Table 1. Plasmids used in this study

Plasmids	Relavant characteristics or construction	Source or reference
pBXMCS-2	High-copy replicating plasmid for xylose-inducible gene expression, kan^R	Thanbichler *et al* (2007)
pBW005	pRVMCS-5 with *clpX** inserted between NdeI and NheI sites	This Study
pBW015	pVGFPC-1 with *clpX* inserted between NdeI and PacI sites	This Study
pBW016	pXMCHYC-4 with *ftsZ* inserted between NdeI and PacI sites	This Study
pBW020	pXSTZN-2 with *ftsZ* inserted between KpnI and SacI sites	This Study
pBW030	pRVGFPC-5 with *clpA* inserted between NdeI and PacI sites	This Study
pBW041	pBXMCS-2 with *ftsA* inserted between NdeI and PacI sites	This Study
pBW045	pXSTZN-2 with $ftsZ_{DD}$ inserted between KpnI and SacI sites	This Study
pBW047	pXSTZC-2 with *ftsZ* inserted between NdeI and AgeI sites	This Study
pBW048	pXSTZC-2 with *ftsA* inserted between NdeI and AgeI sites	This Study
pBW062	pET21B with *tap-*$ftsA_{1236\text{-}1326}$ (MTS) insert from pBW061 inserted in between NdeI and SacI sites	This Study
pEG032	pXSTZN-2 with *ftsQ* inserted between KpnI and AgeI sites	Goley ED, unpublished
pEG033	pXSTZN-2 with *ftsA* inserted between KpnI and SacI sites	Goley ED, unpublished
pET21a	Bacterial vector for expressing N- and/or C-terminal His_6-tagged proteins in *E. coli*	Novagen
pET21b	Bacterial vector for expressing N- and/or C-terminal His_6-tagged proteins in *E. coli*	Novagen
pMT219	pET21a with *ftsZ* inserted between NdeI and SacI sites	Thanbichler *et al* (2006)
pRVGFPC-5	Low-copy replicating plasmid for vanillate-inducible C-terminal eGFP fusions, Tet^R	Thanbichler *et al* (2007)
pRVMCS-5	Low-copy replicating plasmid for vanillate-inducible gene expression, Tet^R	Thanbichler *et al* (2007)
pVGFPC-1	Low-copy replicating plasmid for vanillate-inducible C-terminal eGFP fusions, $Strep^R$/$Spec^R$	Thanbichler *et al* (2007)
pXMCHYC-4	Integrating plasmid for generation of C-terminal mCherry fusions at *xylX* locus, $Gent^R$	Thanbichler *et al* (2007)
pXSTZC-2	Integrating plasmid for generation of C-terminal TAP tag fusions at *xylX* locus, Kan^R	Goley ED, unpublished
pXSTZN-2	Integrating plasmid for generation of N-terminal TAP tag fusions at *xylX* locus, Kan^R	Goley ED, unpublished

