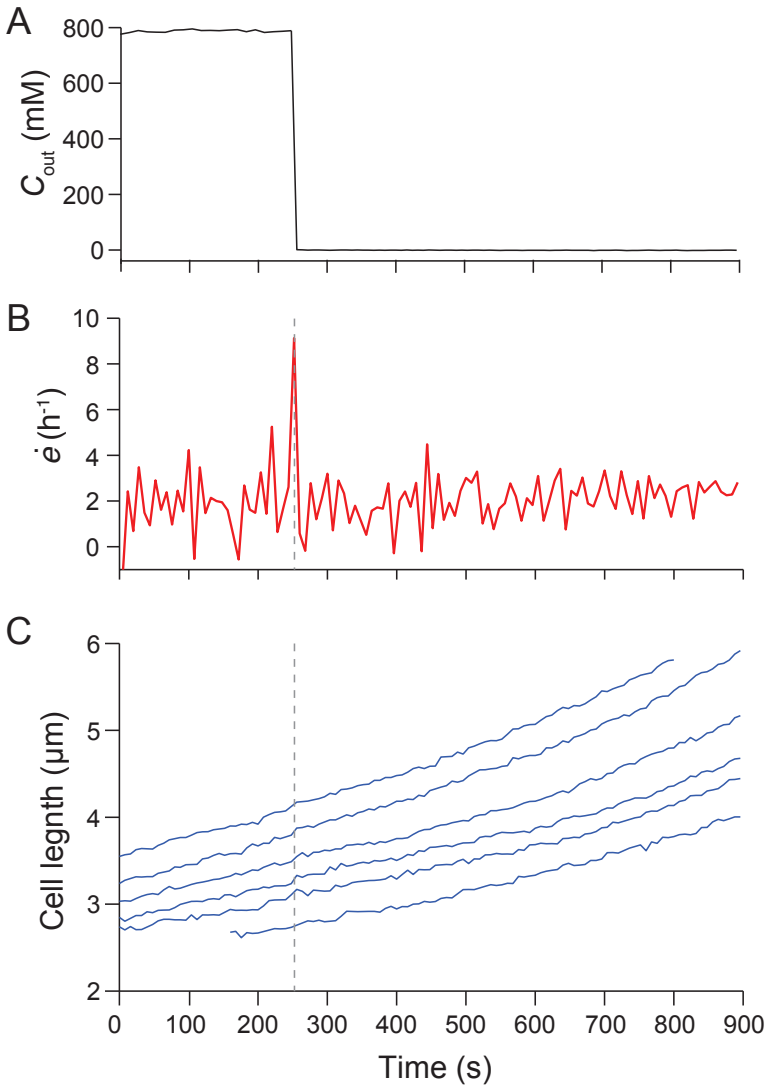
Supplemental Figures



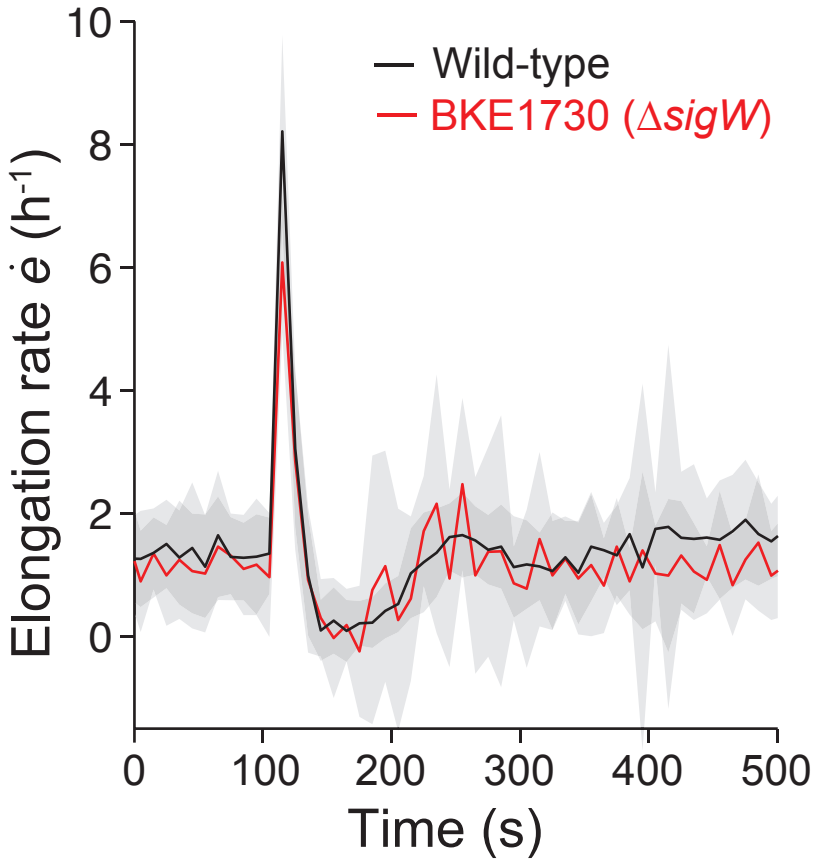
Supplemental Figure 1: Hypoosmotic shock does not cause growth inhibition in *E. coli*.

