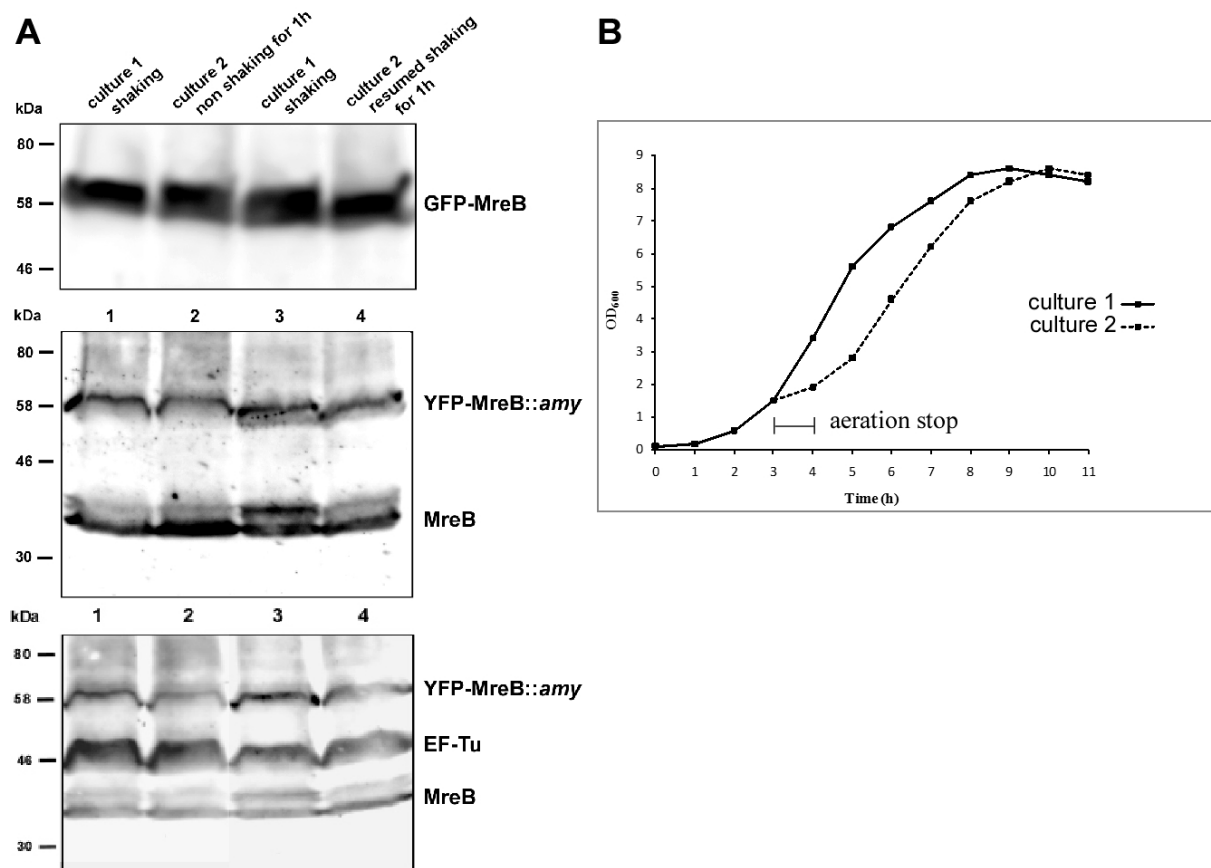


Supplemental Materials

Molecular Biology of the Cell

