SUPPLEMENTARY MATERIAL

Agrobacterium tumefaciens divisome proteins regulate the transition from polar growth to cell division

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1) **SUPPLEMENTAL TABLES S1 and S2**
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1) SUPPLEMENTARY TABLES

Table S1. Bacterial strains and plasmids used in this study.

Strain or plasmid	Relevant characteristics	Reference/ Source
Source Plasmids		
pSRKKm-Plac	Km^r; broad host range vector containing *lacI^q* and lac promoter	(Khan *et al.*, 2008)
pSRKKm-Plac-sf*gfp*	pSRKKm vector containing *lacI^q* and lac promoter with sfGFP	(Figueroa-Cuilan *et al.*, 2016)
pRVMCS-2	Km^r; vector containing vanillate promoter which provides low constitutive expression in *A. tumefaciens*	(Thanbichler *et al.*, 2007)
pMR10	Km^r; mini-RK2 vector; constitutive expression	Brun Lab
pNTPS139	Km^r; Suicide vector containing *oriT* and *sacB*	D. Alley
pUC18-mini-Tn7T-GM-LAC	Ap^r Gm^r; mini-Tn7 vector containing *lacI^q* and tac promoter	(Choi *et al.*, 2010)
pUC18-mini-Tn7T-GM-Plac	Ap^r Gm^r; mini-Tn7 vector containing *lacI^q* and lac promoter	(Figueroa-Cuilan *et al.*, 2016)
pTNS3	Ap^r; helper plasmid encoding the site-specific TnsABCD Tn*7* transposition pathway	(Choi *et al.*, 2010)
pET21a	Ap^r; plasmid for IPTG-inducible expression in *E. coli* for protein overproduction	Novagen
pET21c	Ap^r; plasmid for IPTG-inducible expression in *E. coli* for protein overproduction	Novagen
pTB146	Ap^r; plasmid for IPTG-inducible expression in *E. coli* for overproduction of His_6-SUMO protein fusions	(Bendezu *et al.*, 2009)
pXCFPN-1	Sp^r; integrating plasmid for xylose-inducible expression of N-terminal CFP fusions in *Caulobacter crescentus*	(Thanbichler *et al.*, 2007)
Replicating plasmids		
pRV-*ftsZ_AT*-sf*gfp*	Km^r; Constitutive expression of *ftsZ_AT*-sf*gfp*	(Howell *et al.*, 2017)
pRV-*ftsZ_1*-sf*gfp*	Km^r; Constitutive expression of *ftsZ_1*-sf*gfp*	This Study
pRV-*ftsZ_3*-sf*gfp*	Km^r; Constitutive expression of *ftsZ_3*-sf*gfp*	This Study
pSRKKM-Pqaz-*ftsA*-sf*gfp*	Km^r; vector containing *A. tumefaciens* native *ftsQAZ* promoter followed by *ftsA-sfGFP* coding sequence.	(Howell *et al.*, 2017)
pMR10-PpopZ-*popZ-yfp*	pMR10 vector containing *popZ-yfp* under control of the native promoter	Brun Lab
pRV-*ldtp_0845*-sf*gfp*	Km^r; Constitutive expression of *ldtp_0845*-sf*gfp*	This Study
Deletion plasmids		
pNTPS139Δ*ftsZ_AT*	Km^r Suc^s; deletion plasmid for *ftsZ_AT*	This Study
pNTPS139Δ*ftsZ_1*	Km^r Suc^s; deletion plasmid for *ftsZ_1*	This Study
pNTPS139Δ*ftsZ_3*	Km^r Suc^s; deletion plasmid for *ftsZ_3*	This Study
pNTPS139Δ*ftsA*	Km^r Suc^s; deletion plasmid for *ftsA*	This Study
pNTPS139Δ*ftsW*	Km^r Suc^s; deletion plasmid for *ftsW*	This Study
Depletion plasmids		
pUC18-mini-Tn7T-GM-LAC *ftsZ_AT*	Ap^r Gm^r; mini-Tn7 vector containing *ftsZ_AT* under control of the tac promoter	This Study
pUC18-mini-Tn7T-GM-Plac *ftsA*	Ap^r Gm^r; mini-Tn7 vector containing *ftsA* under control of the lac promoter	This Study
pUC18-mini-Tn7T-GM-Plac *ftsW*	Ap^r Gm^r; mini-Tn7 vector containing *ftsW* under control of the lac promoter	This Study
Plasmids for protein production		
pET21a $FtsZ_{AT}$ (pEG1555)	Ap^r; plasmid for overproduction of $FtsZ_{AT}$ in *E. coli* under control of the T7 promoter and lac operator	This Study

