SUPPLEMENTARY INFORMATION

REFERENCES

1. Levina N, *et al.* (1999) Protection of Escherichia coli cells against extreme turgor by activation of MscS and MscL mechanosensitive channels: identification of genes required for MscS activity. *EMBO J* 18(7):1730-1737.
2. Schumann U, *et al.* (2010) YbdG in Escherichia coli is a threshold-setting mechanosensitive channel with MscM activity. *Proc Natl Acad Sci U S A* 107(28):12664-12669.
3. Edwards MD, *et al.* (2012) Characterization of three novel mechanosensitive channel activities in Escherichia coli. *Channels (Austin)* 6(4):272-281.
4. Li GW, Burkhardt D, Gross C, & Weissman JS (2014) Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. *Cell* 157(3):624-635.

FIGURES

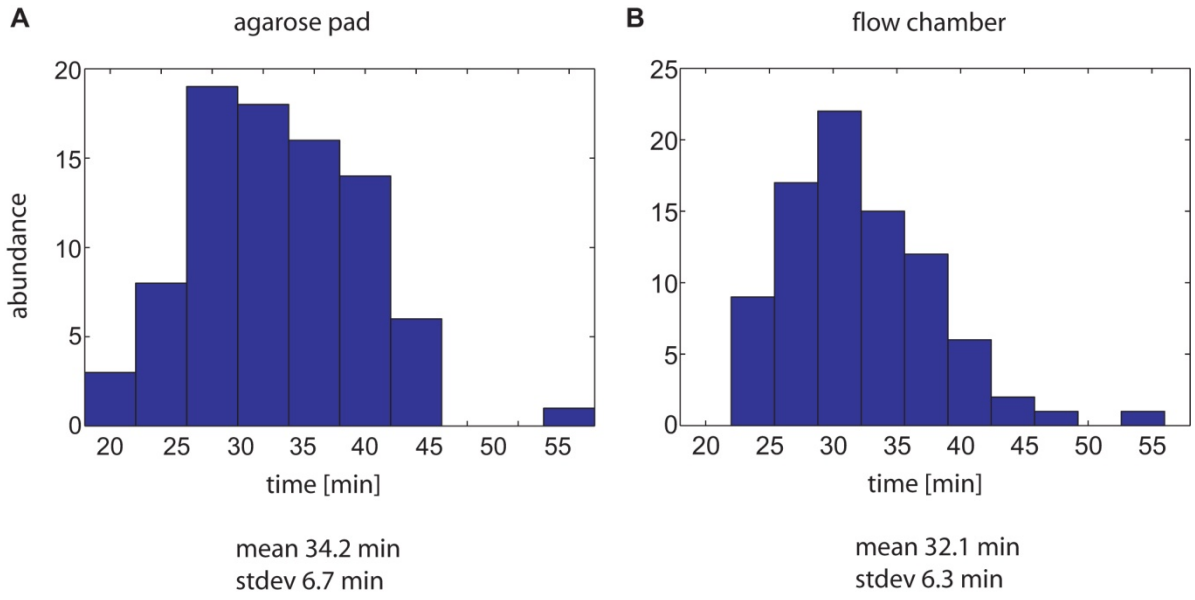

FIGURE S1. Comparison of division times for cells grown under an agarose pad vs. cells grown in a flow chamber. Both samples are imaged under identical environmental

conditions (32° C). Multiple positions are recorded in two minute increments, where at each position, individual cells are tracked. Using image analysis, we determine the division time of each cell from the time between the first appearance of septation (t=0) of an elongated cell and the next division. Shown above are the abundance distribution, mean, and standard deviation of division times for each sample. (A) A ~10000-fold dilution of a cell aliquot is deposited onto a bare, cleaned cover glass to ensure imaging isolated single cells and then covered by a 1.5% agarose-LB pad. (B) An unshocked sample of cells immobilized in a flow chamber, prepared as described in the text, are grown under a steady flow of LB medium. We do not observe any significant difference between the two samples.

