# High Concentrations of the Antimicrobial Peptide Magainin 2 Induce Distinct Biomechanical Changes in *Escherichia coli*

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## SUPPLEMENTAL METHODS

### HEK 293 cell culture

Human Embryonic Kidney 293 (HEK 293) cells were obtained from the American Type Culture Collection (ATCC) and stored at -80 C°. Cells were thawed in warm Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin, and 1% amphotericin. Cells were then transferred to a T75 cell culture flask. Flasks were placed in a $CO_2$ incubator at 37 °C and grown to approximately 75-80% confluence, with the media typically exchanged every two to three days. Once the cells reached the appropriate confluence, they were treated with a 10% trypsin-EDTA solution for 5 minutes at 37 °C. The trypsin was then neutralized with 10 mL of culture medium, and the cells were collected by centrifugation. The cell pellet was resuspended in culture media and split into two flasks. Cells were again allowed to grow to 75-80% confluence before being split or used for downstream experiments.

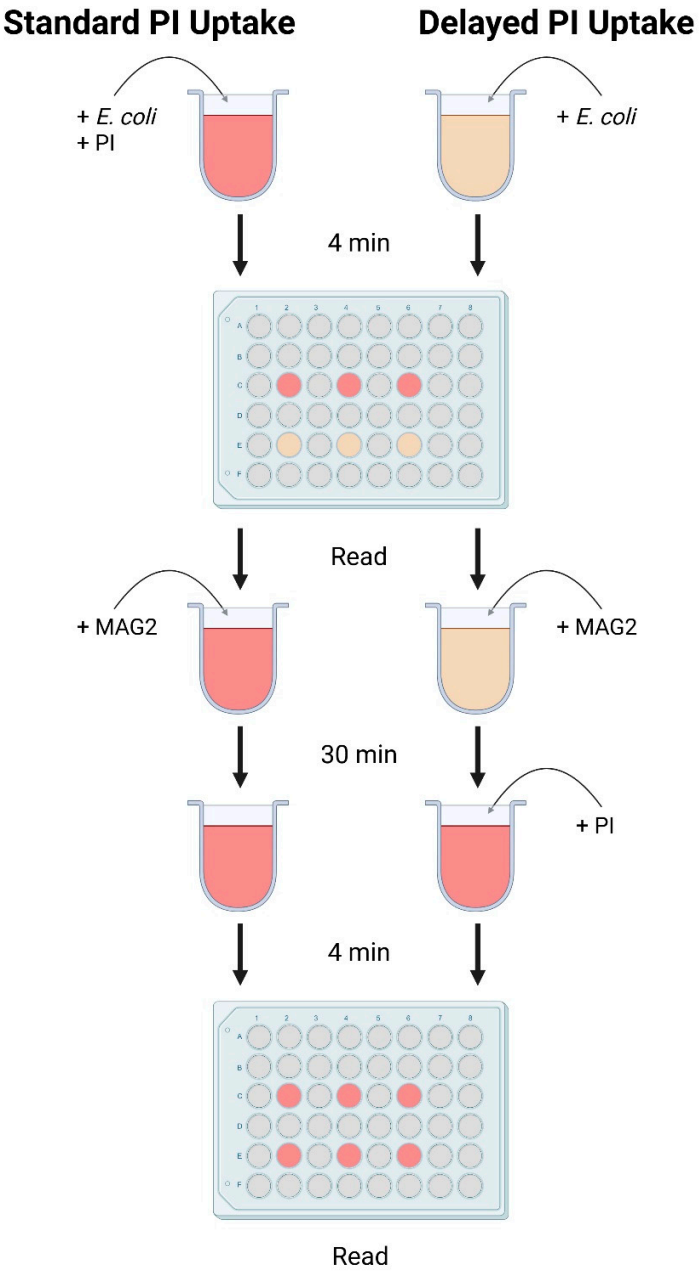
### Propidium iodide uptake assays

Propidium iodide uptake experiments with HEK 293 cells were conducted as described in the main article, with minor modifications. Since growing 3 mL of HEK 293 cells resuspended to an $OD_{600}$ of 0.5 proved impractical, we used a microcuvette with a volume of 500 µL and lowered the $OD_{600}$ to 0.2. To collect HEK 293 cells in suspension, 4-6 flasks were treated with 10% trypsin-EDTA for 5 minutes at 37°C. The trypsin was neutralized with fresh medium, and cells were collected by centrifugation. The cells were washed once with 10 mM sodium phosphate buffer, pH 7.4, and