Related to Figure 1.

A) Concentration of sorbitol in the growth medium during an 800-mM hypoosmotic shock. The medium was exchanged within 3 s during this experiment.

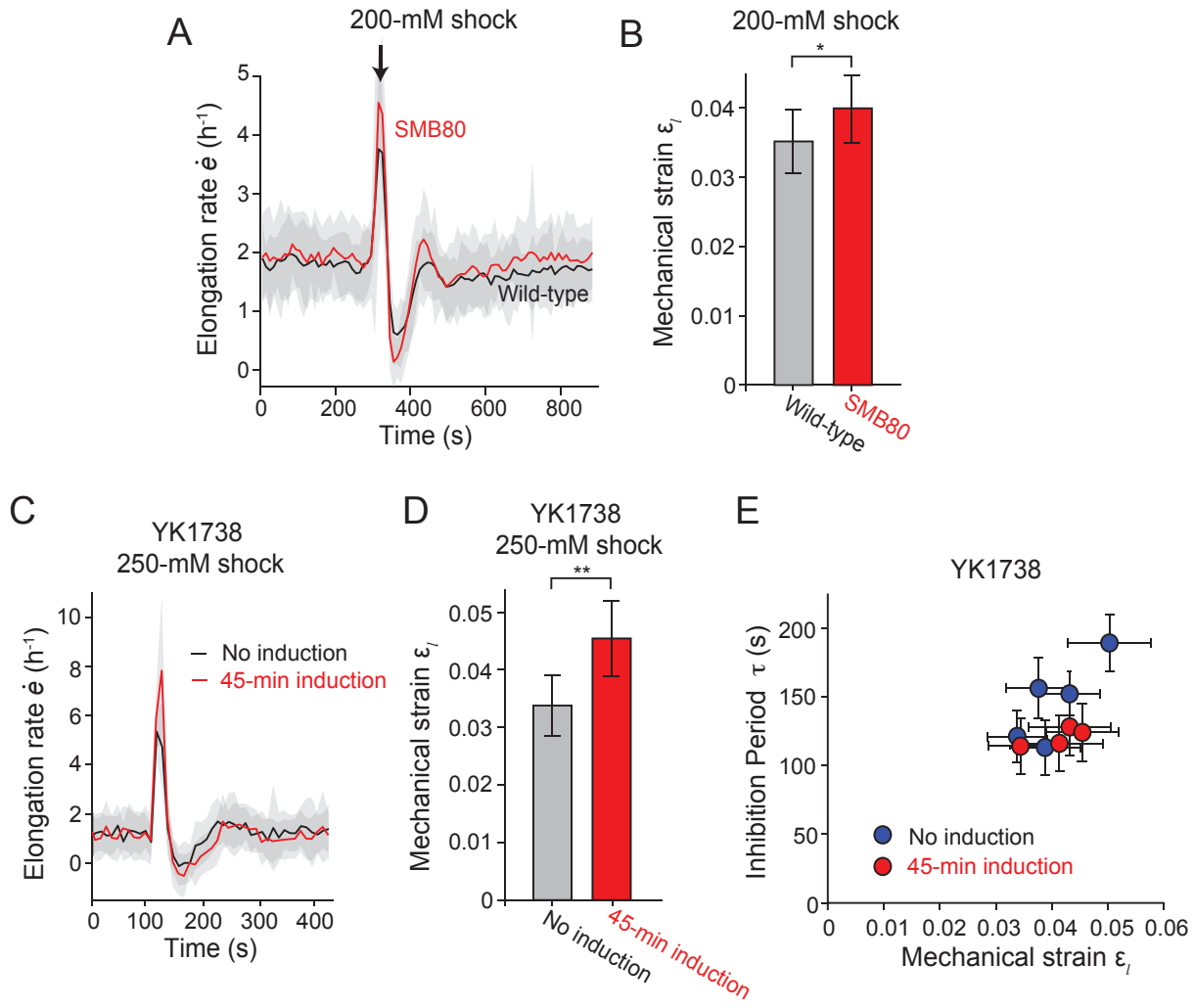
B) Population-averaged elongation rate of *E. coli* cells during the shock (n = 32 cells).

