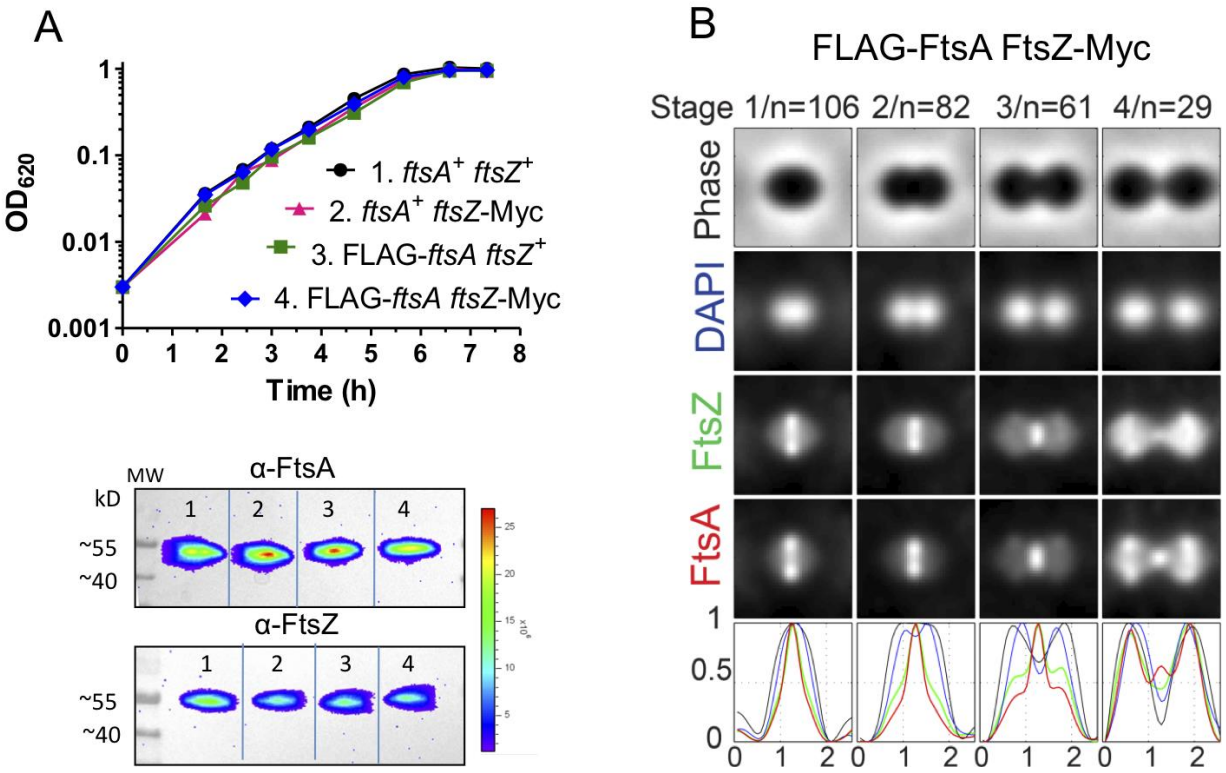

Fig. S3. Comparison of the D39 Δ*cps* FLAG-*ftsA ftsZ*-Myc strain with the wild-type *ftsA*$^+$ *ftsZ*$^+$ strain and colocalization of FtsZ and FtsA. (A) Growth curves (top) and Western blotting signals (bottom) using antibodies against FtsA or FtsZ of (1) D39 WT *ftsA*$^+$ *ftsZ*$^+$ strain IU1945, (2) *ftsA*$^+$ *ftsZ*-Myc strain IU7667, (3) FLAG-*ftsA ftsZ*$^+$ strain IU9969 and (4) FLAG-*ftsA ftsZ*-Myc strain IU10304. (B) Averaged images and fluorescence intensity traces of 2D IFM images obtained with FLAG-*ftsA ftsZ*-Myc strain IU10304. Cells were binned into division stages 1–4, and images of the indicated number of cells (n) from two experiments were averaged and quantified using the graphical user interface program (GUI) described in Materials and Methods. Row 1, cell shapes from phase-contrast images; row 2, DAPI (nucleoid staining) fluorescent signal; row 3, FtsZ-Myc fluorescent signal; row 4, FLAG-FtsA fluorescent signal and row 5, normalized average fluorescence intensity distributions along the horizontal cell axis for each channel (black, phase; blue, DAPI; green, FtsZ; red, FtsA).