Table 2. *Caulobacter crescentus* strains used in this study

Strains	Relevant genotpye	Construction or Source
LS101	CB15N - synchronizable derivative of wild-type strain CB15	Evinger and Agabian (1997)
LS5344	CB15N *vanA*::P_{vanA}-*clpX-egfp*	pBW015 into LS101
LS5345	CB15N *vanA*::P*vanA-clpX-egfp, xylX*::P_{xylX}-*ftsZ-mCherry*	pBW016 intoLS5344
LS5346	CB15N *ftsZ::ftsZΔC xylX::*P_{xylX}-*ftsZ, vanA*::P_{vanA}-*clpX-egfp*	pBW015 into YB1585
LS5347	CB15N *xylX*::P_{xylX}-*tap-ftsZ*	pBW020 into LS101
LS5348	CB15N *xylX*::P_{xylX}-*tap-ftsA*	pBW033 into LS101
LS5349	CB15N pP_{vanA}-*clpX**	pBW005 into LS101
LS5350	CB15N pP_{vanA}-*clpX**, *xylX*::P_{xylX}-*tap-ftsZ*	pBW020 into LS5349
LS5351	CB15N pP_{vanA}-*clpX**, *xylX*::P_{xylX}-*tap-ftsA*	pEG033 into LS5349
LS5352	CB15N pP_{vanA}-*clpX**, *xylX*::P_{xylX}-*tap-ftsQ*	pEG032 into LS5349
LS5353	CB15N *xylX*::P_{xylX}-*ftsZ-tap*	pBW047 into LS101
LS5354	CB15N *xylX*::P_{xylX}-*tap-ftsZ-DD*(TAP-$FtsZ_{A507D\ N508D}$)	pBW045 into LS101
LS5355	CB15N *xylX*::P_{xylX}-*ftsA-tap*	pBW048 into LS101
LS5356	CB15N Δ*clpA*::Ω, *xylX*::P_{xylX}-*tap-ftsZ*	pBW020 into UJ838
LS5357	CB15N Δ*clpA*::Ω, *xylX*::P_{xylX}-*tap-ftsA*	pEG033 into UJ838
LS5358	CB15N Δ*clpA*::Ω, *xylX*::P_{xylX}-*tap-ftsQ*	pEG032 into UJ838
LS5359	CB15N pP_{vanA}-*clpX**, pP_{xylX}-*ftsA*	pBW041 into LS5349
LS5360	CB15N pP_{vanA}-*clpA-egfp*	pBW030 into LS101
LS5361	CB15N *ΔclpA*::Ω, pP_{vanA}-*clpA-egfp*	pBW030 into UJ838
LS5362	CB15N Δ*clpA::Ω ftsZ::ftsZΔC xylX::*P_{xylX}-*ftsZ*	Transduction of Δ*clpA::Ω* $Spec^R$ into YB1585
LS5363	CB15N *ftsZ::ftsZΔC xylX::*P_{xylX}-*ftsZ* pP_{vanA}-*clpX**	pBW005 into YB1585
LS5364	CB15N pP_{xylX}-*ftsA*	pBW041 into LS101
LS5365	CB15N Δ*clpA*::Ω pP_{xylX}-*ftsA*	pBW041 into YB1585
LS5366	CB15N pP_{vanA}-*clpX**, *xylX*::P_{xylX}-*ftsA-tap*	pBW048 into LS5349
LS5367	CB15N Δ*clpA*::Ω, *xylX*::P_{xylX}-*ftsA-tap*	pBW048 into UJ838
UJ838	CB15N Δ*clpA*::Ω	Grunenfelder *et al* (2004)
YB1585	CB15N *ftsZ::ftsZΔC xylX*::P_{xylX}-*ftsZ*	Wang *et al* (2001)

