Electronic Supplementary Material (ESI) for Nanoscale.

Supplementary Files

Conformational equilibrium of MinE regulates allowable concentration ranges of a protein wave for cell division

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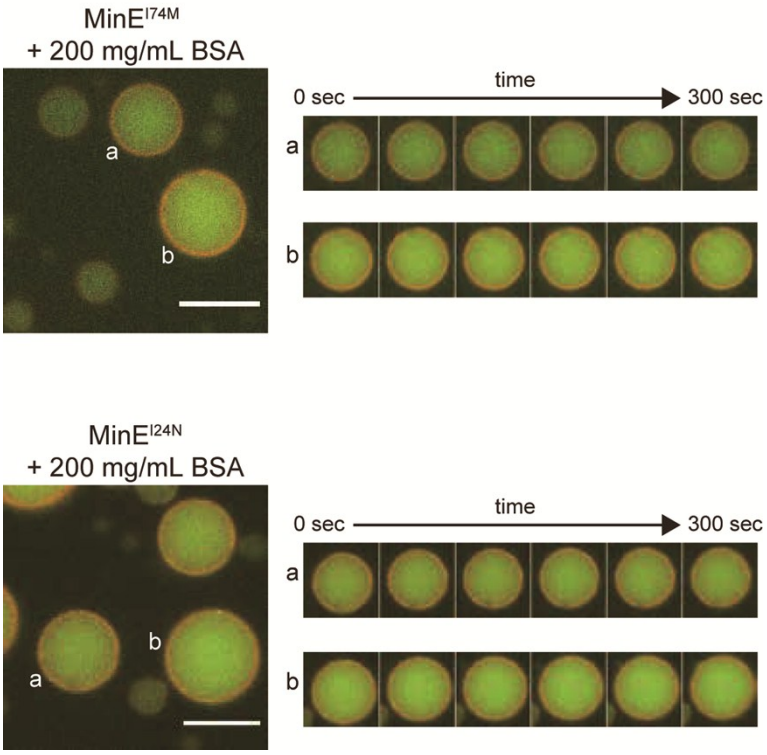
Supplementary Table S1. Periods of Min waves emerged by wildtype and ΔMTS MinE

MinE conc.	Wildtype	ΔMTS
0.3 μM	230 ± 36 (sec)	207 ± 14 (sec)
1 μM	116 ± 6 (sec)	80 ± 12 (sec)
10 μM	No wave	25 ± 4 (sec)