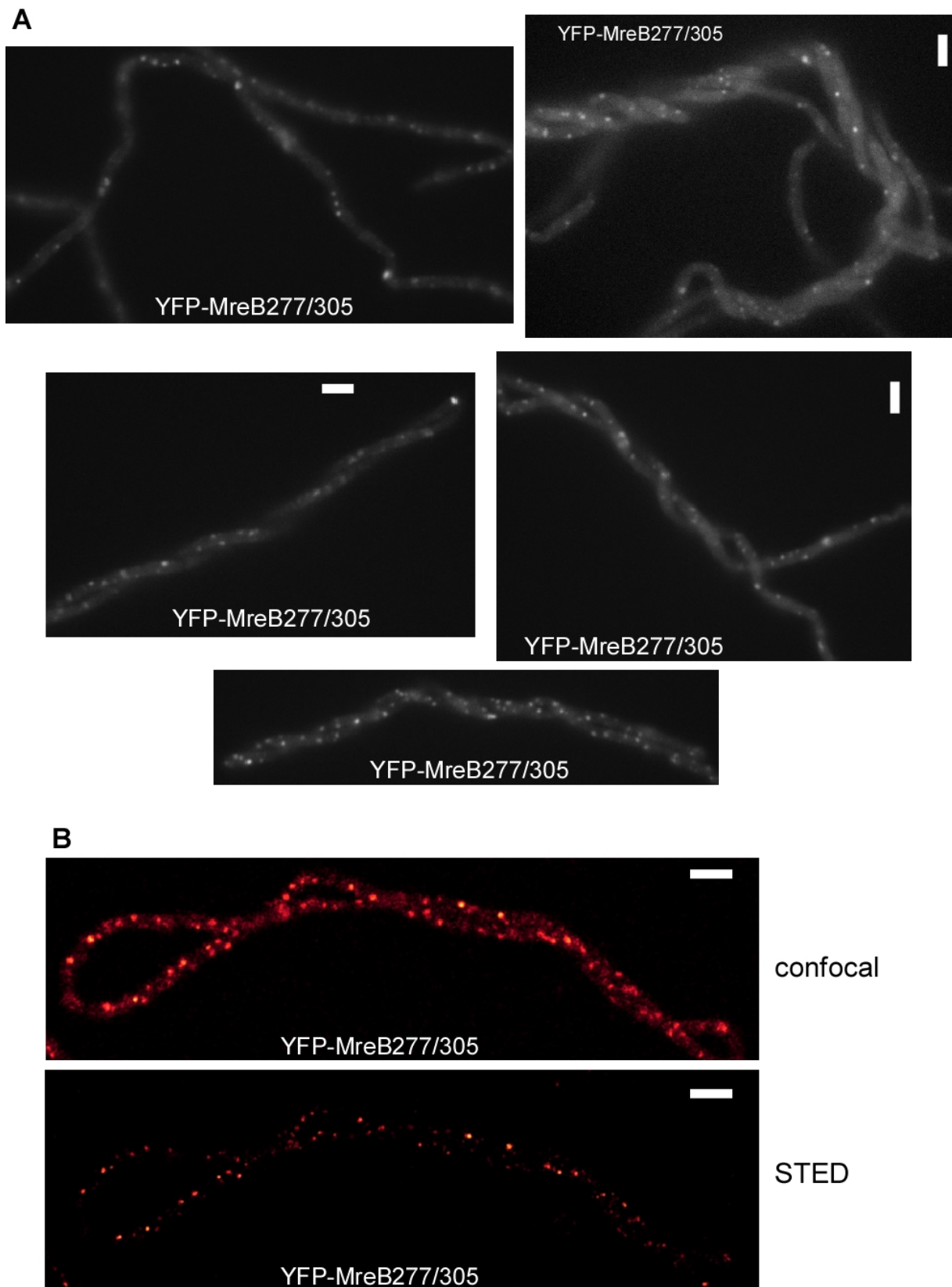
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Suppl. Fig. 1