pET21a FtsZ$_{AT}$-L72W (pEG1556)	Ap^{r}; plasmid for overproduction of FtsZ$_{AT}$-L72W in *E. coli* under control of the T7 promoter and lac operator	This Study
pTB146 FtsZ$_{1}$ (pEG1535)	Ap^{r}; plasmid for overproduction of His$_{6}$-SUMO-FtsZ$_{1}$ in *E. coli* under control of the T7 promoter and lac operator	This Study
pTB146 FtsZ$_{1}$-L71W (pEG1542)	Ap^{r}; plasmid for overproduction of His$_{6}$-SUMO-FtsZ$_{1}$-L71W in *E. coli* under control of the T7 promoter and lac operator	This Study
E. coli strains		
DH5α	Cloning strain	Life Technologies
S17-1	Smr;RP4-2 TC::MU Km-Tn7; for plasmid mobilization	(Simon *et al.*, 1983)
NEB Turbo	Cloning strain	New England Biolabs
Rosetta(DE3)pLysS	Strain for protein overproduction	Novagen
A. tumefaciens strains		
C58	Nopaline type strain; pTiC58; pAtC58	(Watson *et al.*, 1975)
C58ΔtetRA::a-*att*Tn7	Replacement of the *tetRA* locus with an artificial *att*Tn7 site	(Figueroa-Cuilan *et al.*, 2016)
C58ΔtetRA::a-*att*Tn7 pRV-*ftsZ$_{AT}$-sfgfp*	C58ΔtetRA::a-*att*Tn7 with pRV expression FtsZ$_{AT}$-sfGFP constitutively	This Study
C58ΔtetRA::a-*att*Tn7 pRV-*ftsZ$_{1}$-sfgfp*	C58ΔtetRA::a-*att*Tn7 with pRV expression FtsZ$_{1}$-sfGFP constitutively	This Study
C58ΔtetRA::a-*att*Tn7 pRV-*ftsZ$_{3}$-sfgfp*	C58ΔtetRA::a-*att*Tn7 with pRV expression FtsZ$_{3}$-sfGFP constitutively	This Study
C58ΔtetRA::a-*att*Tn7 Δ*ftsZ$_{1}$*	Δ*ftsZ$_{1}$*	This Study
C58ΔtetRA::a-*att*Tn7 Δ*ftsZ$_{3}$*	Δ*ftsZ$_{3}$*	This Study
C58ΔtetRA::a-*att*Tn7 Δ*ftsZ$_{1}$* Δ*ftsZ$_{3}$*	Δ*ftsZ$_{1}$* Δ*ftsZ$_{3}$*	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*	Mini-Tn7T-GM-Ptac-*ftsZ$_{AT}$* inserted into a-*att*Tn7 site	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*, Δ*ftsZ$_{AT}$*	Chromosome-based complementation of Δ*ftsZ$_{AT}$* with C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$* allowing depletion of FtsZ$_{AT}$ under control of the tac promoter	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*, Δ*ftsZ$_{AT}$* Δ*ftsZ$_{1}$*	FtsZ$_{AT}$ depletion background; Δ*ftsZ$_{1}$*	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*, Δ*ftsZ$_{AT}$* Δ*ftsZ$_{3}$*	FtsZ$_{AT}$ depletion background; Δ*ftsZ$_{3}$*	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*, Δ*ftsZ$_{AT}$* Δ*ftsZ$_{1}$* Δ*ftsZ$_{3}$*	FtsZ$_{AT}$ depletion background; Δ*ftsZ$_{1}$* Δ*ftsZ$_{3}$*	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$*; pRV-*ftsZ$_{1}$-sfgfp*	FtsZ$_{AT}$ depletion background with pRV constitutive expression of FtsZ$_{1}$-sfGFP	This Study
C58ΔtetRA::a-*att*Tn7; pSRKKm Pqaz-*ftsA-sfgfp*	constitutive expression of FtsA-sfGFP from Pqaz	This study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ$_{AT}$* ; pSRKKm Pqaz-*ftsA-sfgfp*	FtsZ$_{AT}$ depletion background with constitutive expression of FtsA-sfGFP from Pqaz	This Study
C58ΔtetRA::a-*att*Tn7; pSRKKm PpopZ-*popZ-*	constitutive expression of PopZ-YFP from native promoter	This study

