Supplementary Information

Species- and C-terminal linker-dependent variations in the emergent behavior of FtsZ on supported lipid bilayers

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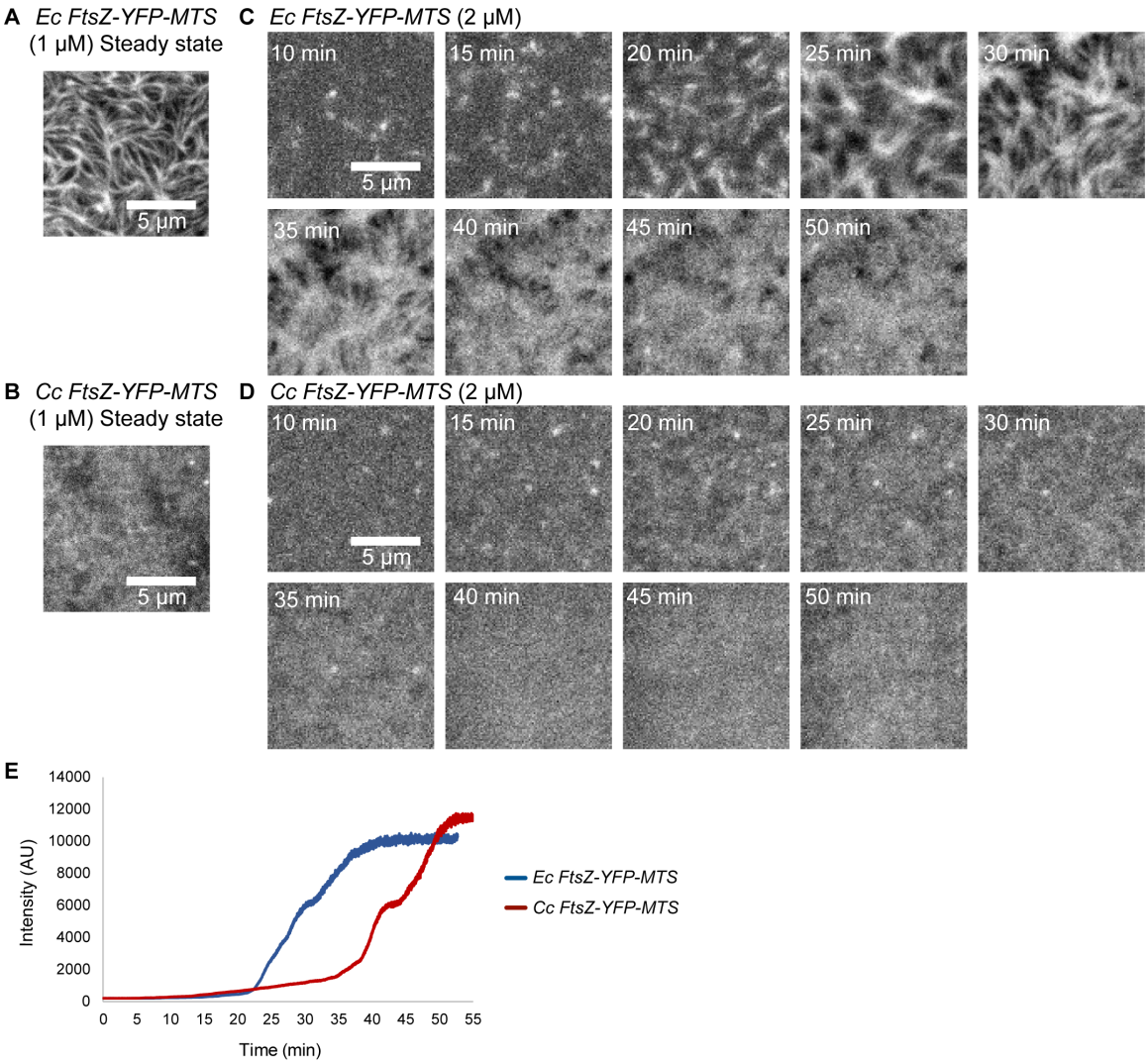
Supplementary figures (1 – 5)

