SUPPLEMENTARY INFORMATION

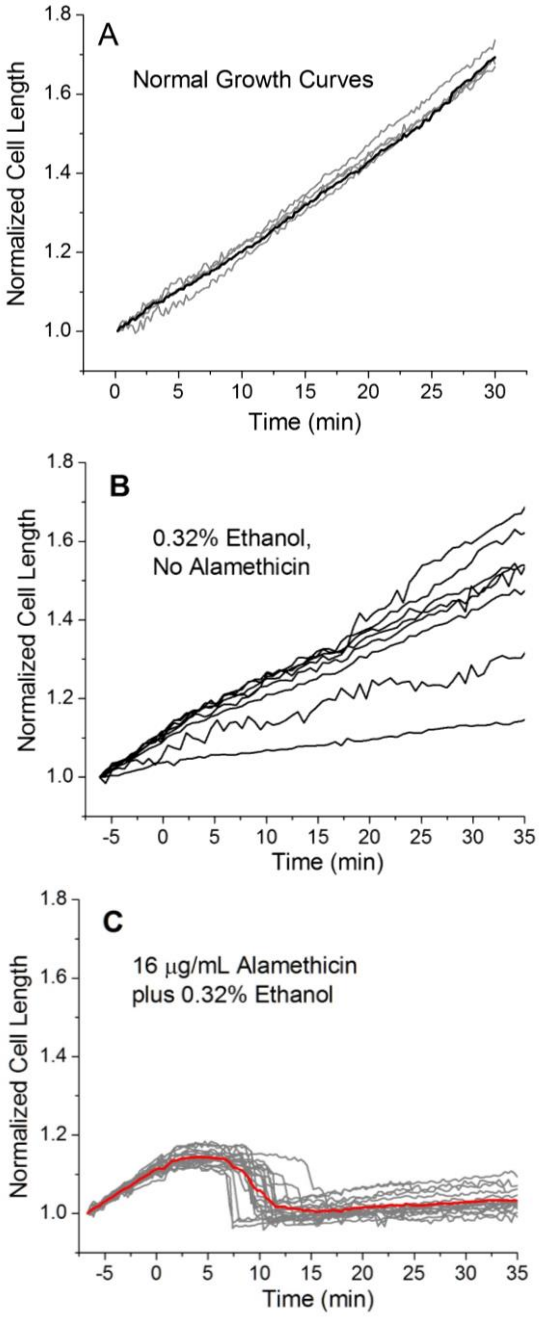

Figure S1. Single-cell length vs time plots at 37°C for cells in (A) *s*-EZRDM, (B) *s*-EZRDM with 0.32% ethanol by volume added at $t = 0$, and (C) *s*-EZRDM with 16 μg/mL alamethcin and 0.32% ethanol added at $t = 0$. Red curve in (C) is mean of all traces.

