Supplementary Material

Supplementary movie S1: Exponentially growing *B. subtilis* cells expressing SftA-YFP from the original gene locus, 30 ms stream acquisition of YFP fluorescence, shown are 33 frames/s

Supplementary movie S2: Exponentially growing *B. subtilis* cells expressing SftA-YFP from the original gene locus, mobile single signals are shown within the cell borders (determined through an overlay of fluorescence with bright field acquisition), 30 ms stream acquisition of YFP fluorescence, shown are 10 frames/s

Supplementary movie S3: Exponentially growing *B. subtilis* cells expressing SftA-YFP from the original gene locus, static single signal is shown within the cell borders, 30 ms stream acquisition of YFP fluorescence, shown are 10 frames/s

Supplementary movie S4: Exponentially growing *B. subtilis* cells expressing SpoIIIE-YFP from the original gene locus, 30 ms stream acquisition of YFP fluorescence, shown are 33 frames/s

Supplementary movie S5: Exponentially growing *B. subtilis* cells expressing SpoIIIE-YFP from the original gene locus, mobile single signals are shown at the cell borders, 30 ms stream acquisition of YFP fluorescence, shown are 10 frames/s

Supplementary movie S6: Exponentially growing *B. subtilis* cells expressing PfkA-YFP from an ectopic gene locus, 30 ms stream acquisition of YFP fluorescence, shown are 33 frames/s

Supplementary movie S7: Exponentially growing *B. subtilis* cells expressing PfkA-YFP from the original gene locus, mobile single signals are shown within the cell borders, 30 ms stream acquisition of YFP fluorescence, shown are 10 frames/s

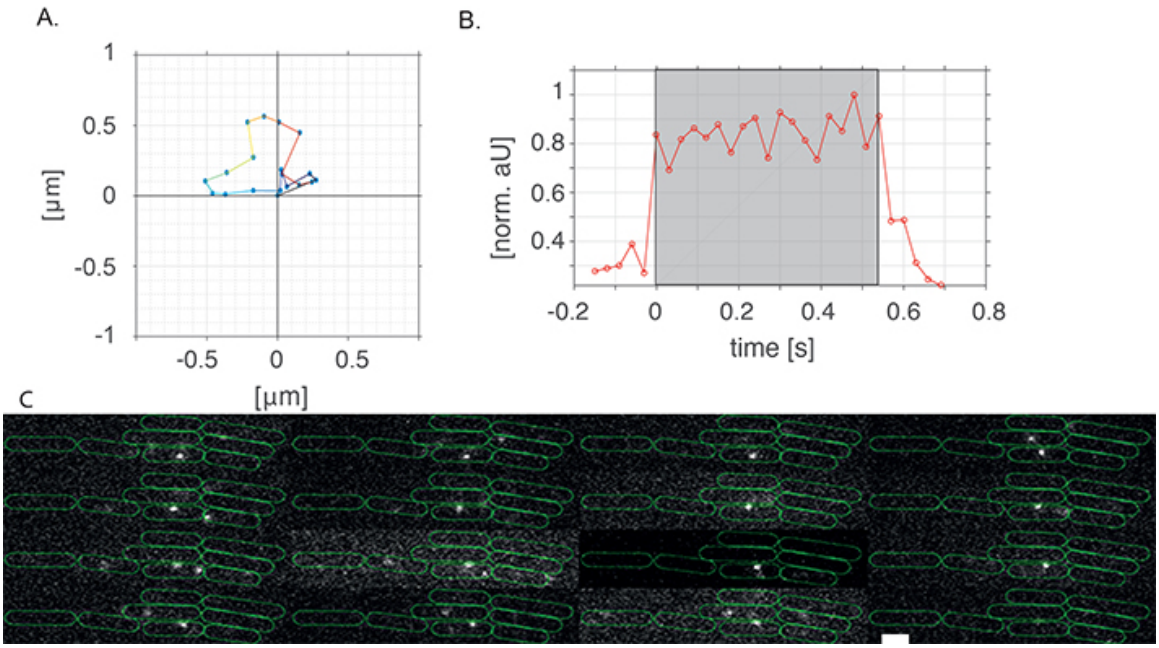

Fig. S1 Movement of a single SftA-YFP molecule within a *B. subtilis* cell. A) Trajectory of a single molecule, shown in panel C. The timing of movement is from blue to red. B) Fluorescence intensity of the single molecules. C) 15 ms stream showing the movement of a dynamic SftA-YFP molecule, left to right, top to bottom. White bar 2 µm.