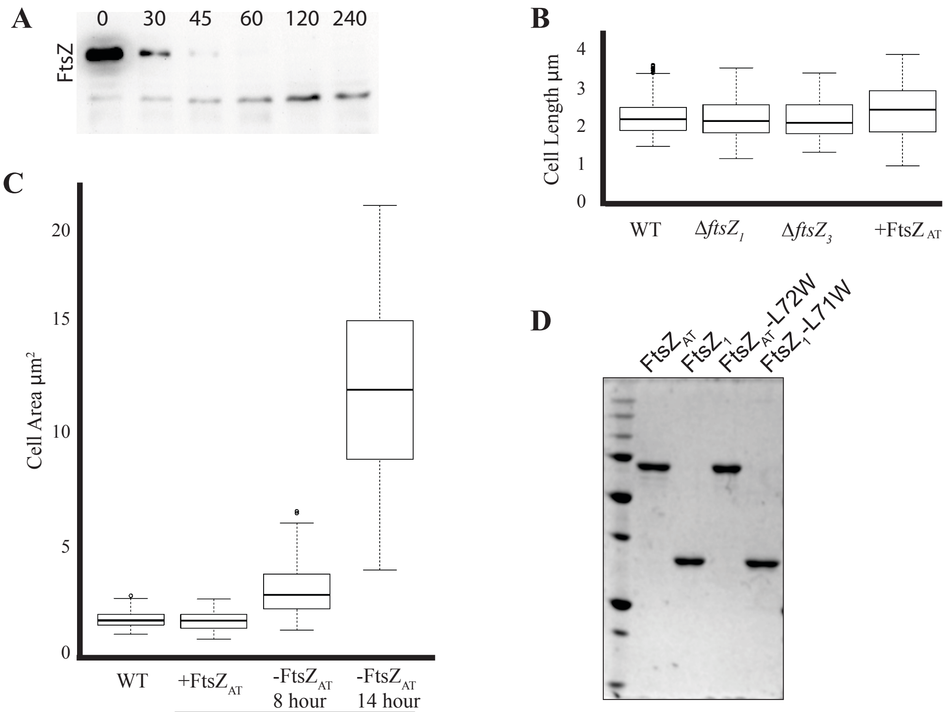
yfp		
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ*$_{AT}$; pSRKKm PpopZ-*popZ-yfp*	FtsZ$_{AT}$ depletion background with constitutive expression of PopZ-YFP from native promoter	This Study
C58ΔtetRA::a-*att*Tn7 pRV-*ldtp*$_{0845}$*-sfgfp*	C58ΔtetRA::a-*att*Tn7 with pRV constitutive expression of LDTP$_{0845}$-sfGFP	This Study
C58ΔtetRA::mini-Tn7-GM-Ptac-*ftsZ*$_{AT}$; pRV-*ldtp*$_{0845}$*-sfgfp*	FtsZ$_{AT}$ depletion background with pRV constitutive expression of LDTP$_{0845}$-sfGFP	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsA*	Mini-Tn7T-GM-Plac-*ftsA*inserted into a-*att*Tn7 site	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsA*, Δ*ftsA*	Chromosome-based complementation of Δ*ftsA* with C58ΔtetRA::mini-Tn7-GM-Plac-*ftsA* allowing depletion of FtsA under control of the lac promoter	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsA*, Δ*ftsA;* pSRKKM PpopZ-*popZ-sfgfp*	FtsA depletion background with constitutive expression of PopZ-sfGFP	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsA*, Δ*ftsA;* pRV-*ftsZ*$_{AT}$*-sfGFP*	FtsA depletion background with constitutive expression of FtsZAT-sfGFP	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsW*	Mini-Tn7T-GM-Plac-*ftsW* inserted into a-*att*Tn7 site	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsW*, Δ*ftsW*	Chromosome-based complementation of Δ*ftsW* with C58ΔtetRA::mini-Tn7-GM-Plac-*ftsW* allowing depletion of FtsW under control of the lac promoter	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsW*, Δ*ftsW;* pSRKKM PpopZ-*popZ-sfgfp*	FtsW depletion background with constitutive expression of PopZ-sfGFP	This Study
C58ΔtetRA::mini-Tn7-GM-Plac-*ftsW*, Δ*ftsW;* pRV-*ftsZ*$_{AT}$*-sfGFP*	FtsW depletion background with constitutive expression of FtsZAT-sfGFP	This Study

Table S2. Synthesized DNA primers and gene fragments used in this study.