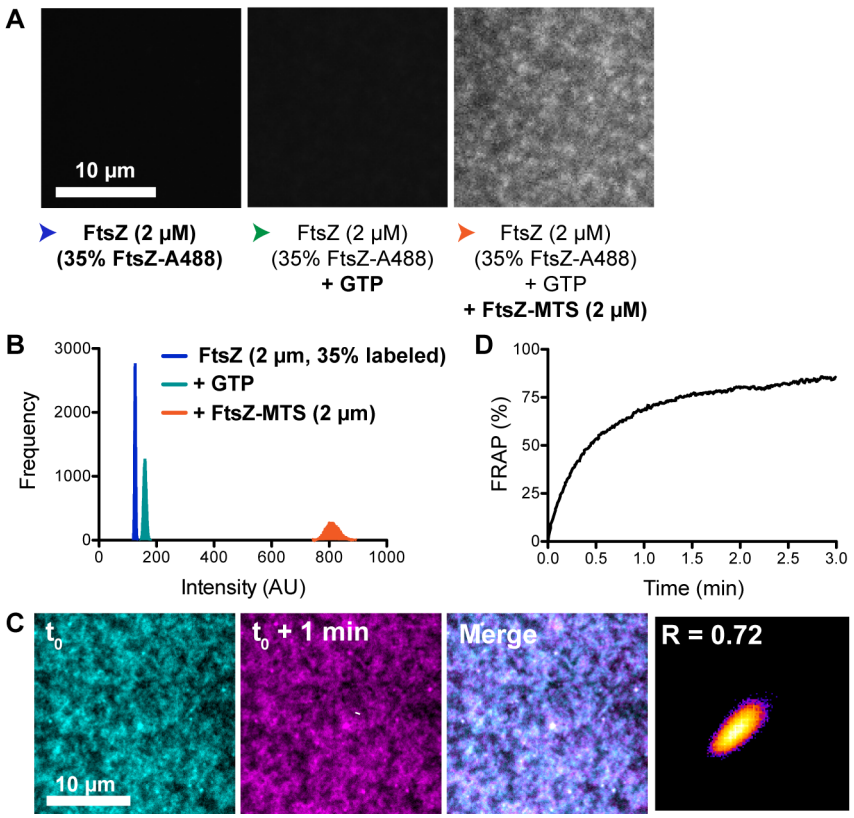
&

Plasmid insert sequences
(pEG658, pEG659, pEG717, pEG1295, pEG1293, pEG1297, pEG1296)