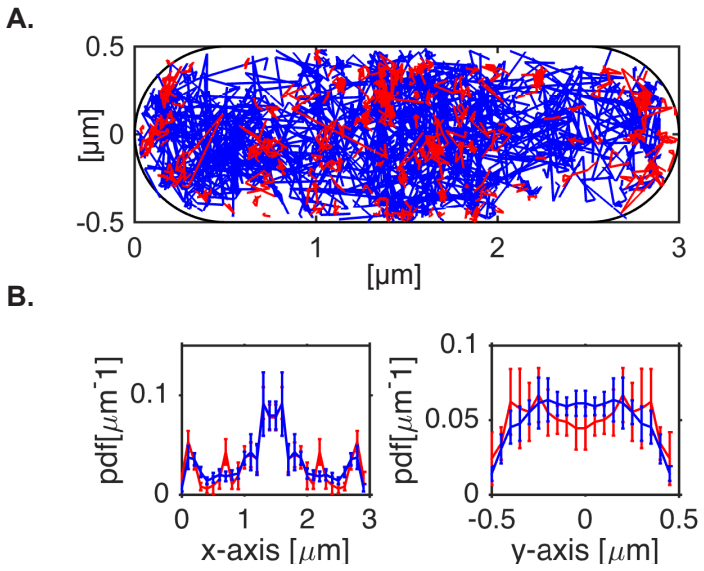

Fig. S2 A) Determination of the subcellular localization of slow and of fast moving molecules of FtsA-YFP, tracked at 30 ms. Tracks were projected into a standardized cell of 3 x 1 µm size, and were sorted into slow moving (not leaving an area of 3 x 3 pixels) molecules indicated by red line, and fast molecules, indicated by blue lines. B) Superimposition of slow (red line) and fast moving (blue line) molecules sorted in x- and y-orientation (long and short axis of the cell). Shown are the mean and the standard deviation of a bootstrap analysis sampling 50 times tracks with replacement.

Figure S2: Superimposition of slow moving (red bars) and fast moving (blue bars) molecules sorted in x-orientation (along the length of cells), data are the same as in Fig. 6.

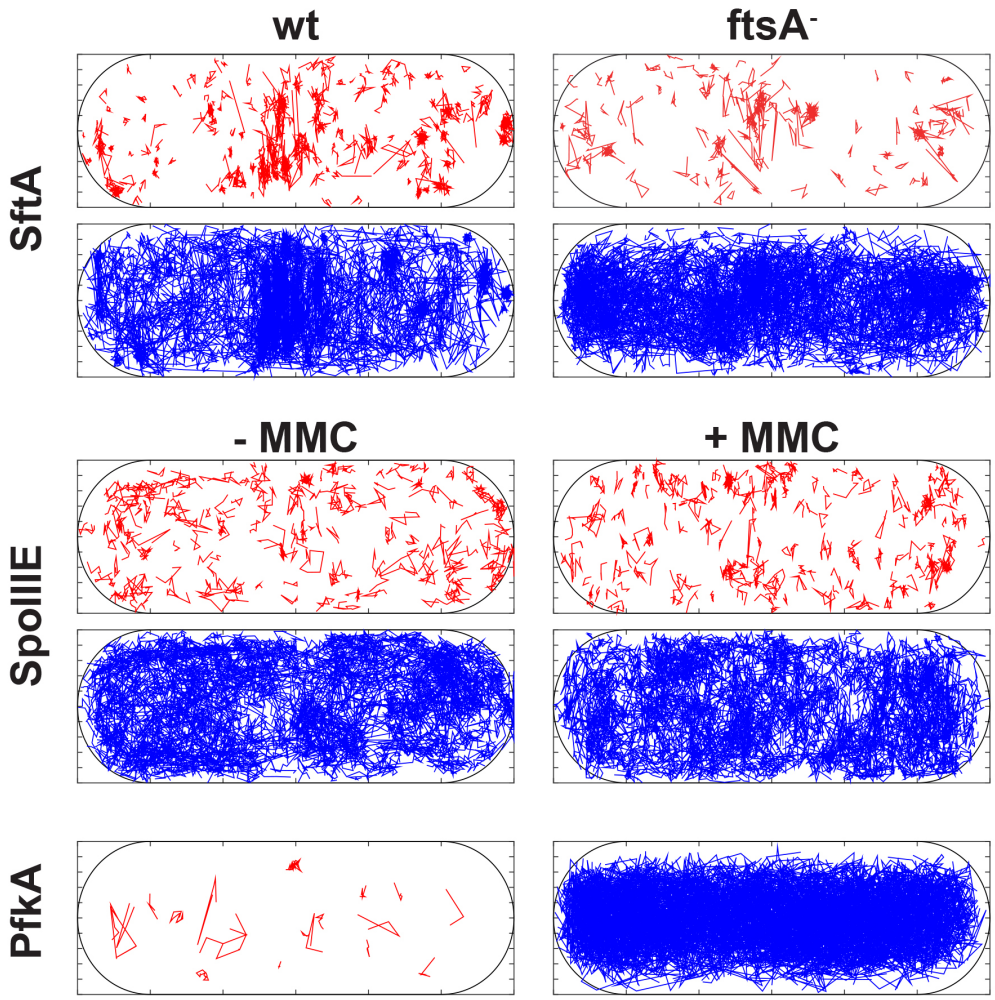

Figure S3 Determination of the subcellular localization of slow and of fast moving molecules. 1000 tracks of SftA-YFP, SpoIIIE-YFP or PfkA-YFP were projected into a standardized cells of 3 x 1 µm size, and were sorted into slow moving (not leaving an area of 3 x 3 pixels) molecules, indicated by the red tracks, and fast molecules, indicated by blue tracks. FtsA$^-$ indicates depletion of FtsA for 1 hour, + MMC the addition of MMC for 1 hour.