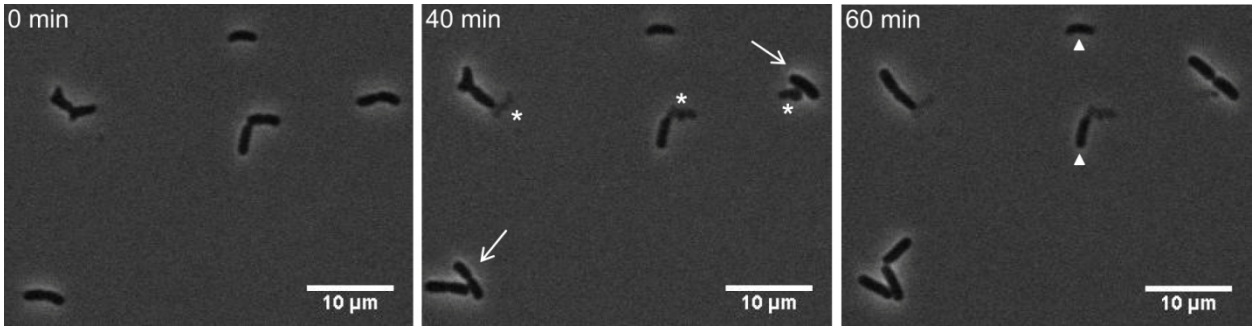

FIGURE S2. Image sequence showing the recovery of MJF465 cells exposed to a 0.5 M NaCl shock at 100 µL/min. Cells can be divided into 3 groups: cells that survived the shock and divide (marked with an arrow), cells which are intact, but do not divide (marked with a triangle), and cells that died as a result of the osmotic challenge (marked with a star). As discussed in the text, the dead cells can be further classified based on their morphology.

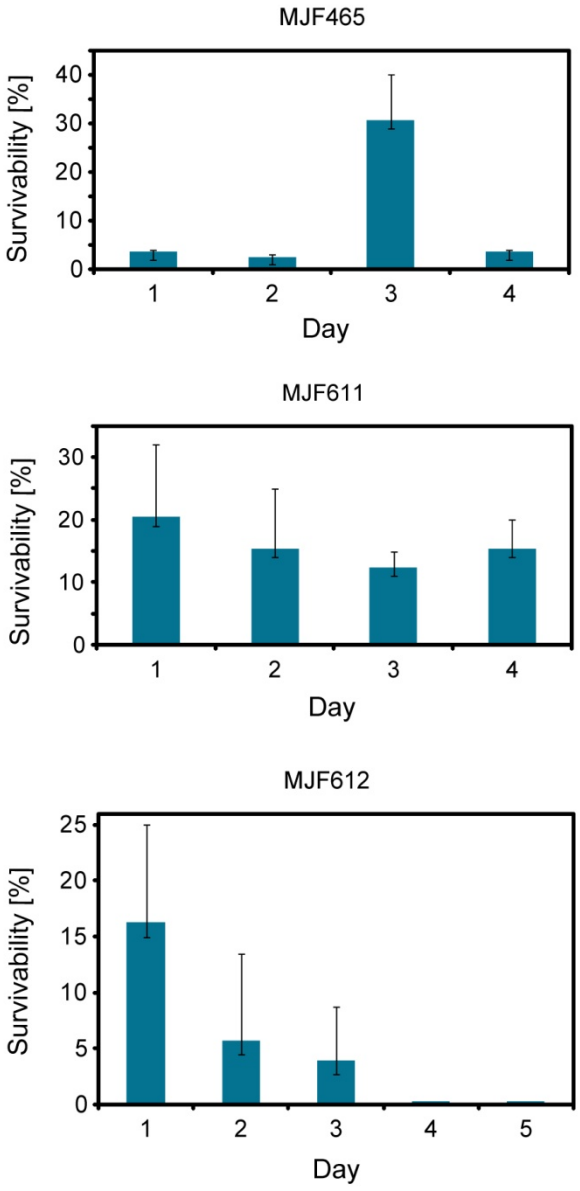

Figure S3: Examples of day-to-day variability of plating assay results for three different strains: MJF465 (ΔmscL ΔmscS ΔmscK), MJF611 (ΔmscS ΔmscK ΔybdG), and MJF612 (ΔmscL ΔmscS ΔmscK ΔybdG). The errors bars are the standard deviation from multiple repeats of the same experiment on a given day.