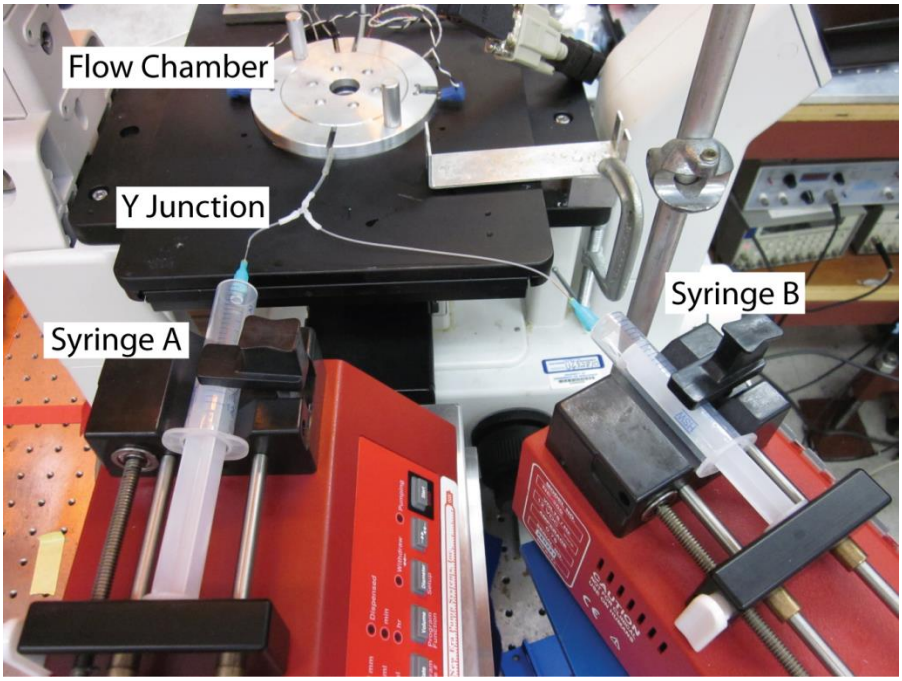

Figure S2. Photograph of the constant flow setup. The setup has two syringes connected by a y-junction that leads to the flow chamber. Syringe A typically contained the alamethicin/Sytox Orange/*s*-EZRDM solution, and Syringe B contained only *s*-EZRDM. To ensure that alamethicin was not being flowed into the chamber due to diffusion at the y-junction, the tubing before the y-junction was filled with *s*-EZRDM only.