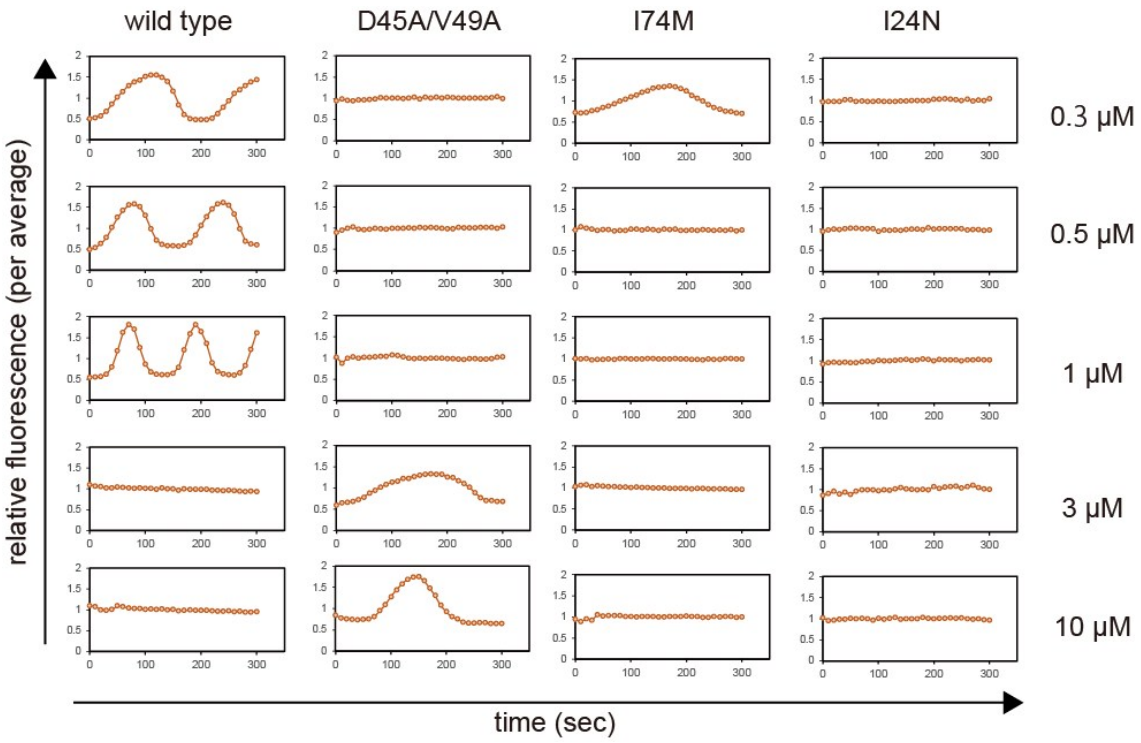
Name	Sequence	note
sfGFP_V206K_fw	CAATCTAAACTTTCGAAAGATCCCAAC	V206K mutation for sfGFP
sfGFP_V206K_rev	CGAAAGTTTAGATTGTGTCGACAGGTA	V206K mutation for sfGFP
MinE_I24N_fw	CTGCAGAATATTGTTGCTGAACGCCG	I24N mutation for MinE
MinE_I24N_rev	AACAATATTCTGCAGCCGTTCTTTTG	I24N mutation for MinE
MinE_Δ2-12_fw	ACATATGAACACAGCCAACATTGCA	MTS deletion for MinE
MinE_Δ2-12_rev	GCTGTGTTCATATGTATATCTCCTTC	MTS deletion for MinE
MinE_V49A_fw	CTTGAGGCGATTTGTAAATACGTACAA	V49A mutation for MinE
MinE_V49A_rev	ACAAATCGCCTCAAGAATATCTTTACG	V49A mutation for MinE
MinE_D45A/V49A_fw	CGTAAAGCGATTCTTGAGGCGATTTGT	D45A mutation for MinE V49A
MinE_D45A/V49A_rev	AAGAATCGCTTTACGCAACTGCGGCAG	D45A mutation for MinE V49A
MinE_I74M_fw	TTTCTATGCTTGAGCTGAACGTGACC	I74M mutation for MinE
MinE_I74M_rev	GCTCAAGCATAGAAATATCGCCATCT	I74M mutation for MinE

Supplementary Table S2. Primers used in this study



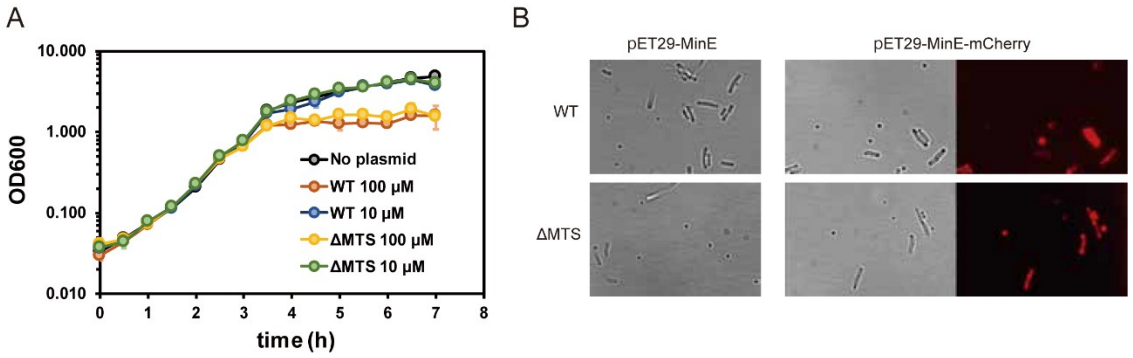
Supplementary Figure S1. Min wave emergence tests using artificial cells entrapping MinDE and higher concentration of BSA

The same experiments as Figure 2 except using 200 mg/mL BSA instead of 100 mg/mL BSA. Only the results of I74M and I24N which show lower c/m of MinE (Figure 2B) are shown. Scale bars indicate 20 µm.