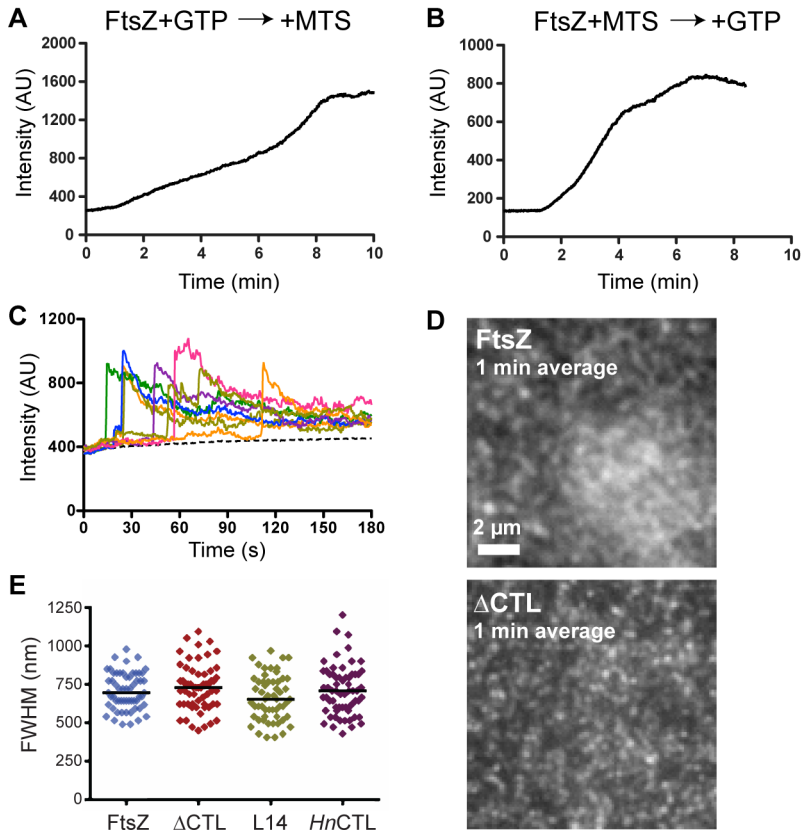


Supplementary Figure S1. FtsZs from *E. coli* and *C. crescentus* form distinct superstructures on SLBs. **A & B.** Contrast adjusted TIRFM images of structures formed by 1 μM *Ec* FtsZ-venus-MTS (A) or 1 μM *Cc* FtsZ-venus-MTS (B) at steady state on SLBs composed of 33% DOPG and 67% DOPC lipids. *Ec* or *Cc* FtsZ-venus-MTS incubated for 15 minutes with 2 mM GTP was flowed into flow cells (25 μL total at 5 μL minute^{-1}). The steady state images were captured after 20 minutes incubation of polymers within flow cells. Representative images of at least 3 replicates. Scale bar – 5 μm. **C & D.** Contrast adjusted TIRFM images formed by 2 μM (solution concentration) of *Ec* FtsZ-venus-MTS (C) or *Cc* FtsZ-venus-MTS (D) on SLBs composed of 33% DOPG and 67% DOPC lipids. *Ec* or *Cc* FtsZ-venus-MTS incubated for 15 minutes with 2 mM GTP was flowed into flow cells at 0.5 μL minute^{-1}. Time indicated represents time passed since the beginning of flow (i.e. 25 μL of reaction mixture introduced at time = 50 minutes). Scale bar – 5 μm. **E.** Plots showing fluorescence intensity (averaged over the frame) vs. time corresponding to experiments in C & D. Reaction buffer contains

