

Supplementary Material

Movie S1: Single molecule tracking of SecA-mNeonGreen expressed from the original gene locus in *B. subtilis* cells, grown to transition phase. Frames are shown in 20 fps, which is a 2.5 fold slow-down of real time motion.

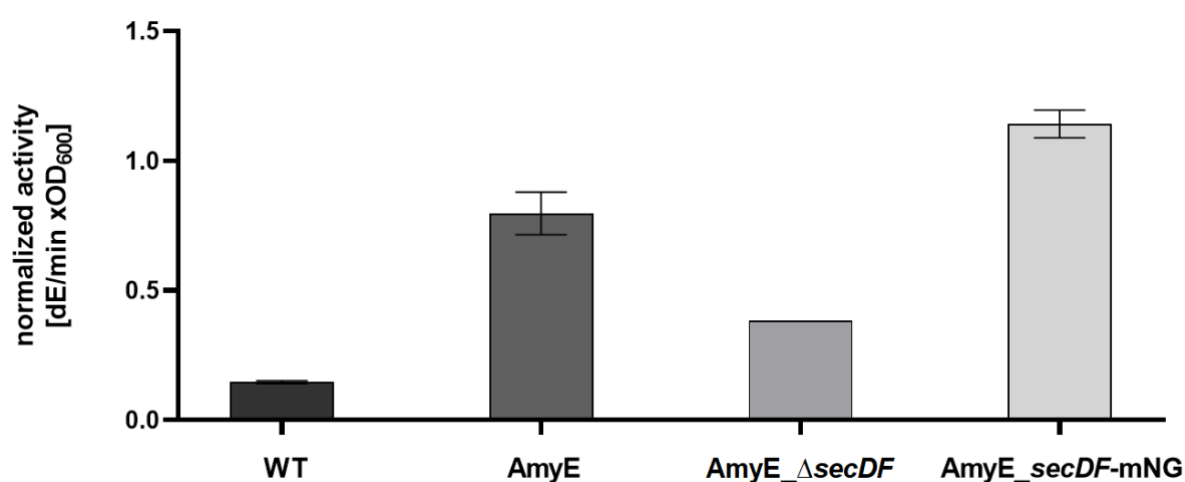


Fig. S1 Amylase activity in culture supernatant of *B. subtilis*. AmyE: plasmid based expression of *amyE*, WT: native genomic expression of *amyE*, Δ*secDF*: deletion strain, *secDF*-mNG: native genomic expression of the SecDF-mNeonGreen fusion protein. Activity is given as change in absorbance per min and cell density.