C) Length of representative *E. coli* cells during the shock. The dotted lines indicate the time of shock.



Supplemental Figure 2: SigmaW has no effect on the growth-inhibition response. Related to Figure 2.

Population-averaged elongation rate of wild-type *B. subtilis* 168 and BKE1730 cells lacking the sigma factor σ_w (n = 25 and n = 68 chains for strains 168 and BKE1730, respectively). Shading indicates ±1 standard deviation (s.d.).



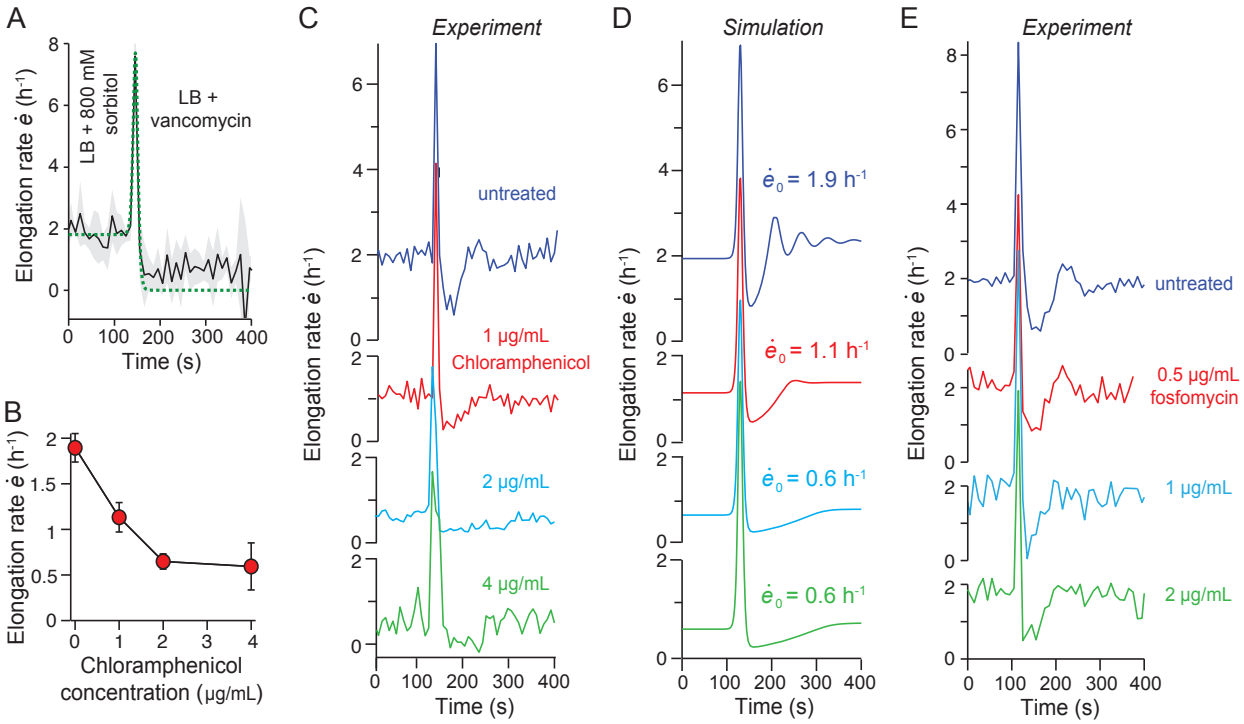
Supplemental Figure 3: Membrane overproduction causes increased swelling. Related to Figure 5.

A,B) Population-averaged elongation rate (A) and mechanical strain (B) of wild-type *B. subtilis* JH642 (black line) and *B. subtilis* SMB80 lacking stretch-activated ion channels (red line) during a 200-mM hypoosmotic shock (from LB + 500 mM sorbitol to LB + 300 mM sorbitol; n = 19 and n = 20 chains for wild-type and SMB80, respectively). Shading in (A) and error bars in (B) indicate ±1 s.d. *: Student's t-test, p = 0:0029.