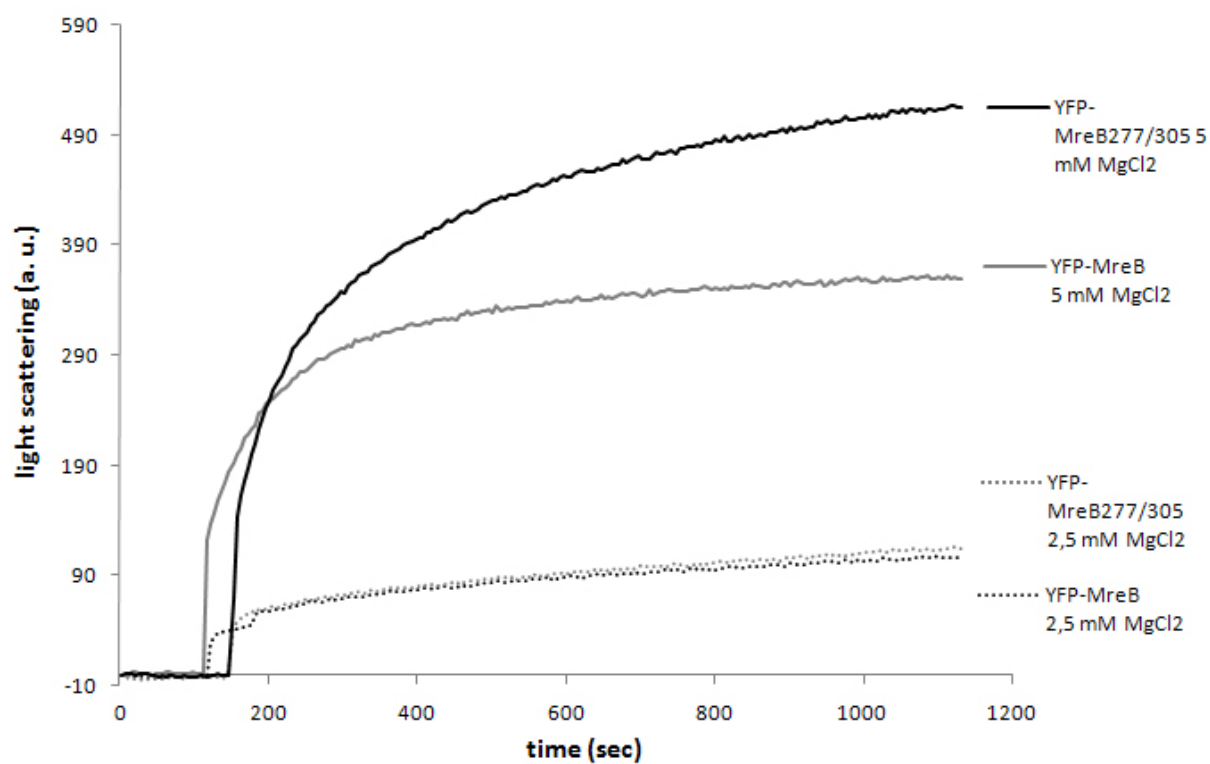


A) Western Blot analysis: *B. subtilis* cells expressing GFP-MreB from the original gene locus as sole copy of MreB (upper panel), or YFP-MreB from an ectopic site (middle and lower panels), using anti MreB serum and additionally anti EF-Tu serum (lower panel) as a loading control. Cells were grown at 30°C until early exponential phase and were split into two cultures. One fraction remained constantly shaking, while the other fraction was incubated without shaking. After one hour, when YFP-MreB filaments from the non-shaking culture were fragmented into short filaments or spotty structures, equal amounts of cells were harvested from both cultures (lane 1: culture 1 (shaking); lane 2: culture 1 (non shaking for 1h)). Subsequently, both cultures were incubated for one more hour under shaking condition and second fractions with equal amounts of cells were harvested (lane 3: culture 2 (shaking); lane 4: culture 1 (resumed shaking for 1h)). Proteins were separated by SDS PAGE and transferred to a nitrocellulose membrane via Western blotting. MreB was visualized by chemiluminescence using a primary rabbit-anti-MreB-IgG and a secondary goat-anti-rabbit-IgG, fused to horseradish peroxidase. The nature of the different fusions is indicated on the right. YFP-MreB expressed from the amylase locus showed lowered concentration under non-shaking conditions in some experiments, and no change in other independent experiments, therefore both cases are shown.