resuspended to an $OD_{600}$ of 0.2. Bacteria were grown, collected, and washed as described in the main article, and likewise resuspended in phosphate buffer to an $OD_{600}$ of 0.2. The rest of the assay was completed as described in the main article using a final concentration

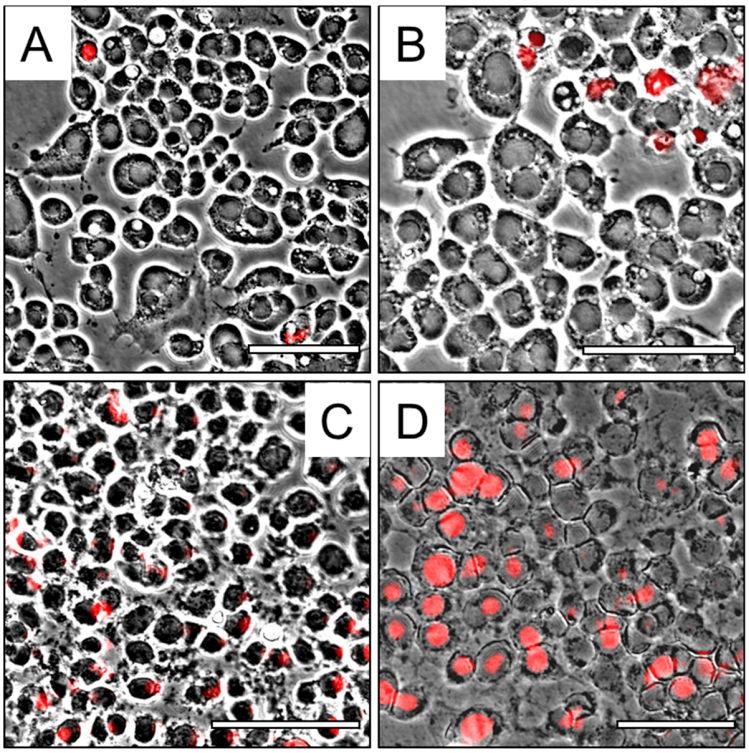
of 500 μM magainin 2 (MAG2). Assays were performed at least three times each on bacteria and HEK 293 cells.

**Propidium iodide uptake fluorescence microscopy**

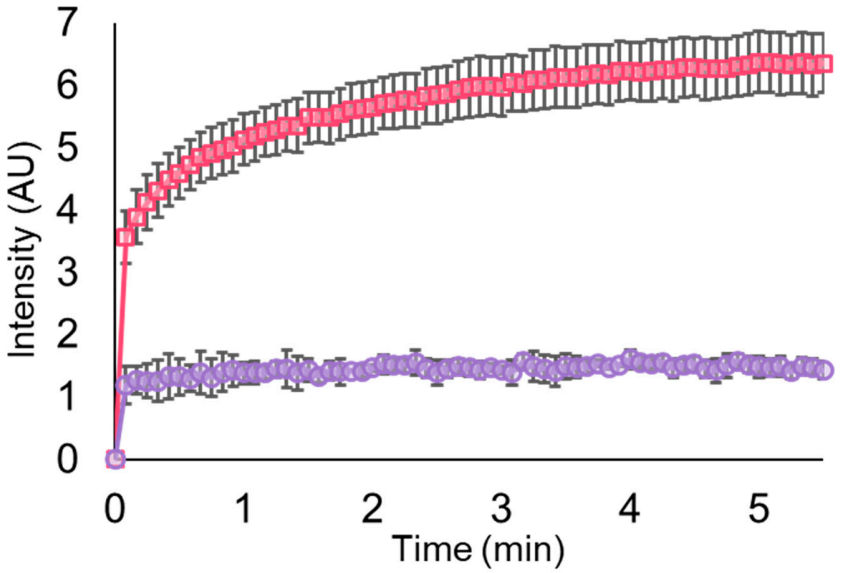
To assess membrane permeability in eukaryotic cells during treatment with MAG2, we monitored PI uptake in HEK 293 cells using fluorescence microscopy, analogous to live/dead staining in bacteria. Once flasks with 75-80% confluent cells were available, the culture medium was aspirated, and the cells were washed with fresh medium or 10 mM phosphate buffer, pH 7.4. Five milliliters of fresh media or buffer were added to the flask, and propidium iodide (PI) was added to a final concentration of 2 μg/mL. The mixture was then allowed to equilibrate for five minutes at room temperature. Performing the experiment in medium or buffer did not affect the outcome. The flask was placed on an Olympus ix70 inverted fluorescence microscope with an Olympus Plan C 20X lens. Cells were imaged in phase contrast and checked for PI fluorescence with an Olympus ET-TRITC filter. After initial images of untreated cells were acquired, MAG2 was added at final concentrations of 25, 125, or 500 μM. Cells were incubated at room temperature for 30 minutes before images were captured, as described above. Files were imported into ImageJ and overlaid. Experiments were performed at least four times for each concentration of MAG2.