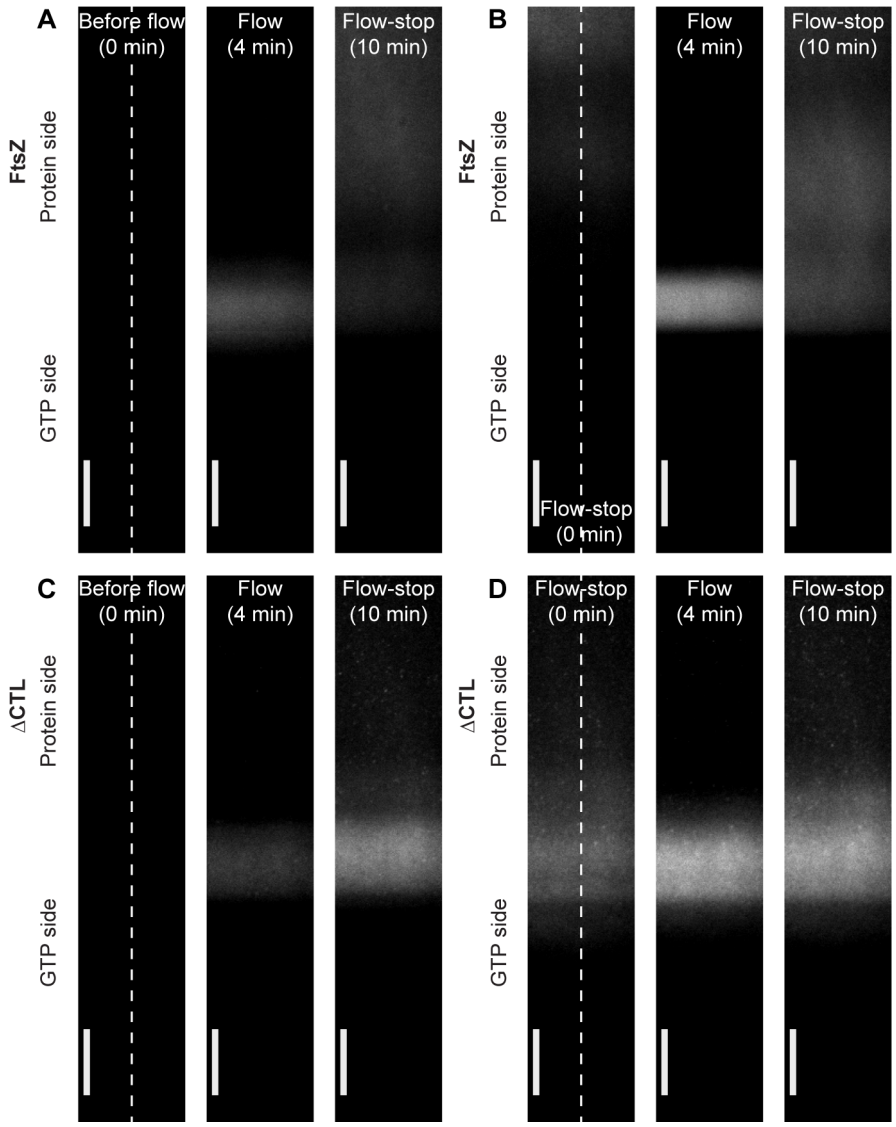
50 mM HEPES pH 7.3, 5 mM $Mg(CH_3COO)_2$, 300 mM KCH_3COO, 50 mM KCl, 10% glucose, 0.1 mg mL^{-1} casein (blocking agent).