C,D) Population-averaged elongation rate (C) and mechanical strain (D) of *B. subtilis* YK1738 during a 250-mM hypoosmotic shock (from LB + 1M sorbitol to LB + 750

mM sorbitol) with wild-type levels of AccDA (black line) and induction of AccDA overexpression for 45 min (red line; $n = 54$ and $n = 104$ chains for uninduced and induced, respectively). Shading in (C) and error bars in (D) indicate ±1 s.d. **: Student's t-test, $p < 0{:}0001$.

E) Inhibition period versus mechanical strain of *B. subtilis* YK1738 across a range of shock magnitudes (150 mM < C_{out} < 750 mM), with wild-type levels of AccDA (blue dots) and induction of AccDA overexpression for 45 min (red dots; each point represents the average of 19-164 chains). Error bars indicate ±1 s.d.



Supplemental Figure 4: Response to hypoosmotic shock in the presence of antibiotics.

Related to Figure 4.

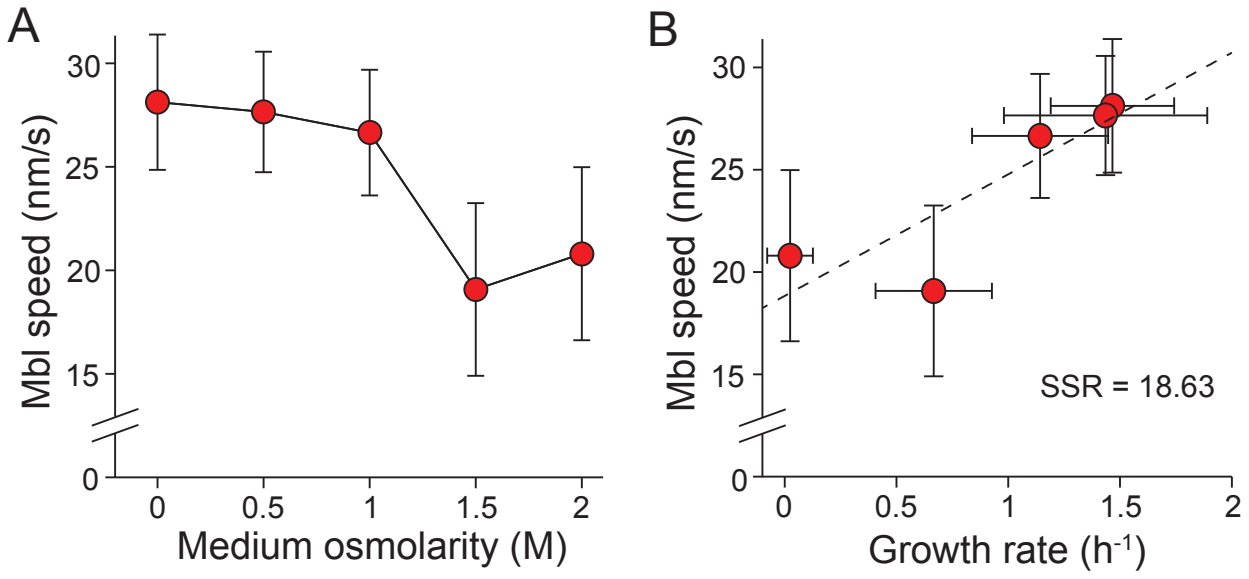
A) Population-averaged elongation rate in response to 800-mM hypoosmotic shock, where the medium was switched from LB + 800 mM sorbitol to LB + 10 μg/mL vancomycin (*n* = 5 chains).

B) Population-averaged elongation rate decreased with increasing concentration of chloramphenicol in the growth medium (each point represents the average of 15-37 cell chains). Error bars indicate ±1 s.d.

C) Population-averaged elongation rate versus time during a 500-mM hypoosmotic shock (from LB + 500mMsorbitol to LB) from experiments in which 0, 1, 2, or 4 μg/mL chloramphenicol was included in the growth medium (each trace represents the average of 15-37 cell chains).

D) Elongation rate during a 500-mM hypoosmotic shock predicted by the tension-inhibition model when the initial elongation rate ($\dot{e}$) was specified to the experimental value observed when 0, 1, 2, or 4 μg/mL chloramphenicol was included in the growth medium.