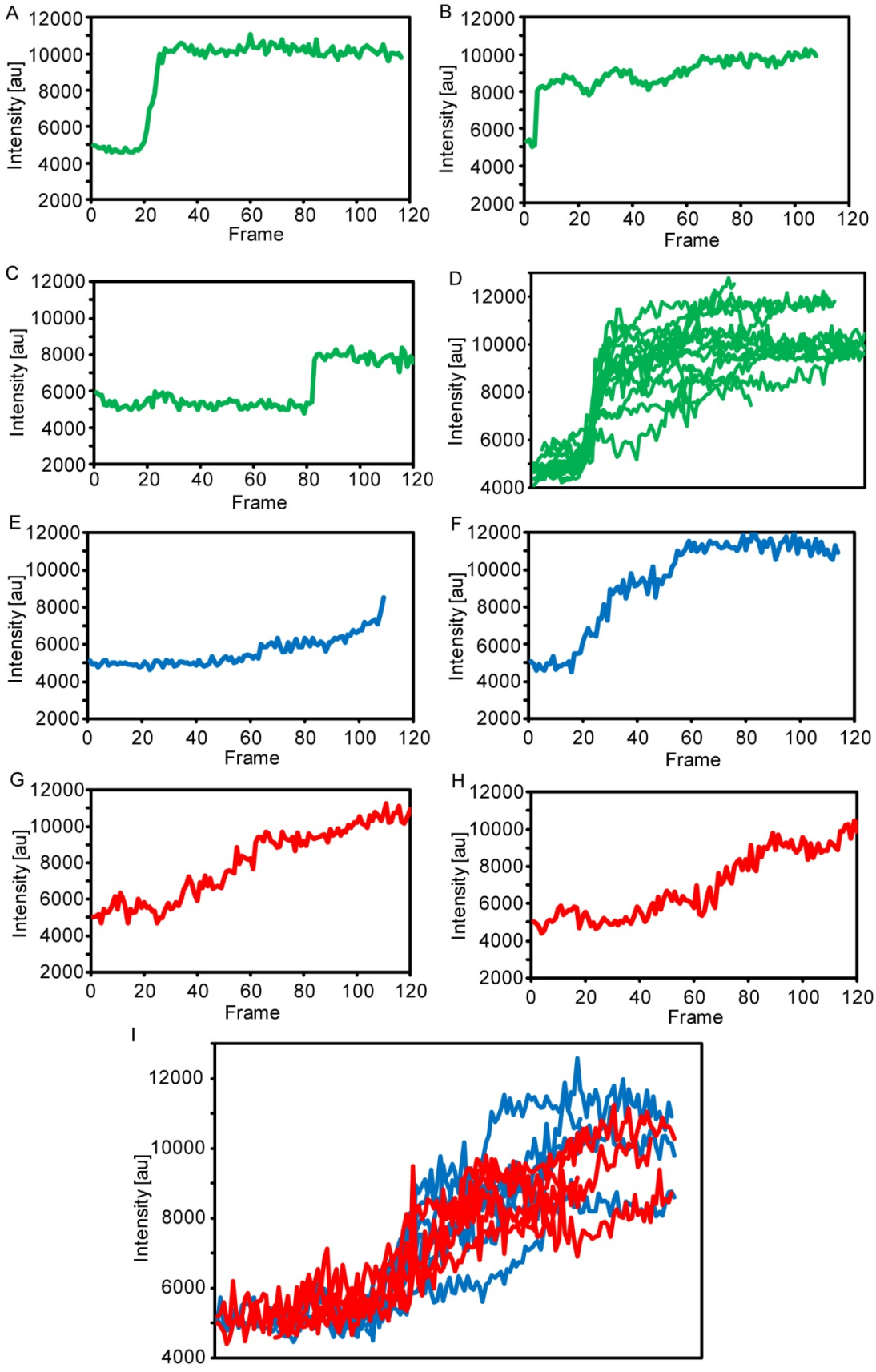
A
B
C
D
E
F
G
H
I
Intensity [au]
Frame
12000
10000
8000
6000
4000
2000
0
20
40
60
80
100
120