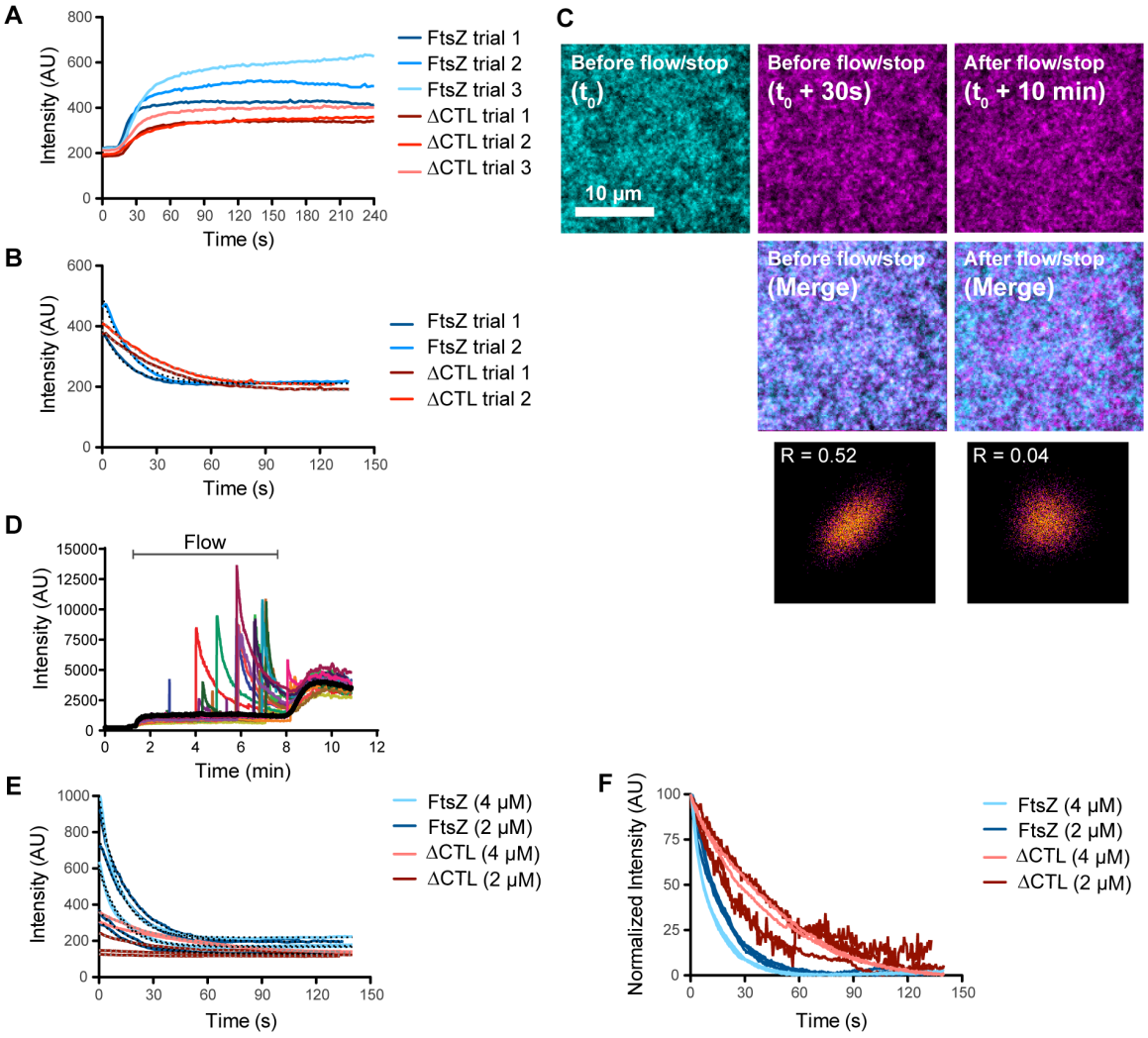
Supplementary Figure S2. FtsZ/FtsZ-MTS protofilaments form dynamic superstructures on SLBs. **A.** TIRFM images corresponding to images shown in Figure 2D and 2E with identical contrast adjustments in all three images. **B.** Histogram of fluorescence intensity corresponding to micrographs shown in Figure 2D and 2E (and Supplementary Figure S2A). **C.** Individual contrast enhanced frames and merged TIRFM images showing overlay of structures formed by 2 µM FtsZ (35% FtsZ-Alexa488) and 2 µM FtsZ-MTS in the presence of GTP at steady state spaced 1 minute apart (cyan – time 't_0', magenta – time 't_0 + 1 minute', white regions in the merged image represent colocalization of signal). Scale bar – 10 µm. 2-D intensity histograms and Pearson's coefficient (R) values shown correspond to overlaid images. **D.** Fluorescence intensity at the region of photobleaching over time showing fluorescence recovery after photobleaching (FRAP) for structures formed by 2 µM FtsZ (35% FtsZ-Alexa488) and 2 µM FtsZ-MTS with GTP on SLB at steady state. Plot shows average of 3 replicates. Scale bar – 10 µm. Reaction buffer contains 50 mM HEPES pH 8.0, 0.1 mM EDTA, 2.5 mM $MgCl_2$, 300 mM KCl, 1% glycerol, 0.1 mg/mL casein (blocking agent), 0.5 mg/mL ascorbate.