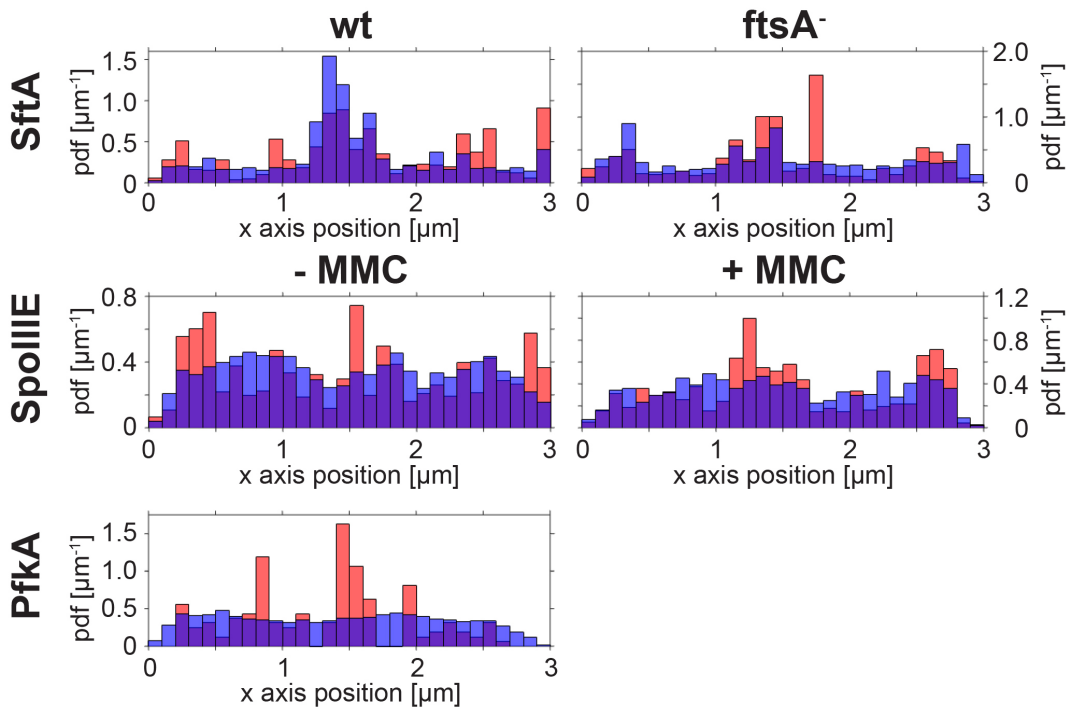

Figure S4 Superimposition of slow moving (red bars) and fast moving (blue bars) molecules sorted in x-orientation (along the length of cells), data are the same as in Fig. S2.

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Table S1

Oligonucleotide	Sequence	Construct
2622	5’-CATGGGCCCAATCATCATGAAAA-3’	1164-sftAN-tap
2624	5’-CATATCGATCGGAAATACATAGCTGCC-3’	
4406	5’-CATGAATTCATGAACAACAATGAACT TTAC-3’	pPR-IBA 101-FtsA$_{1-425}$
4407	5’-CATGGATCCATGTTCGGCTTGTGTTTTTT-3’	
5509	5’-CATGGTACCATGTTGGAGTTCGAAAC AAAC-3’	pFD1-ftsZ
5510	5’-CATACTAGTGCCGCGTTTATTACGGTT-3'	
5517	5’- CATCTCGAGGCCGCGTTTATTACGGTT-3’	pFD1-ftsZ-yfp
5456	5’-CATGGTACCATGAACAACAATGAACT	pFD1-ftsA
5457	TTAC-3’ 5’-CATACTAGTTTCCCAAAACATGCTTAA TAG-3’	
4281	5’-CATGGGCCCATGAACAACAATGAACT TTAC-3’	pFD1-ftsA-yfp
4295	5’-CATCTCGAGTTCCCAAAACATGCTTA ATAG-3’	
2342	5’-CATGGGCCCGACGAACCGAAATCCGCG-3’	pFD1-sftA-yfp
4088	5’-CATGATATCTTCGTTTATTAAATCACT TGC-3’	
2523	5’-CATGGGCCCCGGAAATACATAGCTGCC-3’	pFD1-sftAN-yfp
2624	5’-CATATCGATCGGAAATACATAGCTGCC-3’	

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