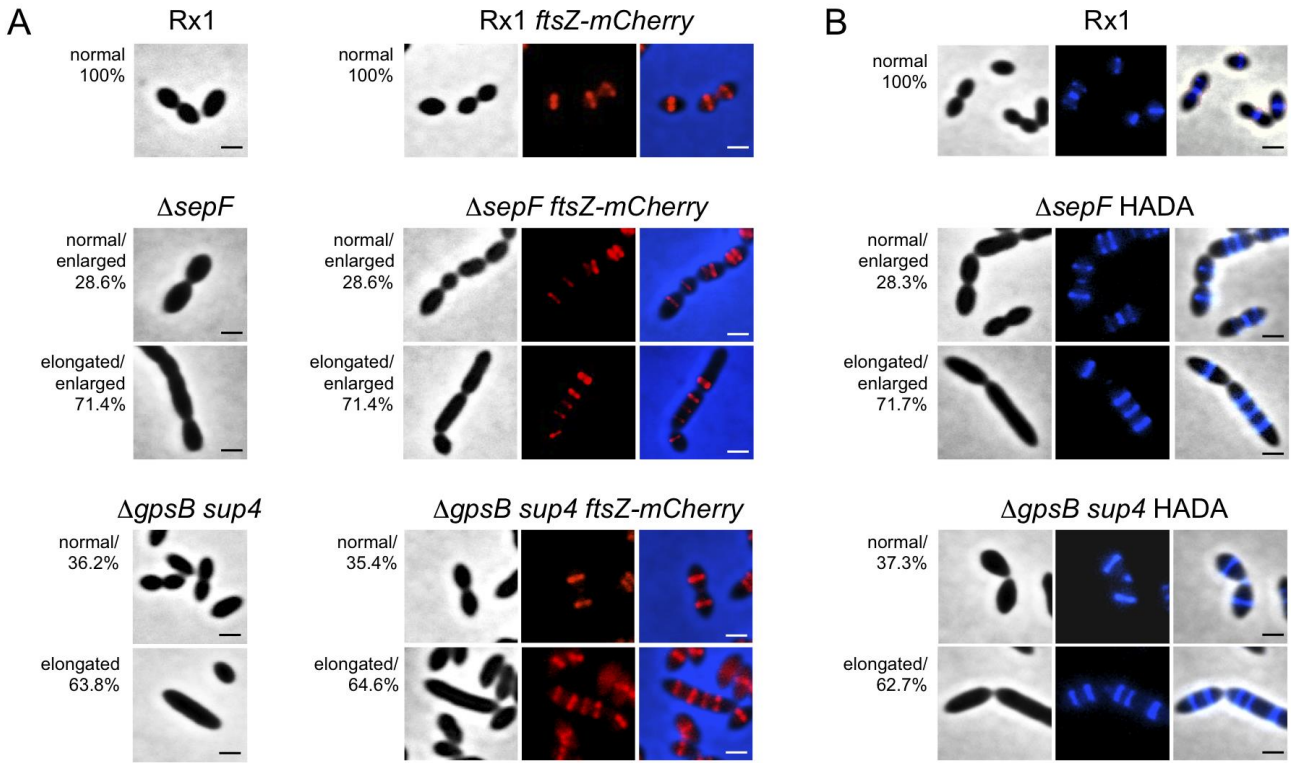

Fig. S4. Localization of FtsZ and PG synthesis in cells lacking SepF and GpsB. Cells were grown in TSB (or C+Y) medium at 28°C (or 37°C) to mid-exponential phase (0.3 OD). Fluorescence microscopy and HADA staining were performed as described in Materials and Methods. A minimum of 100 cells were counted and classified and the percentage (%) is indicated beside each frame. (A) Localization of FtsZ in Rx1 *ftsZ-mCherry*, Rx1 Δ*sepF ftsZ-mCherry* and Rx1 Δ*gpsB sup4 ftsZ-mCherry*. Upper panels, Rx1 and Rx1 *ftsZ-mCherry*; Middle panels, Rx1 Δ*sepF* and Rx1 Δ*sepF ftsZ–mCherry*; Lower panels, Rx1 Δ*gpsB sup4* and Rx1 Δ*gpsB sup4 ftsZ–mCherry.* The frames shown, from left to right, are: phase-contrast, FtsZ-mCherry fluorescence and phase-contrast/FtsZ-mCherry fluorescence overlay. Size bar, 1 µm. (B) Localization of PG synthesis in Rx1, Rx1 Δ*sepF* and Rx1 Δ*gpsB sup4.* Upper panels, Rx1; Middle panels, Rx1 Δ*sepF*; Lower panels, Rx1 Δ*gpsB sup4.* The frames shown, from left to right, are: phase-contrast, HADA labelling fluorescence and phase-contrast/HADA labelling fluorescence overlay. Size bar, 1 µm.