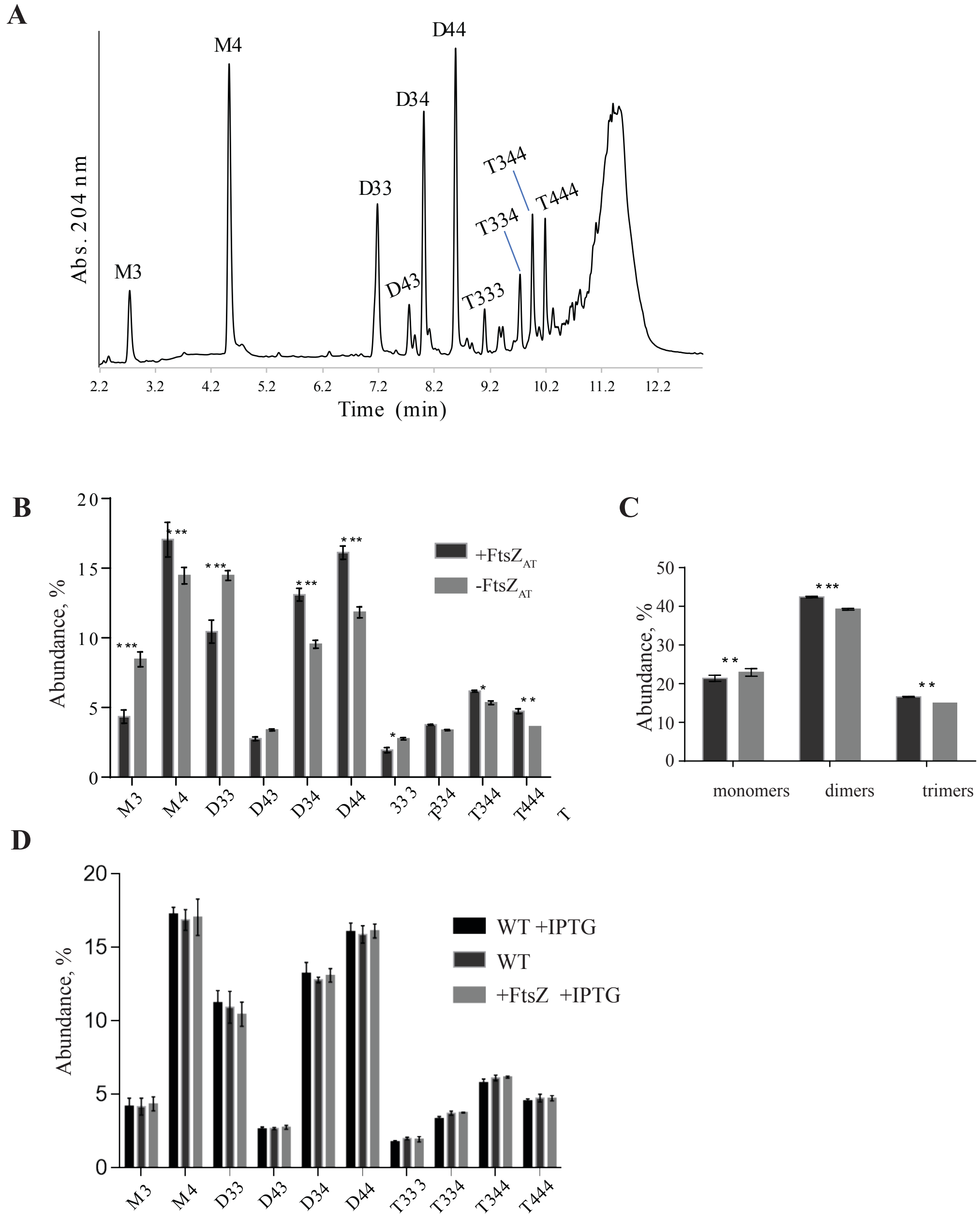
Synthesized DNA	Sequence (5' – 3')
Primers for gene amplification	
FtsZ$_{AT}$ For NdeI	CGA CGC CAT ATG ACG ATA CAG CTG C
FtsZ$_{AT}$ Rev BamHI for *gfp* fusion	GCG TGA GGA TCC GTT GGA CTGGCG GCG C
FtsZ$_{AT}$ Rev BamHI stop codon	CGG CAC GGA TCC TTA GTT GGA CTG GC
FtsZΔCTP Rev NheI	GCT AGC TTA GTG ATG GCT GGA CGA GGA AAG
FtsZ$_{1}$ For NdeI	GCA CGT CAT ATG ACC CAG TCT CCG CGC GCC
FtsZ$_{1}$ Rev BamHI for *gfp* fusion	GCA CGT GGA TCC GTT ACG CAA GCC GGT GGC CAC
FtsZ$_{1}$ Rev BamHI stop codon	GCA CGT GGA TCC TCA GTT ACG CAA GCC GGT GGC CAC
FtsZ$_{3}$ For AseI	GCA CGT ATT AAT ATG AAA CCA ACA ATG GCC ACA
FtsZ$_{3}$ Rev BamHI for *gfp* fusion	GCA CGT GGA TCC TGT CGG CTC CGC GTT CTC AA
FtsZ$_{3}$ Rev BamHI stop codon	GCA CGT GGA TCC TCA TGT CGG CTC CGC GTT CTC AA
FtsA For NdeI	CGA CGC CAT ATG AGC TTT TTT GGT TC
FtsA Rev BamHI	CGG CAC GGA TCC AAA ACT TTC TTT CAG CC
PopZ XmaI For	GCA TTA CCC GGG ATG GCT CAG CCA AGT
PopZ BamHi Rev	GTC GCT GGA TCC GCG GCG CGA GCC GCG CGC CAC A
Ldtp0845 NdeI for	GCC TAC ATA TGT ACA GCG GCG ATA TAG CCG
Ldtp0845 BamHI rev	CGC TCG GAT CCT TAC TGC CAC ACC ACC ACA CG
FtsW For AseI for	GCA CGT ATT AAT ATG GTA AGC CGA GTT GAT CGC G
FtsW Rev BamHI	GCA CGT GGA TCC CTC CGC AGG AAC GCC GGA ACC G
FtsZ$_{AT}$ GTPase Rev KpnI SacI (oEG827)	GAT CGA GCT CGG TAC CGT CGA TGC CGG TGG CGA C
FtsZ$_{AT}$ For NdeI (oEG789)	GAT CCA TAT GAC GAT ACA GCT GCA AAA G
FtsZ$_{AT}$ Rev EcoRI (oEG1160)	GAT CGA ATT CTT AGT TGG ACT GGC GGC
FtsZ$_{1}$ For SapI (oEG1132)	GAC TGC TCT TCT GGT ATG ACC CAG TCT CCG CG
FtsZ$_{1}$ Rev BamHI (oEG1133)	GAC TGG ATC CTT AGT TAC GCA AGC CGG TG
Oligos for mutagenesis	
FtsZ$_{AT}$-L72W (oEG1134)	GGC GTC AAC GTC ACC GAA GGT TGG GGC GCC GGT TC
FtsZ$_{1}$-L71W (oEG1135)	CTG ACC GGC GGC TGG GGC GCA GGC GCC
Primers for deletion vectors and deletion confirmation	
FtsZ$_{1}$ P1	GGA TCC CCT CTT CTT CCG CCT G
FtsZ$_{1}$ P2	AAG CGC CCC GTC TTA TTC TCT TTC
FtsZ$_{1}$ P3	TAA GAC GGG GCG CTT TCG CAG C
FtsZ$_{1}$ P4	ACT AGT CCA TGG TCG AAC GGT TCG
FtsZ$_{1}$ P5	CTA CGG CCT GAT CCA GCC