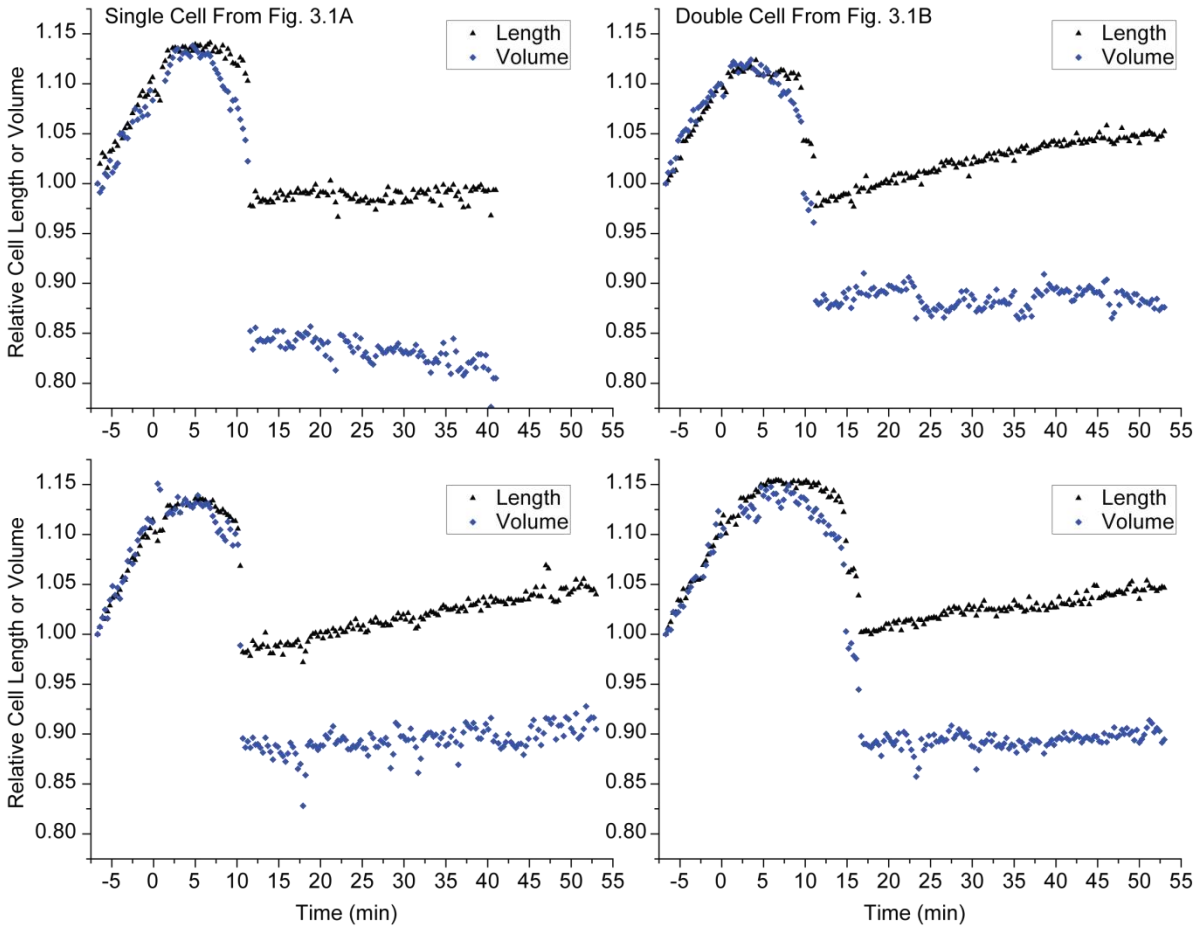

Figure S3. Plots of relative cell length (black triangles) and relative cell volume (blue diamonds) during exposure to 16 µg/mL alamethicin. Time zero is the time of injection of the alamethicin solution. The lengths and volumes were normalized to one to show the curves on the same y-axis, and for ease of comparison. The plots at top left and top right are for the cells in Fig. 1 and Fig. 3, respectively. At the bottom are shown two additional double cells. Each cell in the figure is from a different experimental run.