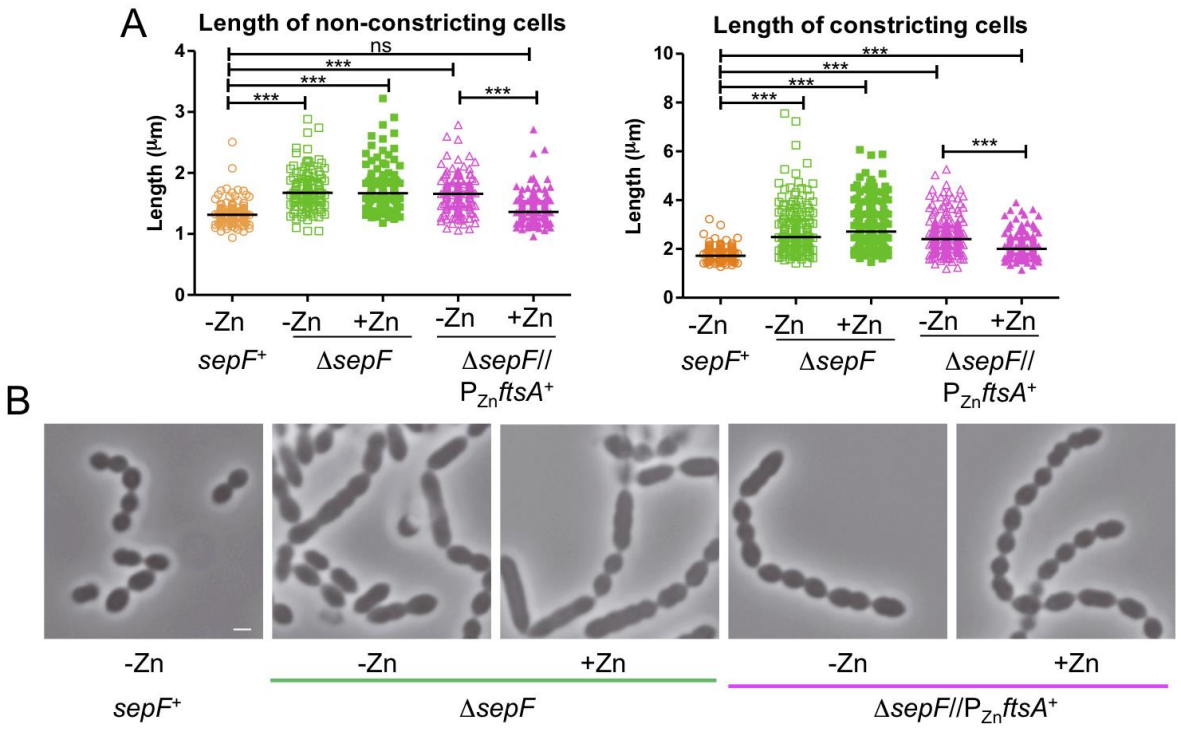

Fig. S5. Overproduction of FtsA partially reverses cell-length defects in Δ*sepF* mutants of *S. pneumoniae* D39. Exponentially growing strains of IU1824 (*sepF*$^+$; wild-type parent strain), IU8039 (Δ*sepF*), and IU12326 (Δ*sepF*//P_{Zn}*ftsA*$^+$) were diluted into BHI broth (starting OD_{620} ~ 0.005) supplemented with no zinc/manganese (-Zn) or 0.4 mM $ZnCl_2$ and 0.04 mM $MnSO_4$ (+Zn) and grown at 37°C for 3 h prior to fixing with 4% paraformaldehyde for direct imaging via phase-contrast microscopy. (A) Scatter plot analysis of cell lengths in non-constricting cells (left graph) or constricting cells (right graph) with the median shown as the black bar. Over 100 cells from two independent experiments were analyzed per strain in each experiment. Open shapes indicate the absence of Zinc inducer (-Zn) while closed shapes indicate the presence of Zinc inducer (+Zn). The following symbols indicate the respective strain: orange circles are IU1824, green squares are IU8039, magenta triangles are IU12326. P values were obtained by one-way ANOVA analysis (GraphPad Prism, nonparametric Kruskal-Wallis test). ($P < 0.001$ indicated by ***). (B) Phase-contrast representative images of strains listed in A. Size bar, 1 μm.