Supplementary Figures

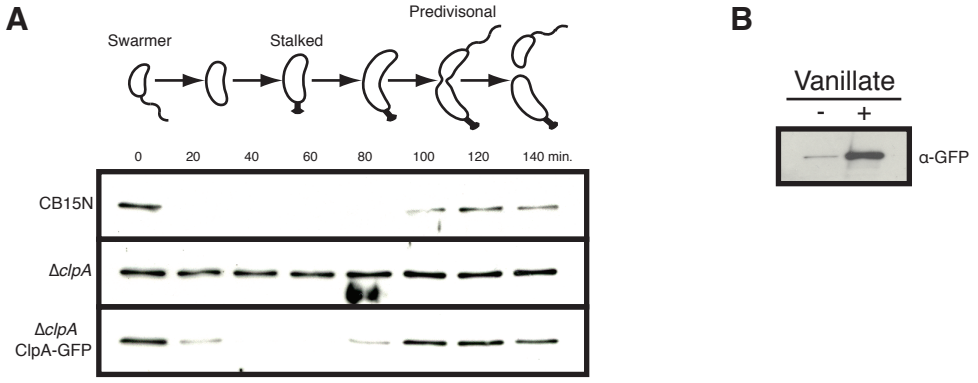

Fig. S1. Intracellular levels of Flif and ClpA-GFP. (A) ClpA-GFP rescues cell cycle dependent degradation of the FliF ClpA substrate. Swarmer cells from strains CB15N wild type, Δ*clpA or* Δ*clpA:* pP_{vanA}-*clpA-egfp* induced with 50 mM vanillate for two hours, were isolated and allowed to progress synchronously through the cell cycle. Aliquots were taken at 20-minute intervals and subjected to immunoblot analysis using FliF-specific antiserum. (B) Intracellular levels of ClpA-GFP. Cells of the strain Δ*clpA* p*PvanA-clpA-gfp* were grown to exponential phase in M2G medium, induced with 0.5 mM vanillate for 2 h, and analyzed by immunoblotting using anti-GFP antiserum (+ van). As a control, uninduced cells were subjected to the same analysis (- van).

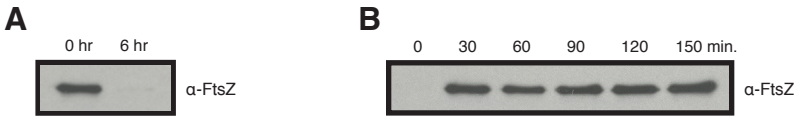

Fig. S2. Intracellular levels of FtsZ. (A) Immunoblots of samples taken concurrently with the fluorescence microscopy. A sample of cells from a mixed population of the strain *ftsZ::ftsZΔC xylX::*P_{xylX}-*ftsZ* P_{vanA}-*clpX-egfp* grown in minimal media supplemented with 0.3% xylose was taken before the population of cells was washed and resuspended in minimal media lacking xylose. A sample of cells was subsequently taken again after growth in the absence xylose for six hours. Each sample was then subjected to immunoblot analysis with anti-sera specific for FtsZ. (B) FtsZ levels upon reinduction with xylose after a six-hour depletion. A population of cells from the same population used above was resuspended in media supplemented with 0.3% xylose. A sample of cells was taken every 30 minutes (starting with t = 0) and subjected to immunoblot analysis with antisera specific to FtsZ.

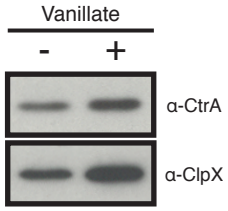

Fig. S3. Levels of CtrA and ClpX in the presence or absence of vanillate. (A) Cells from the strain pP_{vanA}-*clpX** were grown in rich PYE media in the presence of the vanillate inducer for two hours. Aliquots were taken before and after the addition of vanillate and subjected to immunoblot analysis using CtrA or ClpX-specific antiserum.