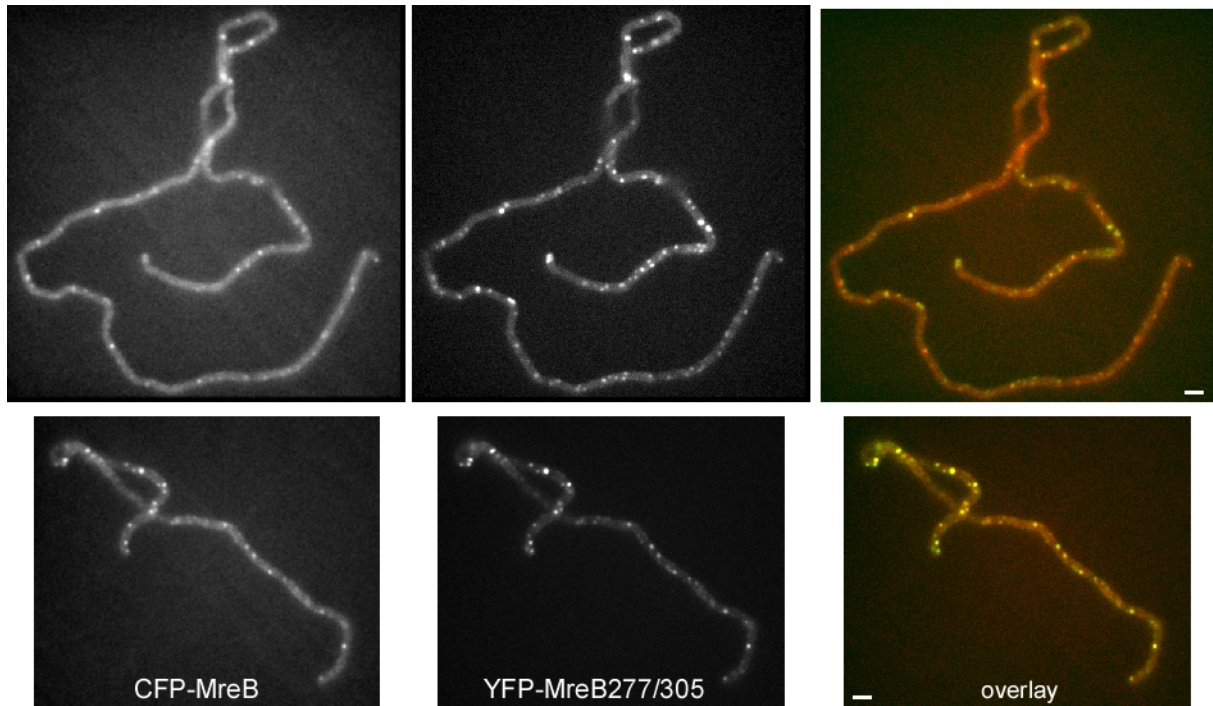
B) Growth curves of YFP-MreB expressing *B. subtilis* cells grown at 37°C under different aeration conditions. The initial culture was grown until early exponential phase (OD₆₀₀ of 1.5) and concomitantly split into two cultures. Culture 1 (solid line) remained shaking, whereas culture 2 (dashed line) was incubated without shaking for 1 hour and subsequently resumed growth under shaking condition.



Suppl. Fig. 2 A) Epifluorescence microscopy of *B. subtilis* cells expressing xylose-inducible YFP-MreB277/305 from an ectopic locus for 8 hours. 20 to 40% show irregular cell shape (upper left panel), the remaining cells double or triple coiled super structures. B) confocal (upper panel) and STED images (lower panel) of YFP-MreB277/305 expressing cells. White bars 2 μm .



Suppl. Fig. 3: Light scattering analysis of filament formation of YFP-MreB and of YFP-MreB277/305. Proteins were purified in the presence of ATP, and filament formation was induced by the addition of magnesium.



Suppl. Fig. 4: Epifluorescence microscopy of *B. subtilis* cells expressing CFP-MreB from the threonine locus (IPTG induction, red in the overlay) and YFP-MreB277/305 from the amylose locus (xylose induction, green in the overlay) for 6 hours. White bars 2 μ m.

Supplementary movies:

Note that the size of the movie player may have to be adjusted (reduced or enlarged) to properly see the filamentous structures (especially for the *E. coli* movies)

Movie 1: *B. subtilis* cells expressing YFP-MreB, Z-stack acquired by SIM (Zeiss), step size 150 nm, going from top to bottom, shown are 6 frames/s.

Movie 2: *B. subtilis* cells expressing YFP-MreB, time lapse N-SIM acquisition (exposure time 1.2 s, 3D SIM, 5 s intervals), 6 frames/s

Movie 3: same field as in movie 2 and same experimental setups, movie acquired 5 min later than movie 2.

Movie 4: *B. subtilis* cells expressing YFP-MreB, time lapse N-SIM acquisition (exposure time 1.2 s, 3D SIM, 5 s intervals), 6 frames/s

Movie 5: *B. subtilis* cells expressing YFP-MreB, 1 h after arrest of aeration, time lapse epifluorescence acquisition, 5 s intervals, 6 frames/s

Movie 6: *E. coli* cells expressing MreB-RFP^{sw}. Time lapse SIM (Zeiss) acquisition (exposure time 1 s, 2D SIM, 5 s intervals), 6 frames/s (note that the size of the movie must be adjusted to properly see the filamentous structures).