Supplementary Figure S3: ΔCTL/ΔCTL-MTS forms extended bundles and sparse superstructures on SLBs. **A & B.** Average fluorescence intensity over time following the introduction (by flow at 0.5 µL/minute) of 2 µM FtsZ-MTS into a flow cell equilibrated with 2 µM FtsZ (6% FtsZ-Alexa488) incubated with 2 mM GTP (A) or 2 mM GTP into flow cell equilibrated with 2 µM FtsZ (6% FtsZ-Alexa488) and 2 µM FtsZ-MTS (B). **C.** Plot of average fluorescence intensity over time of the extended structures observed for ΔCTL/ΔCTL-MTS on SLBs. Black dotted line represents fluorescence intensity averaged over the frame (~1000 μm^2). Colored lines each indicate average fluorescence intensities of regions of interests (ROIs) where individual bright extended structures appear. **D.** Time averages for frames corresponding to changes in structures on the SLB over 1 minute for 2 µM FtsZ or ΔCTL with corresponding 2 µM MTS fusions at steady state. Scale bar – 10 µm. **E.** Full width at half-maximum intensity (FWHM) of fluorescent clusters observed at steady state in flow cells containing FtsZ/FtsZ-MTS (2 µM FtsZ (6% FtsZ-Alexa488) and 2 µM FtsZ-MTS), ΔCTL/ΔCTL-MTS, L14/L14-MTS, or *Hn*CTL/*Hn*CTL-MTS in the presence of 2 mM GTP. Bar indicates median (n > 50). Reaction buffer contains 50 mM HEPES pH 8.0, 0.1 mM EDTA, 10 mM $MgCl_2$, 300 mM KCl, 1% glycerol, 0.1 mg/mL casein (blocking agent), 0.5 mg/mL ascorbate.



Supplementary Figure S4: Characterization of differences in large-scale polymer assembly between FtsZ and ΔCTL. **A – D.** Contrast adjusted TIRFM images of distribution of polymers corresponding to kymographs shown in Figure 4B – 4E respectively, at times indicated. (A – D correspond to Movies 4.1 – 4.4). Scale – 100 μm. Dashed line indicates region of the frame used for making corresponding kymographs. A & B – FtsZ/FtsZ-MTS polymers. C & D. ΔCTL/ΔCTL-MTS polymers.



Supplementary Figure S5: Two-inlet setup allows rapid depletion/repletion of GTP for following disassembly/reassembly of FtsZ or ΔCTL superstructures. **A.** Fluorescence intensity profiles on the protein side showing assembly after stoppage of flow for 2 μM FtsZ (6% Alexa488 labeled) and 2 μM FtsZ-MTS (unlabeled) or 2 μM ΔCTL (6% Alexa488 labeled) and 2 μM ΔCTL-MTS (unlabeled). **B.** Fluorescence intensity profiles on the protein side showing disassembly after restarting flow to deplete GTP in flow cells with 2 μM FtsZ (6% Alexa488 labeled) and 2 μM FtsZ-MTS (unlabeled) or 2 μM ΔCTL (6% Alexa488 labeled) and 2 μM ΔCTL-MTS (unlabeled). Overlaid dotted lines indicate non-linear fit assuming one-phase exponential decay. Curves in A and B were obtained from experiments using 10x objective magnification. **C.** Contrast-adjusted TIRFM images (at 100x magnification) and merged images showing overlay of structures formed on SLBs by FtsZ/FtsZ-MTS corresponding to experiment in C at steady state before flow at time = 0 minutes (cyan) and at time = 30 seconds (magenta) or at steady state after reinitiating and stopping flow at time = 10 minutes (magenta). White regions in the merged image represent colocalization of signal. Scale bar – 10 μm. 2-D intensity histograms and

Pearson's coefficient (R) values shown correspond to overlaid images. **D.** Plots of fluorescence intensity over time of large fluorescent clusters (aggregates) observed on the protein side when flowing 4 μM ΔCTL (6% Alexa488 labeled) and 4 μM ΔCTL-MTS (unlabeled) in the protein channel and 4 mM GTP in the GTP channel. Black curve indicates fluorescence intensity averaged over the entire frame (~32 μm x ~32 μm). **E.** Fluorescence intensity profiles on the protein side showing disassembly after restarting flow to deplete GTP in flow cells with FtsZ (6% Alexa488 labeled) and FtsZ-MTS (unlabeled) or ΔCTL (6% Alexa488 labeled) and ΔCTL-MTS (unlabeled), obtained from experiments using 100x objective magnification. Overlaid dotted lines indicate non-linear fit assuming one-phase exponential decay. **F.** Normalized fluorescence intensity profiles of plots shown in D. Reaction buffer contains 50 mM HEPES pH 8.0, 0.1 mM EDTA, 10 mM $MgCl_2$, 300 mM KCl, 1% glycerol, 0.1 mg/mL casein (blocking agent), 0.5 mg/mL ascorbate.