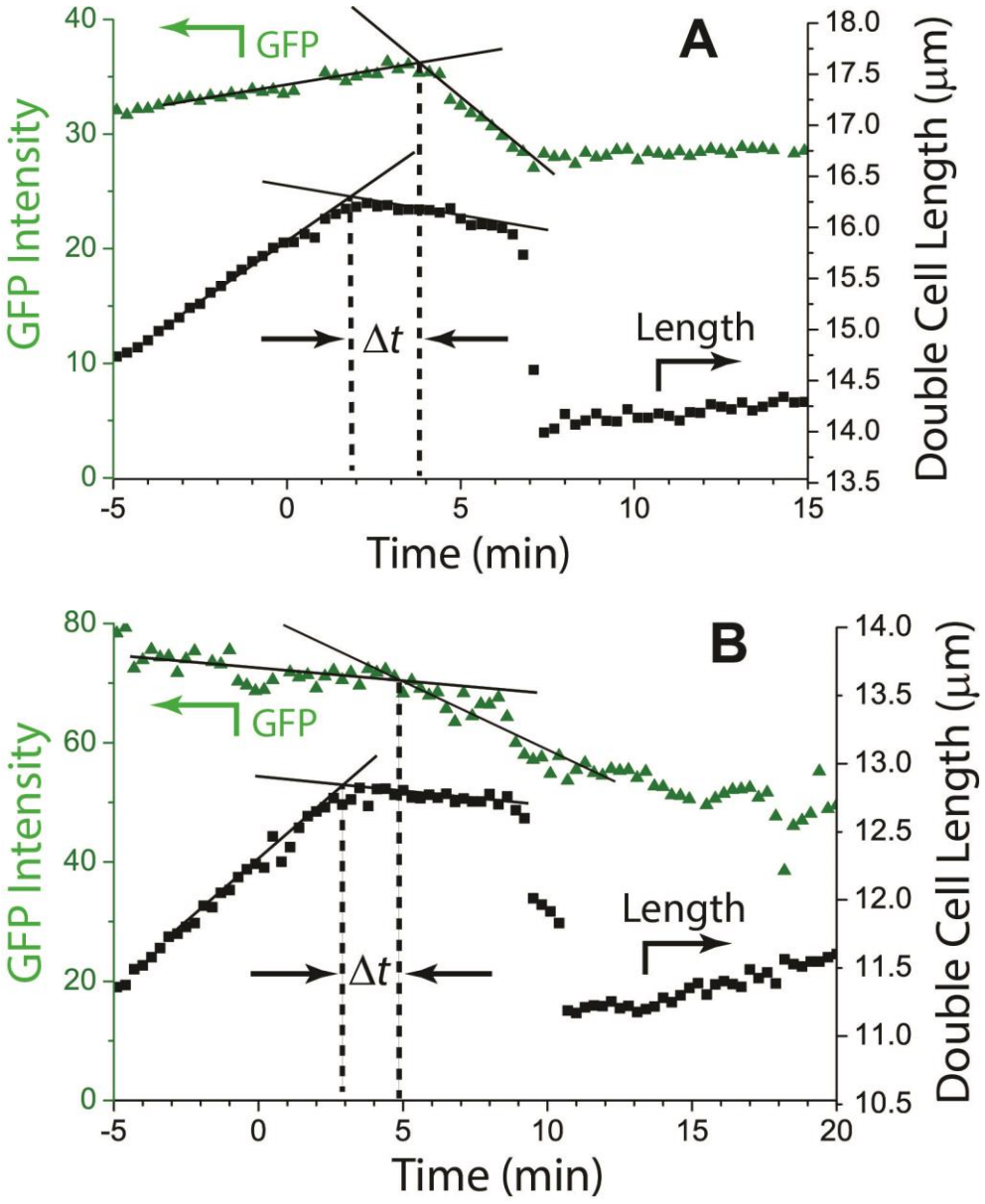

Figure S4. Two additional examples of measurements of the halting of cell growth (based on length vs time plots) and the onset of decreasing GFP fluorescence intensity after initiation of flow of 16 μg/mL alamethicin at $t = 0$. In panel (A), the GFP baseline is increasing prior to $t = 0$, but there is an abrupt change in slope at $t = 3.8$ min. In panel (B), the time of changing slope of GFP intensity is less certain. The estimated lag time Δt is shown in both cases.

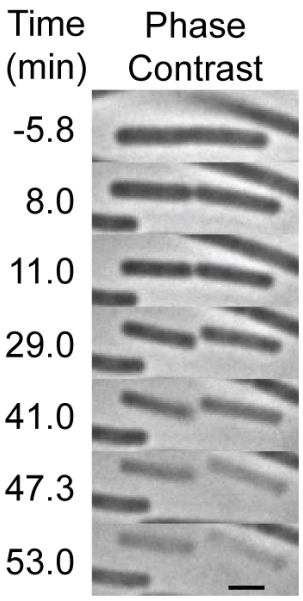

Figure S5. Example of cell losing phase contrast ~40 min after injection of alamethicin at 16 μg/mL. Time zero is the time of injection of alamethicin. Cell shrinkage occurs between 8 and 11 min. Loss of phase contrast occurs from 41 to 53 min, which is the end of data acquisition. The nucleiod was stained with Sytox Orange after shrinkage, and the cell lost cytoplasmic GFP before 41 min.

Supplementary Movie Information

Movie 1. Single-cell *B. subtilis* response to alamethicin at 16 μg/mL (1X MIC) and 2 nM Sytox Orange, injected at $t = 0$. Three imaging channels are cycled with 6 s between camera frames (18 s complete cycle time): GFP fluorescence (top images), Sytox Orange fluorescence (center), and phase contrast (bottom). This is the companion movie to Fig. 1 in the main text.

Movie 2. Double-cell *B. subtilis* response to alamethicin at 16 μg/mL (1X MIC) and 2 nM Sytox Orange, injected at $t = 0$. Three imaging channels are cycled with 6 s between camera frames (18 s complete cycle time): GFP fluorescence (top images), Sytox Orange fluorescence (center), and phase contrast (bottom). This is the companion movie to Fig. 3 in the main text.

Movie 3. Fast, single-channel imaging of Sytox Orange staining of *B. subtilis* nucleoids after attack of 2 μM of LL-37 (2X MIC), injected at $t = 0$. Interval between camera frames is 0.5 s. Initial permeabilization is localized. This is the companion movie to the top of Fig. 5 in the main text.

Movie 4. Fast, single-channel imaging of Sytox Orange staining of *B. subtilis* nucleoids after attack of 16 μg/mL alamethicin (1X MIC), injected at $t = 0$. Interval between camera frames is 0.5 s. Initial permeabilization is apparently global. This is the companion movie to the bottom of Fig. 5 in the main text.