$FtsZ_1$ P6	GTA CAT CAG CAC CAG CGC ATC
$FtsZ_3$ P1	GCT GCA ACT AGT AAG TGG GGC ACG CAG GGC CAT
$FtsZ_3$ P2	AAG CAT GGT ACC GAA TTC GGA AGC CGA TCT CCT GGC AA
$FtsZ_3$ P3	GAA TTC GGT ACC ATG CTT TGA GTG AGA CAA CGT TCT A
$FtsZ_3$ P4	GCA CGT AAG CTT CCG GTC TTG CGG TCT TGT CC
$FtsZ_3$ P5	GAG CCA CCC CGA TTG CCG AAC
$FtsZ_3$ P6	CTC ACG CGC TAT CGT GCG TTG
$FtsZ_{AT}$ P1	GCA ACT AGT GAT CGC GAT CAA CCA TCG
$FtsZ_{AT}$ P2	AAG CTT GGT ACC GAA TTC TTT ACC ATT CCT TCT TTC
$FtsZ_{AT}$ P3	GAA TTC GGT ACC AAG CTT AGG CGA TTC CAT ACG CTC
$FtsZ_{AT}$ P4	GCA GGA TCC GAT AGC GAA CGG CCG AGC
$FtsZ_{AT}$ P5	AAT CGA AGA TGC GGA GAG
$FtsZ_{AT}$ P6	AGG TTG TCG AGG CCC ATC
FtsA P1	GCA TAG ACT AGT GAA CTG TCG CTG ATC GAA
FtsA P2	AAG CTT GGT ACC GAA TTC GAC GAT GTG GCT GCG CTT
FtsA P3	GAA TTC GGT ACC AAG CTT GGA ATG TTT TCG CCG TTT GG
FtsA P4	GCA TAG GGA TCC GCC GCC GCC CAT GCC GGC
FtsA P5	CGA AGA CGG TGG AAG TTC GCC TG
FtsA P6	CGA TGC CCT GTT CGG CCA GAC
FtsW P1	GCT GCA ACT AGT GGA AAT TCG TGC CGG TCT CAA GTC
FtsW P2	AAG CAT GGT ACC GAA TTC GGC CTC AGC CCC CTT TTC CAG
FtsW P3	GAA TTC GGT ACC ATG CTT TAA ACG ATC ATG AAC AAG GGT A
FtsW P4	GCA CGT GGA TCC ACC GGA TTG CCG GTC GCA ACC GT
FtsW P5	GAC GGT ATC CAG ACG CTT CGC GGC AGC CA
FtsW P6	GGT CTC GCC CGT CTG TGA AGG CGT ATA G
Primers for confirmation of Mini-Tn7 vector insertion	
Tet forward	ACA TGT TGT ATA CCG GAA ACT GAT TGC AC
Tn7R109	CAG CAT AAC TGG ACT GAT TTC AG