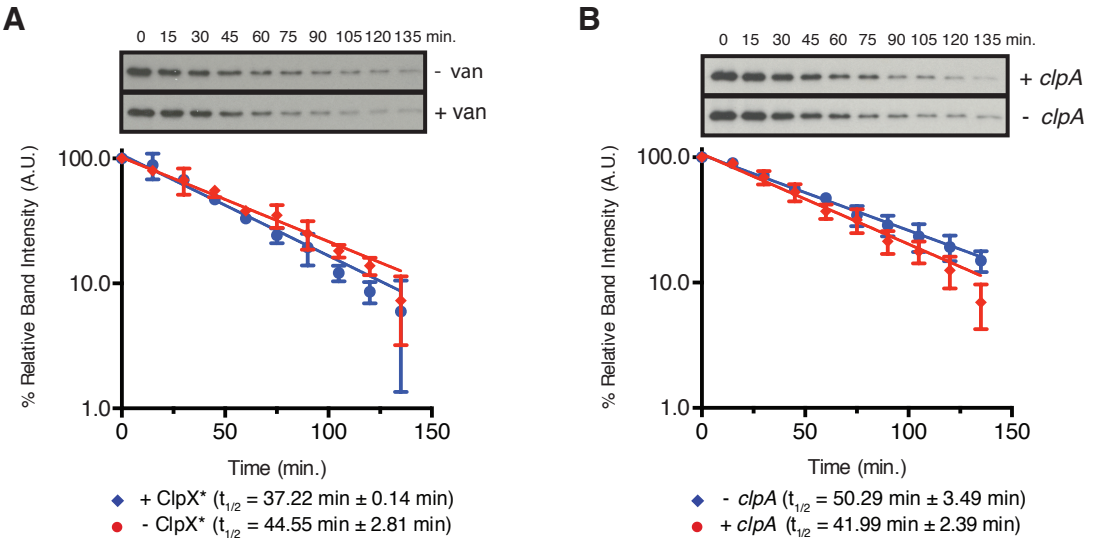

Fig. S4. FtsQ is not a substrate of ClpXP and ClpAP *in vivo*. (A) Levels of TAP-FtsQ in the presence or absence of ClpX*. ClpX* is a dominant, catalytically inactive mutant of ClpX. Cells from the strain pP$_{vanA}$-*clpX* xylX*::P$_{xylX}$-*tap-ftsQ* were grown in rich PYE media in the presence of vanillate and xylose for two hours. Cells were then washed and resuspended in media lacking xylose. Aliquots were taken at 15-minute intervals and subjected to immunoblot analysis using TAP-specific antiserum in the presence or absence of the vanillate inducer. (B) Levels of TAP-FtsQ in the presence or absence of ClpA. Cells from the strain Δ*clpA*::Ω *xylX*::P$_{xylX}$-*tap-ftsQ* were grown in rich PYE media in the presence of xylose for two hours, washed and resuspended in media lacking xylose. Aliquots were then taken at 15-minute intervals and subjected to immunoblot analysis using TAP-specific antiserum in the presence or absence of clpA allele. A graph of the relative band intensity as a function of time after the cessation of TAP-FtsQ synthesis is shown below each immunoblot.

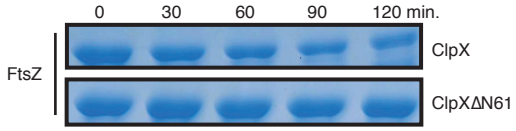

Fig. S5. ClpX N-terminal domain is important for proteolysis of FtsZ. Representative images of reactions showing purified *Caulobacter* FtsZ in the presence of ClpXP or ClpX(ΔN61)P and ATP *in vitro*. Reaction conditions consist of 1 μM ClpX$_6$ or 1 μM ClpX(ΔN61)$_6$, 1 μM ClpP$_{14}$ and 1 μM FtsZ.

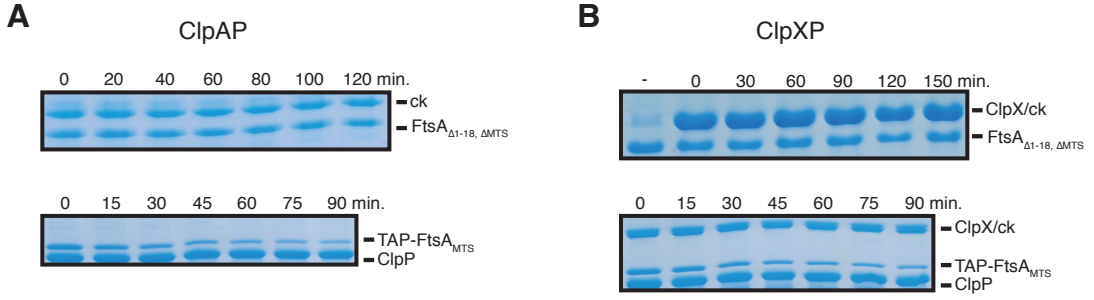

Fig. S6. The N- and C-terminus of FtsA is important for proteolysis by ClpAP and ClpXP. (A and B) Representative images of reactions showing purified FtsA$_{Δ1\text{-}18,\ ΔMTS}$ or TAP-FtsA$_{MTS}$ in the presence of ClpAP or ClpXP and ATP *in vitro*. Reaction conditions consist of 1μM ClpA$_6$ or 1μM ClpX$_6$, 1μM ClpP$_{14}$ and 1μM FtsA$_{Δ1\text{-}18,\ ΔMTS}$ (or 1.5 FtsA$_{Δ1\text{-}18,\ ΔMTS}$ in Fig. S6B) and 1 μM TAP-FtsA$_{MTS}$.