Figure S4: Phase contrast intensity change as a function of time (frames) for cells demonstrating the exploder (A-C), fading (E & F), and membrane rupture phenotypes (G & H). Panels A-C illustrate step-like changes in the cytoplasmic which are characteristic of the exploder phenotype (green). Panel D overlays the intensity traces of several exploder cells. The individual traces have been offset in time so that steps begin to increase in intensity at the same time. Panels E&F and G&H show typical intensity traces of fading (blue) and membrane rupture (red) phenotypes, respectively. Panel I overlays the intensity traces of several fading and membrane rupture cells to demonstrate the similarity of their time responses to each other and to contrast them against the step-like response of the exploders. The traces have been time aligned so that the traces begin increasing in intensity at the same time.

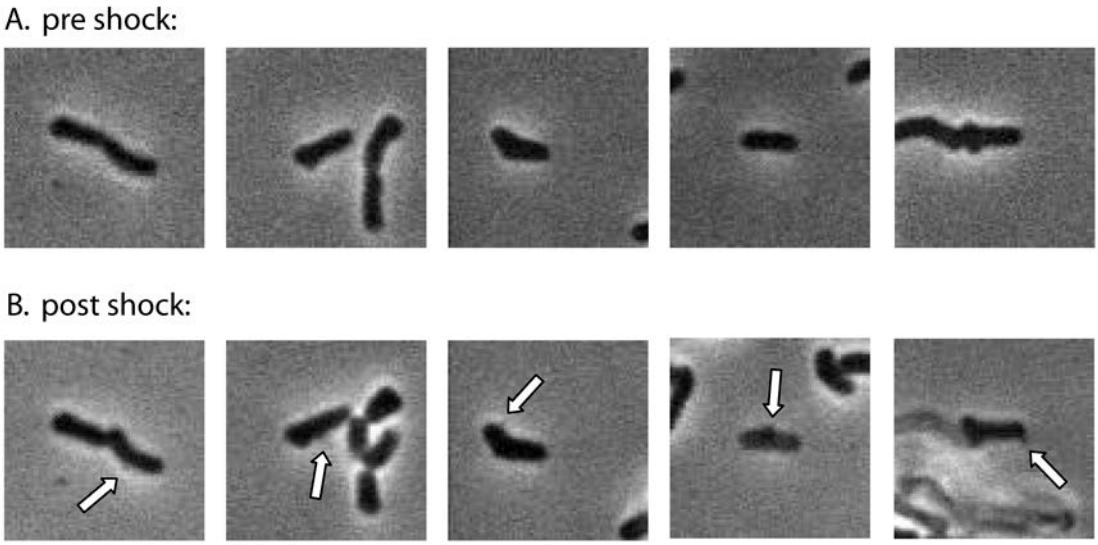

Figure S5. Examples of the rupture phenotype. Before the shock (A) the cells can have different initial morphologies. After the shock (B), we identify small structures that emerge from the cell wall, which were not there before the shock (white arrows). These structures do not appear to be blebs because they do not change in size during the lysis and remain after the cell has lysed.