Plasmid insert sequences:

Nucleotide sequence for inserts corresponding to plasmids in Supplementary table 1. All the sequences were confirmed by sequencing.

pEG658 and pEG659 (*Ec* FtsZ-venus-MTS) insert:

nnnATGTTTGAACCAATGGAACTTACCAATGACGCGGTGATTAAAGTCATCGGCGTC
GGCGGCGGCGGCGGTAATGCTGTTGAACACATGGTGCGCGAGCGCATTGAAGGT
GTTGAATTCTTCGCGGTAAATACCGATGCACAAGCGCTGCGTAAAACAGCGGTTGG
ACAGACGATTCAAATCGGTAGCGGTATCACCAAAGGACTGGGCGCTGGCGCTAAT
CCAGAAGTTGGCCGCAATGCGGCTGATGAGGATCGCGATGCATTGCGTGCGGCGC
TGGAAGGTGCAGACATGGTCTTTATTGCTGCGGGTATGGGTGGTGGTACCGGTAC
AGGTGCAGCACCAGTCGTCGCTGAAGTGGCAAAAGATTTGGGTATCCTGACCGTT
GCTGTCGTCACTAAGCCTTTCAACTTTGAAGGCAAGAAGCGTATGGCATTCGCGGA
GCAGGGGATCACTGAACTGTCCAAGCATGTGGACTCTCTGATCACTATCCCGAACG
ACAAACTGCTGAAAGTTCTGGGCCGCGGTATCTCCCTGCTGGATGCGTTTGGCGC
AGCGAACGATGTACTGAAAGGCGCTGTGCAAGGTATCGCTGAACTGATTACTCGTC
CGGGTTTGATGAACGTGGACTTTGCAGACGTACGCACCGTAATGTCTGAGATGGG
CTACGCAATGATGGGTTCTGGCGTGGCGAGCGGTGAAGACCGTGCGGAAGAAGCT
GCTGAAATGGCTATCTCTTCTCCGCTGCTGGAAGATATCGACCTGTCTGGCGCGCG
CGGCGTGCTGGTTAACATCACGGCGGGCTTCGACCTGCGTCTGGATGAGTTCGAA
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CAGCCAGTGATGGATCGCTACCAGCAGCATGGGATGGCTCCGCTGACCCAGGAGC
AGAAGCCGGTTGCTAAAGTCGTGAATGACAATGCGCCGCAAACTGCGCCTCCACG
TCCTGCAGGCGGCCGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGT
GCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCC
GGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGCTGATCTGC
ACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTAC
GGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCA
AGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGA
CGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAA
CCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAC
AAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCACCGCCGACAAGCAGAA
GAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTG
CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTG
CTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCCAACG
AGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCT
CGGCATGGACGAGCTGTACAAGGGCGGCCGCATTGAAGAAGAGAAGAAAGGCTTC
CTCAAACGCTTGTTCGGAGGATAActcgag

Features :
Ec FtsZ (ΔCTC) : [4 : 1101]
Venus : [1126 : 1842]
MTS : [1852 : 1896]

(In pEG658, 'nnn' [1:3] is 'cat'; In pEG659, 'nnn' [1:3] is 'ggt')

pEG717 (*Cc* FtsZ-venus-MTS) insert:

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GGCCTCGAAGGTGTGGAGTTCGTGGTGGCCAACACTGACGCTCAACAGCTTCAGT
TCGCCAAGACGGACCGTCGCATCCAGCTGGGCGTGCAAATCACCCAGGGCCTGG
GCGCCGGCGCGCACCCCGAGGTGGGCATGAGCGCGGCCGAAGAGAGCTTCCCC
GAGATCGGCGAGCACCTCGACGGCGCCCACATGGTCTTCATCACCGCCGGTATGG
GCGGCGGCACCGGCACCGGCGCGGCCCCGATCATCGCCAAGTGCGCCCGTGAG
CGCGGCATCCTGACCGTCGGCGTCGTGACCAAGCCCTTCCACTTCGAAGGCCGTC
ACCGCATGCGCCTGGCCGACAGCGGCATCCAGGAGCTGCAGCGCTATGTCGACA
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CTTCGCCGAAGCCTTCGGCATGGCCGACCAGGTGCTGCACTCGGGCGTCCGCTC
GATCACCGACCTGATGGTCCTGCCGGGCCTGATCAACCTCGACTTCGCCGACGTC
CGCACGGTCATGACCGAGATGGGCAAGGCGATGATGGGCACCGGCGAGGGCACC
GGCGAAGACCGCGCCCTGATGGCCGCTCAGAACGCCATCGCTAACCCGCTGCTG
GACGAAGTCTCGCTGAAGGGCGCCAAGGCCGTTCTGGTCAACGTGACCGGCGGC
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CCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTG
ACCACCCTGGGCTACGGCCTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGC
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CAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCC
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AGAAGAAAGGCTTCCTCAAACGCTTGTTCGGAGGATAActcgag

Features :
Cc FtsZ (ΔCTC) : [4 : 1476]
Venus : [1501 : 2217]
MTS : [2227 : 2271]

pEG1295 (FtsZ-MTS) insert:

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GGCCTCGAAGGTGTGGAGTTCGTGGTGGCCAACACTGACGCTCAACAGCTTCAGT
TCGCCAAGACGGACCGTCGCATCCAGCTGGGCGTGCAAATCACCCAGGGCCTGG
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GAGATCGGCGAGCACCTCGACGGCGCCCACATGGTCTTCATCACCGCCGGTATGG
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CGTCATCCGCGTGTCGGTCGTCGCCACCGGCATGGACGGTACCGGCGCCTCGAT
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gctcactcgaattc

Features :
Cc FtsZ GTPase : [4 : 963]
Cc FtsZ CTL : [970 : 1485]
MTS : [1501 : 1545]

pEG1293 (ΔCTL-MTS) insert:

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GACGAAGTCTCGCTGAAGGGCGCCAAGGCCGTTCTGGTCAACGTGACCGGCGGC
ATGGACATGACCTTGCTGGAAGTCGACGAGGCCGCCAATGCGATCTCCGACCAGG
TCGATCCGGAAGCCAACATCATCTTCGGCGCGGCCTTCGATCCGTCGCTGGAAGG
CGTCATCCGCGTGTCGGTCGTCGCCACCGGCATGGACGGTACCGCTAGCGGCGG
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cttaatcgccttgcagcacatccccctttcgccagcggtatcagctcactcgaattc

Features :
Cc FtsZ GTPase : [4 : 963]
MTS : [985 : 1029]

pEG1297 (L14-MTS) insert:

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GGCCTCGAAGGTGTGGAGTTCGTGGTGGCCAACACTGACGCTCAACAGCTTCAGT
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CGTCATCCGCGTGTCGGTCGTCGCCACCGGCATGGACGGTACCCTGCAGCCGATC
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tatcagctcactcgaattc

Features :
Cc FtsZ GTPase : [4 : 963]
14 aa (*Cc*) CTL: [964 : 1005]
MTS : [1015 : 1059]

pEG1296 (*Hn*CTL-MTS) insert:

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CCTTCGCAGATCGTCACATCGCCCGAGGACCACCCCAAACCGGCCCCGTTTGCTA
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GCTGAcccgggggatccactagttctagagcggccattcactggccgtcgttttacaacgtcgtgactgggaaaaccct
ggcgttacccaacttaatcgccttgcagcacatccccctttcgccagcggtatcagctcactcgaattc

Features :
Cc FtsZ GTPase : [4 : 963]
Hn FtsZ CTL : [970 : 1464]
MTS : [1480 : 1524]