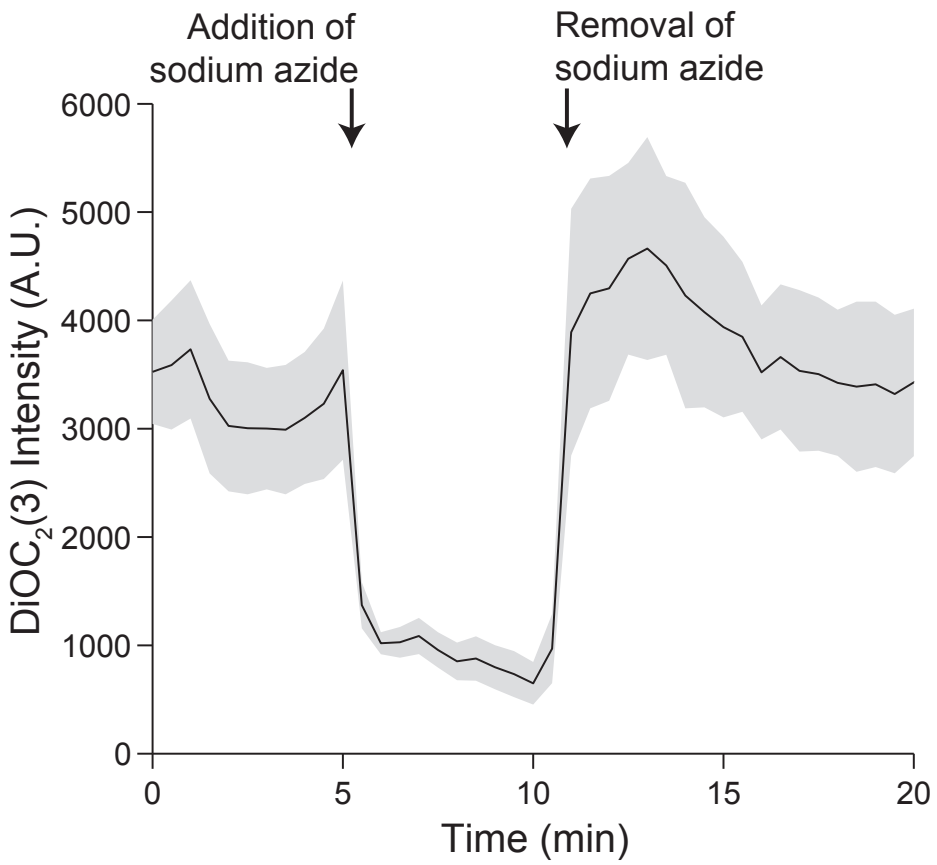
E) Population-averaged elongation rate versus time during a 500-mM hypoosmotic shock (from LB + 500 mM sorbitol to LB) from four experiments in which 0, 0.5, 1, or 2 μg/mL fosfomycin was included in the growth media (each trace represents the average of 34-70 cell chains).



Supplemental Figure 5: Mbl speed depends on medium osmolarity. Related to Figure 6.

A) Ensemble-averaged speed of Mbl puncta decreases with increasing medium osmolarity during steady-state growth (each point represents the average of 491-1742 puncta). Error bars indicate ±1 standard error of the mean (s.e.m.).

B) Ensemble-averaged speed of Mbl puncta increases with increasing growth rate during steady-state growth, when growth rate was modulated by medium osmolarity (each point represents the average of 491-1742 puncta). Error bars indicate ±1 s.e.m. SSR: Sum of squared residuals.



Supplemental Figure 6: $DiOC_2(3)$ reports membrane depolarization by sodium azide.

Related to Figure 7.

Population-averaged fluorescence intensity of cells stained with $DiOC_2(3)$ during a 5-min treatment with 1 mg/mL sodium azide (n = 12 chains). Shading indicates ±1 s.d.

Supplemental Tables

Supplemental Table 1: List of strains used in this study. Related to STAR Methods.

Strain	Species	Genotype	Reference
JH642	*B. subtilis*	Wildtype, *trpC2 pheA1*	*Bacillus* Genetic Stock Center 1A96
BMD122	*B. subtilis*	168, *sfGFP-mbl::spec*	Gift from the Ethan Garner Lab
YK1738	*B. subtilis*	168CA Ω*amyE::*P_{xyl}*-accDA spc*	Gift from the Jeff Errington Lab (Mercier et al., *Cell* 2013)
SMB80	*B. subtilis*	JH642, *mscL::spc*, Δ(*ydhY::ery*), Δ(*yfkC::tet*), Δ(*ykuT::cat*)	Gift from the Erhard Bremer Lab (Hoffmann et al., *Appl Env Microbiol* 2008)
BKE01730	*B. subtilis*	168, *sigW::MLS*	Gift from the Carol Gross lab (Koo et al., *Cell Syst* 2017)
JAT150	*Listeria monocytogenes*	1040βS, *actA::comK*	Laboratory stock
CN1491	*Clostridium perfringens*	Wildtype	ATCC13124, gift from the Justin Sonnenburg Lab