2) SUPPLEMENTAL FIGURES



Supplemental Figure 1. Cell growth and morphology of *ftsZ* mutants. A.) Western blot analysis showing the depletion of FtZ_{AT} after the removal of inducer. B) Cell lengths of WT, $\Delta ftsZ_1$, $\Delta ftsZ_3$, and induced $ftsZ_{AT}$. are indistinguishable. C) Cell area in WT and induced $ftsZ_{AT}$ are the same while cells depleted of $FtsZ_{AT}$ for 8 and 14 hours accumulate cell area. D) Coomassie Blue stained protein gel showing the purification of $FtsZ_{AT}$, $FtsZ_1$, $FtsZ_{AT}$-L72W, and $FtsZ_1$-L71W.



Supplemental Figure 2. Peptidoglycan analysis of WT and *ftsZ* depletion strains. A) UPLC spectra of muropeptides derived from WT cells. Major muropeptides are labeled. M= monomers, D= dimers, T= trimers. Numbers indicate the length of the muropeptide stems and the position of crosslink in dimers and trimers. B) Quantitation of the major muropeptide peaks in $ftsZ_{AT}$ depletion strain induced (black) and depleted for 14 hours (gray). C) Abundance of total monomers, dimers, and trimers in the muropeptide profile in $ftsZ_{AT}$ depletion strain when induced (black) and after 14 hours of depletion (gray). D) Quantitation of the major muropeptide peaks in WT with IPTG (black), WT without IPTG (black with gray outline), and $ftsZ_{AT}$ depletion strain induced with IPTG (gray). Data shown is the average abundance of each muropeptide and is taken from analysis of three independent biological samples. Statistical significance is indicated with an asterisk.

3) SUPPLEMENTAL MOVIE LEGENDS

Movie 1. Growth and morphological changes during $FtsZ_{AT}$ depletion. Cells were washed to remove inducer and grown in liquid ATGN for 4 hours before spotting on an ATGN pad. Images were acquired every ten minutes and movie is played at 10 frames per second for a total of 145 frames.

Movie 2. Growth and morphological changes during FtsA depletion. Cells were washed to remove inducer and grown in liquid ATGN for 2 hours before spotting on an ATGN pad. Images were acquired every ten minutes and movie is played at 10 frames per second for a total of 97 frames.

Movie 3. Growth and morphological changes during FtsW depletion. Cells were washed to remove inducer and grown in liquid ATGN for 4 hours before spotting on an ATGN pad. Images were acquired every ten minutes and movie is played at 10 frames per second for a total of 85 frames.

4) SUPPLEMENTAL REFERENCES

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