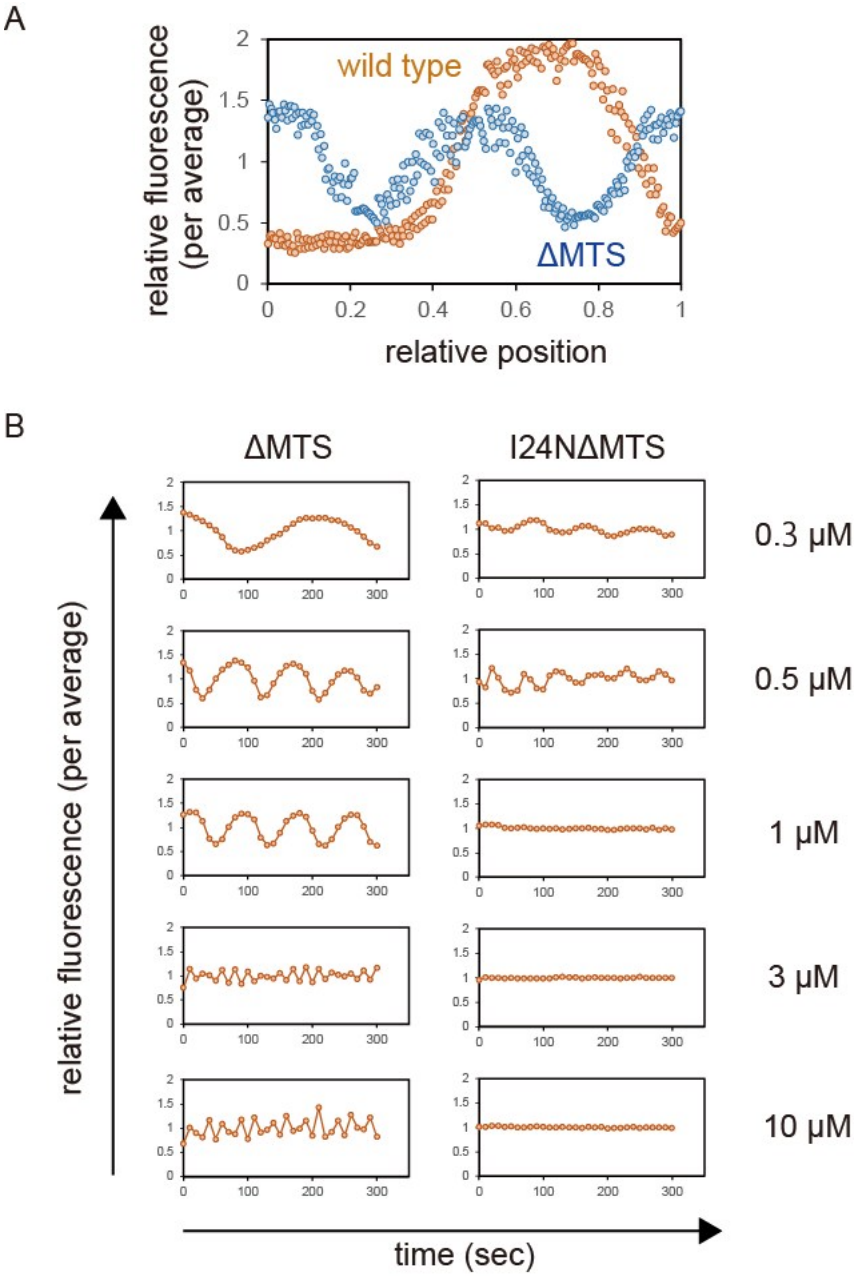


Supplementary Figure S2. Time plots of Min waves by various concentrations of MinE wildtype and mutants at fixed points

Representative time plots of MinD localization under each condition are shown. Time intervals are 10 sec (total 300 sec). Relative fluorescent intensities normalized by its average intensity during 300 sec are the vertical axis.



Supplementary Figure S3. Overexpression assays for the MTS deletion mutant of MinE
A: Growth curves of *E. coli* harboring the plasmid encoding WT MinE or MinEΔMTS are shown. Average and standard deviation of triplicates are shown. IPTG concentrations (10 µM and 100 µM) are also shown. B: A minicell phenotype observed by microscope. IPTG was supplied to *E. coli* harboring the plasmid encoding WT MinE or MinEΔMTS, and cell morphology was observed by miroscope. The red color at the right panel indicate mCherry fluorescence of MinE-mCherry and MinEΔMTS-mCherry. IPTG was supplied at 100 µM.



Supplementary Figure S4. Position and time plots of Min waves by ΔMTS mutants of MinE
A: Representative position plots of Min waves by wildtype MinE and its ΔMTS mutant. Relative fluorescent intensities normalized by the average intensity on the surface of each microdroplet are the vertical axis. Positions were normalized by 1 circle as 1 (namely, 0 and 1 is very near). Orange and blue points indicate the position intensities of the wildtype or the ΔMTS mutant, respectively.
B: Representative time plots under each condition are shown. Time intervals are 10 sec (total 300 sec). Relative fluorescent intensities normalized by its average intensity during 300 sec are the vertical axis.