Movie 7: *E. coli* cells expressing MreB-RFP^{sw}. Time lapse SIM (Zeiss) acquisition (exposure time 1 s, 2D SIM, 5 s intervals), 6 frames/s.

Movie 8: *B. subtilis* cells expressing YFP-MreBD158A, time lapse SIM (Zeiss) acquisition (exposure time 0.5 s, 2D SIM, 5 s intervals), 6 frames/s

Movie 9: *B. subtilis* cells expressing YFP-MreBD158A, time lapse SIM (Zeiss) acquisition (exposure time 0.5 s, 2D SIM, 5 s intervals), 6 frames/s

Movie 10: *B. subtilis* cells expressing YFP-MreB277/305, Z-stack acquired by SIM (Zeiss), step size 150 nm, going from top to bottom, shown are 3 frames/s.

Movie 11: *B. subtilis* cells expressing YFP-MreB277/305 for 2 hours, time lapse acquisition by SIM (Zeiss), (exposure time 0.5 s, 2D SIM, 5 s intervals), 6 frames/s

Movie 12: *B. subtilis* cells expressing YFP-MreB277/305 for 4 hours, time lapse acquisition by epifluorescence, 5 s intervals, 6 frames/s

Suppl. table 1. Oligonucleotides

Name	Sequence	Restrictionsite
2390	ACTAGATCTGCATTTAAGAGGAGGAGAAG	<i>Bgl</i> II
2391	ACTGGTACCCATCTATTTATATCCTCCTTG	<i>Kpn</i> I
2515	GTCATCAGCGAAGAAACAGACATGCCGGTCCTTATCGC	
2516	GCGATAAGGACCGGCATGTCTGTTTCTTCGCTGATGAC	
2563	CCGCCTGAGCTTGCACAAGATATCATGGACAGAGG	
2564	CCTCTGTCCATGATATCTTGTGCAAGCTCAGGCGG	
2616	ACTGCATGCTTATCTAGTTTTCCCTTTGAAA	<i>Sph</i> I
2861	ACTGCTAGCAAGGAGATTCTAGGATGTTGGATTCAATAGAAAAGGTAAG	<i>Nhe</i> I
2962	ACTGCTAGCAAGGAGATTCTAGGATGTTGGAATTGGTGCTAG	<i>Nhe</i> I

Suppl. table 2. Plasmids

Name	Genotype*	Reference
pDP150	(<i>P_{hyperspank}</i> , <i>lacI</i> :: <i>thrC</i> (<i>bla</i> ^r , <i>mls</i> ^r , <i>spc</i> ^r)	(Kearns and Losick, 2005)
pJS13	<i>P_{xyI}</i> - <i>gfp-mbl</i> (<i>bla</i> ^r , <i>cm</i> ^r)	(Defeu Soufo and Graumann, 2004)
pJS152	<i>P_{mbl}</i> - <i>gfp-mbl</i> (<i>bla</i> ^r , <i>cm</i> ^r)	this work
pJS24	<i>P_{xyI}</i> - <i>yfp-mreB::amyE</i> (<i>bla</i> ^r , <i>spc</i> ^r)	(Defeu Soufo and Graumann, 2006)
pJS64	<i>P_{hyperspank}</i> - <i>strep-yfp-mreB</i> (<i>sm</i> ^r)	(Defeu Soufo <i>et al.</i> , 2010)
pCR4	<i>P_{xyI}</i> - <i>yfp-mreB277/305::amyE</i> (<i>bla</i> ^r , <i>spc</i> ^r)	this work
pCR5	<i>P_{hyperspank}</i> - <i>strep-yfp-mreB277/305</i> (<i>sm</i> ^r)	this work
pCR6	(<i>P_{hyperspank}</i> - <i>mreB277/305</i> , <i>lacI</i> :: <i>thrC</i> (<i>bla</i> ^r , <i>mls</i> ^r , <i>spc</i> ^r)	this work
pCR7	(<i>P_{hyperspank}</i> - <i>mCer-mreB</i> , <i>lacI</i> :: <i>thrC</i> (<i>bla</i> ^r , <i>mls</i> ^r , <i>spc</i> ^r)	this work

* Resistance (^r) gene abbreviations: *bla*, ampicillin, *cm*, chloramphenicol, *spc*, spectinomycin, *mls*, macrolide-lincosamide-streptogramin, *sm*, streptomycin.

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