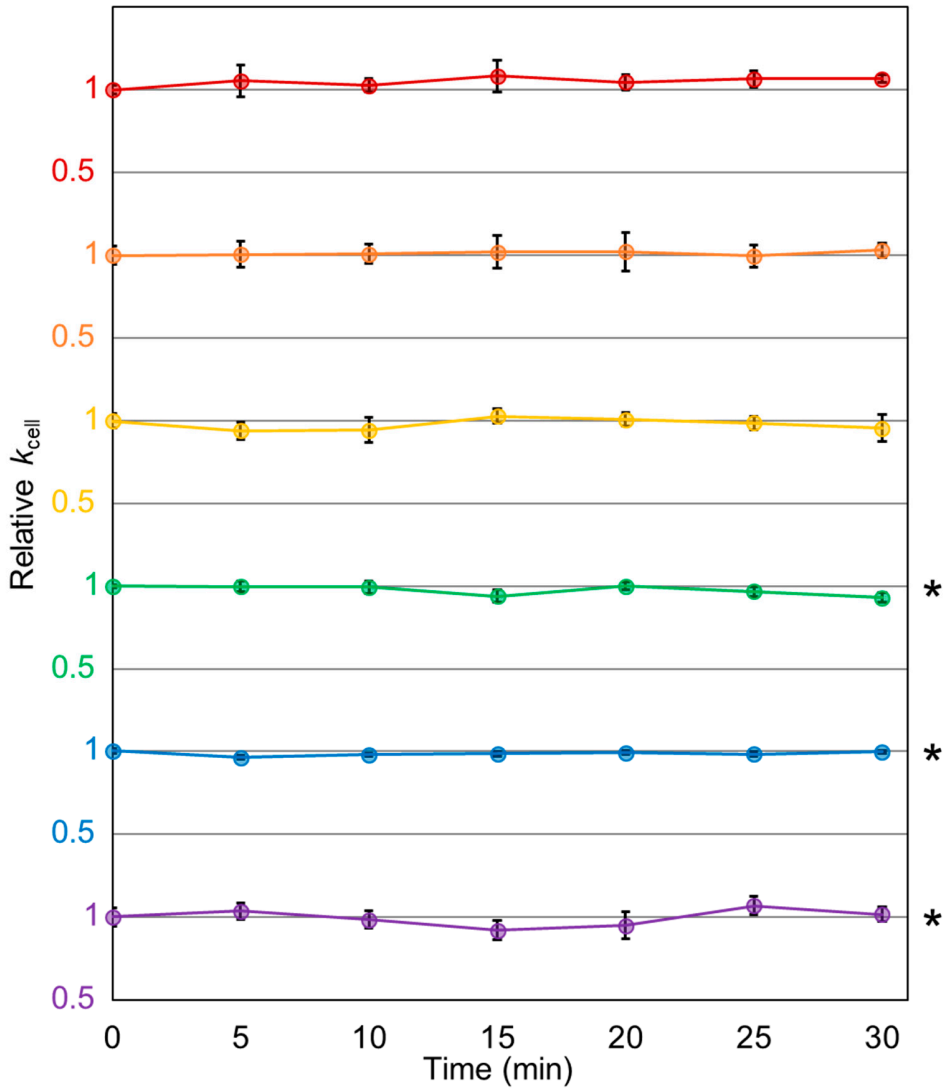

**Supplemental Figure S1:** Standard and delayed PI uptake experiments were performed in tandem to measure PI uptake at the beginning and end of MAG2 treatment. Created in https://BioRender.com.



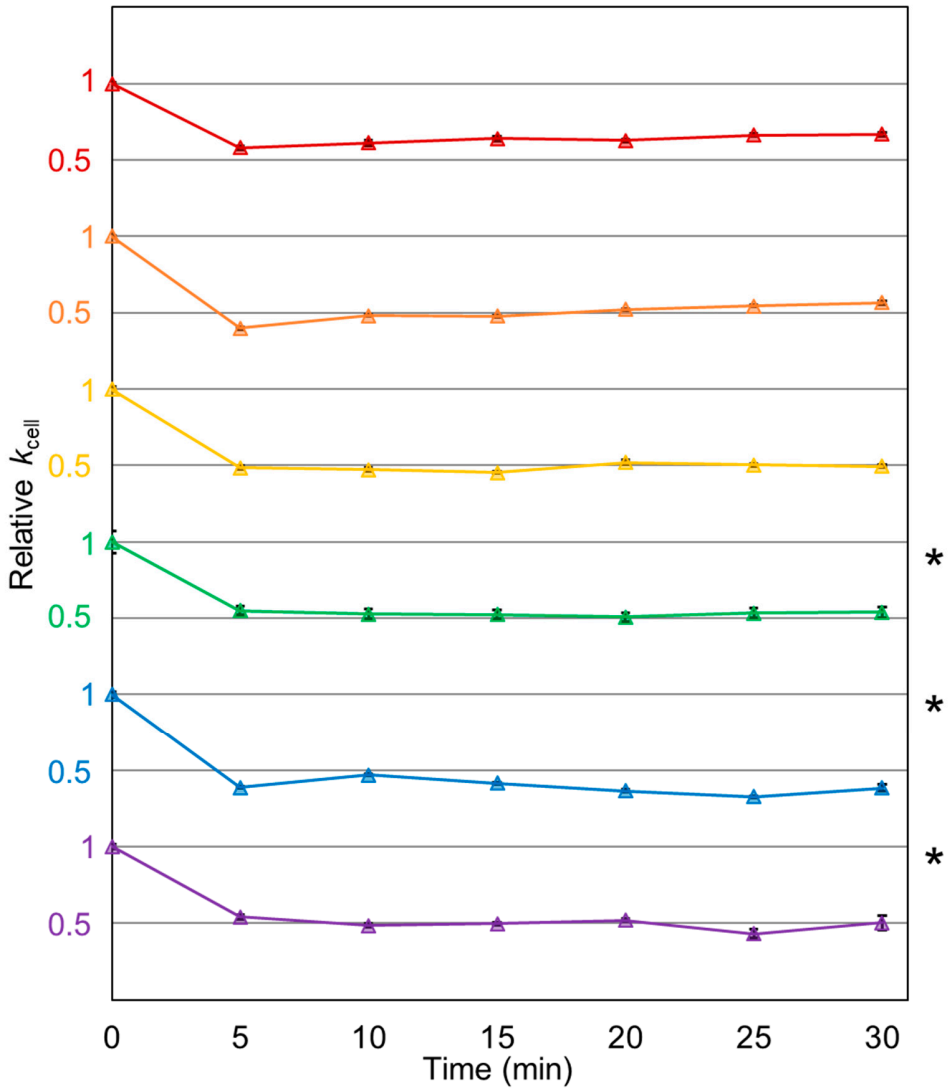

**Supplemental Figure S2:** Fluorescence microscopy reveals limited permeabilization of HEK 293 cells, even at high concentrations of MAG2. Representative images of untreated cells (A) and cells treated with 25 µM MAG2 (B), 125 µM MAG2 (C), and 500 µM MAG2 (D) for 30 minutes are shown. The scale bar is 100 microns.