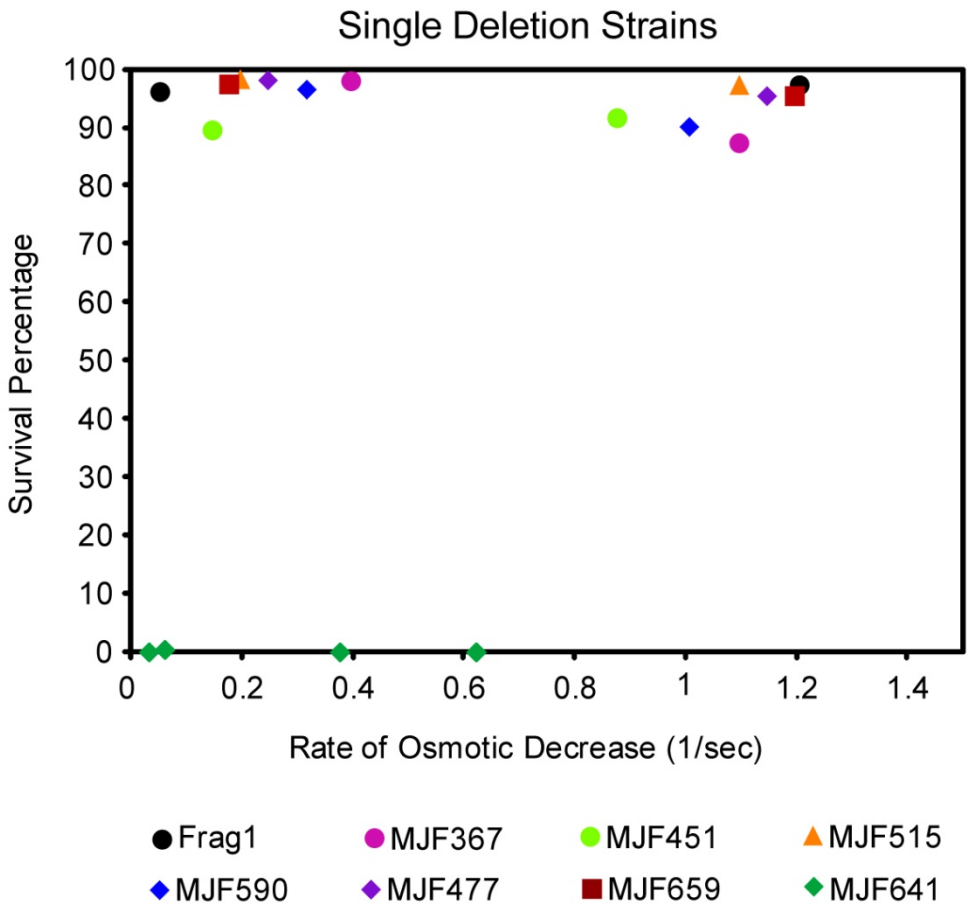

Figure S6: Survival percentage vs. rate of osmotic decrease for single deletion mutants MJF367 (Δ*mscL*), MJF451 (Δ*mscS*), MJF515 (Δ*ybiO*), MJF590 (Δ*yjeP*), MJF477 (Δ*ynaI*), MJF659 (Δ*ybdG*). Strains Frag1 (wild-type) and MJF641 (Δ*mscL* Δ*mscS* Δ*mscK* Δ*ybdG* Δ*ybiO* Δ*yjeP*Δ*ynaI*) are included for reference. There is less than 10% difference among the single deletion strains, where the largest effects may be for strains missing MscL (MJF367), MscS (MJF451), and YjeP (MJF590). However, these difference levels are roughly at the resolution of our experiment.

TABLES

TABLE S1. Previously published survival results for strains used in this study.

Strain	Genes deleted	Osmotic shock [M NaCl]	Survival [%]	Reference
Frag1		0.5	94 ± 1	(1)
MJF367	*mscL*	0.5	84 ± 6	(1)
MJF451	*mscS*	0.5	90 ± 4	(1)
MJF379	*mscK*	0.5	84±10	(1)
MJF429	*mscS, mscK*	0.5	82 ± 3	(1)
MJF465	*mscL, mscS, mscK*	0.5	8 ± 1	(1)
		0.25	6 ± 6	(2)
MJF612	*mscL, mscS, mscK, ybdG*	0.25	4 ± 4	(2)
MJF641	*mscL, mscS, mscK, ybdG, ynaI, ybiO, yjeP*	0.3	0.6 ± 0.3	(3)

TABLE S2. Abundances of MS channels adapted from Li et al. (4).

MS channel in *E. coli*	**# of channels (in supplemented MOPS medium)**	**# of subunits**	**# subunits per channel**
MscL	560	2802	5
MscS	610	4271	7
MscK	106	744	7
YbdG	51	357	7
YnaI	31	214	7
YjeP	21	146	7
YbiO	0.3	2	7

VIDEO LEGENDS

Video S1. A “blebbing” cell minutes after hypoosmotic downshock.

Video S2. A “rupturing” cell minutes after hypoosmotic downshock.

Video S3. A “fading” cell minutes after hypoosmotic downshock.

Video S4. A “bursting” cell minutes after hypoosmotic shock.

Video S5. Unshocked test cells growing in a flow chamber for multiple divisions.

Video S6. Unshocked control cells growing under an agarose pad for multiple divisions.