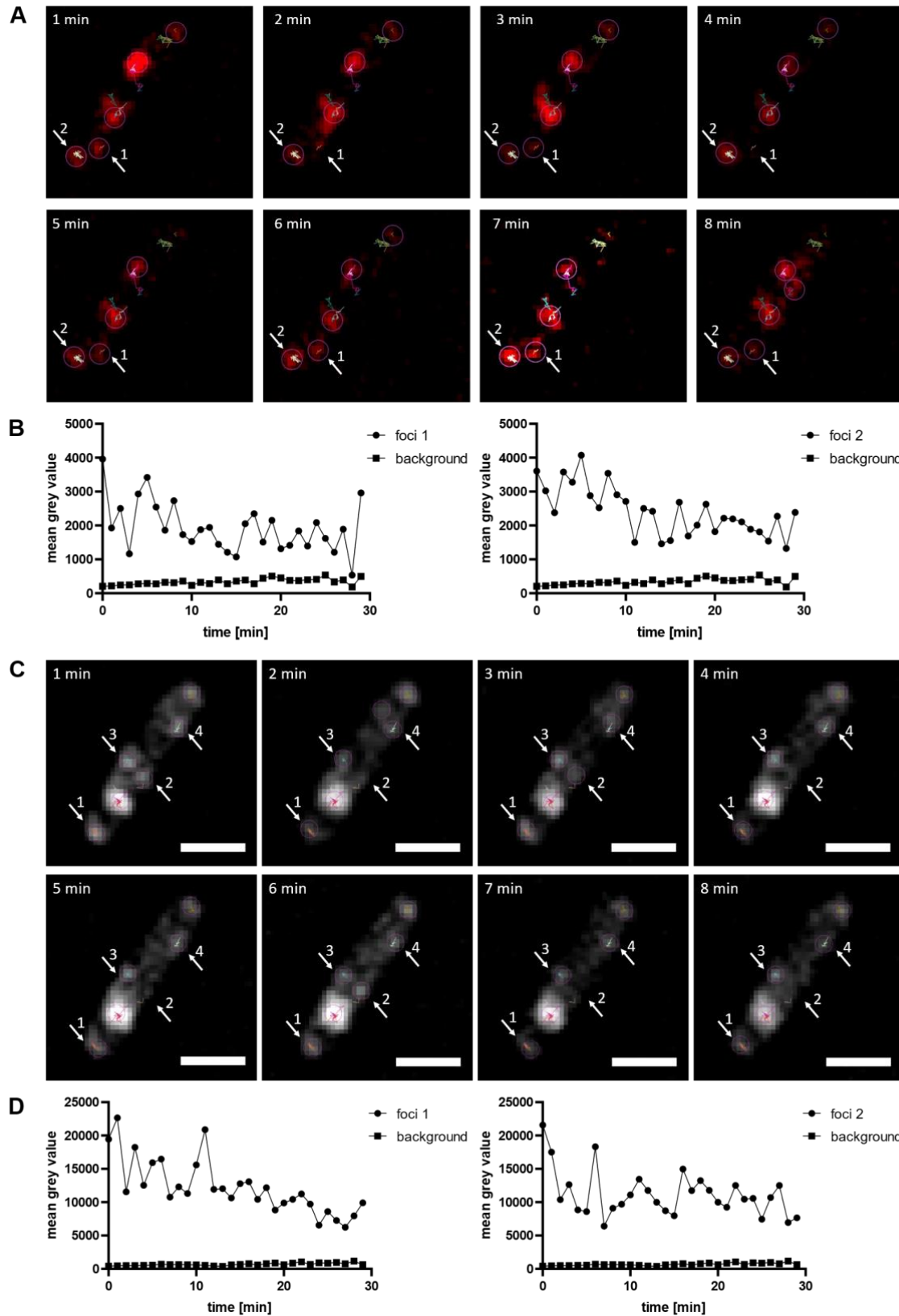


Fig. S2 Analysis of fluctuating AmyE-mCherry foci in two *B. licheniformis* cells. (A, C) SIM time lapse images showing cells with AmyE-mCherry foci fluctuating in fluorescence intensity over time. Images in A are shown as RGBs, while C images are shown as greyscale. (B, D) Fluorescence intensity analysis of foci compared to background fluorescence and calculation of the relative change in fluorescence intensity compared to the mean.

Table S1: Strains used in this study

<i>Strain</i>	<i>Relevant features</i>	<i>Reference of source</i>
<i>B. subtilis</i> PY79	Wild type	Richard Losick Harvard University
<i>B. subtilis</i> MS1	pM11K_amyEBs	This study
<i>B. subtilis</i> MS2	pM11K_amyE-mCherry	This study
<i>B. subtilis</i> MS3	pM11K_Δsp-amyE-mCherry	This study
<i>B. subtilis</i> MS7	ΔsecDF	This study
<i>B. subtilis</i> MS8	secDF-mNeonGreen	This study
<i>B. subtilis</i> MS9	secDF-mNeonGreen pM11K_amyEBs	This study
<i>B. subtilis</i> MS10	secDF-mNeonGreen pM11K_amyE-mCherry	This study
<i>B. subtilis</i> MS11	secA-mNeonGreen	This study
<i>B. subtilis</i> MS12	secA-mNeonGreen pM11K_amyE-mCherry	This study
<i>B. licheniformis</i> MC26	comP::IS3Bli1, ΔhsdR1, ΔhsdR2, Δupp, degU32, ΔaprE, Δpga, Δeps, ΔyqfD	B.R.A.I.N. Biotech AG (Zwingenberg, Germany)
<i>B. licheniformis</i> MC28	comP::IS3Bli1, ΔhsdR1, ΔhsdR2, Δupp, degU32, ΔaprE, Δpga, Δeps, ΔyqfD, ΔamyS	B.R.A.I.N. Biotech AG (Zwingenberg, Germany)
<i>B. licheniformis</i> MS4	MC28, pM11K_amyEBs	This study
<i>B. licheniformis</i> MS5	MC28, pM11K_amyE-mCherry	This study
<i>B. licheniformis</i> MS6	MC28, pM11K_Δsp-amyE-mCherry	This study

Table S2 Primers used in this study

<i>Primer</i>	Sequence 5'→ 3'
<i>SecA-UP</i>	AGGATGGGTACCGGGCCCATATCTTCGGCAAAGAACCGGA
<i>SecA-Down</i>	AATTGCTGCGGCCGTACTGAAGGTATCGATAAGCTTGATA
<i>SecDF-for</i>	CCTAGGATGGGTACCGAATTCCTGTACGCAGTTGCTATAGC
<i>SecDF-rev</i>	CCAGGCCAGATAGGCCGGGCCCTTGCGCCGAATCTTTTTTCAG
<i>noSP-blunt</i> <i>dw</i>	TAATGTATTCCTCCCTAATGTGAG
<i>noSP-blunt</i> <i>up</i>	CATATGGAAACGGCGAACA
<i>mC-up01</i>	TAAGCGGTTCTCTTCCCCATAAGCTTGGCAGCGGCAGCGGCAGCATG GTGAGCAAGGGCGAGGA
<i>mC-dw01</i>	GGTGGTGGTGGTGGTGCTCGAGCTACTTGTACAGCTCGTCC