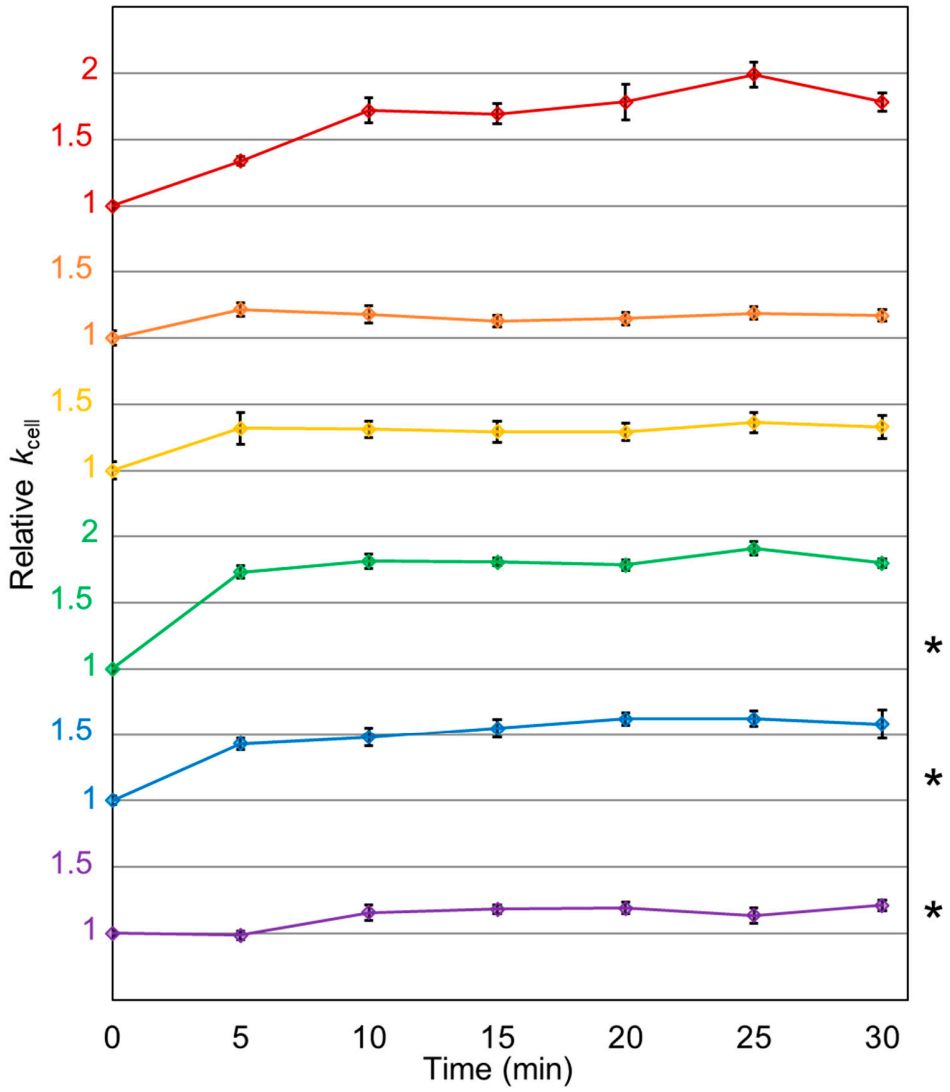

**Supplemental Figure S3:** Propidium iodide fluorescence from *E. coli* treated with 500 µM MAG2 is approximately seven times greater than that of HEK 293 cells treated with the same concentration. The fluorescence intensity was determined every five seconds for *E. coli* (pink squares) or HEK 293 cells (purple circles) treated with 500 µM MAG2. The average intensity and standard error were determined from at least 3 individual experiments. Error bars are present for each point, but many are not distinguishable from the marker.



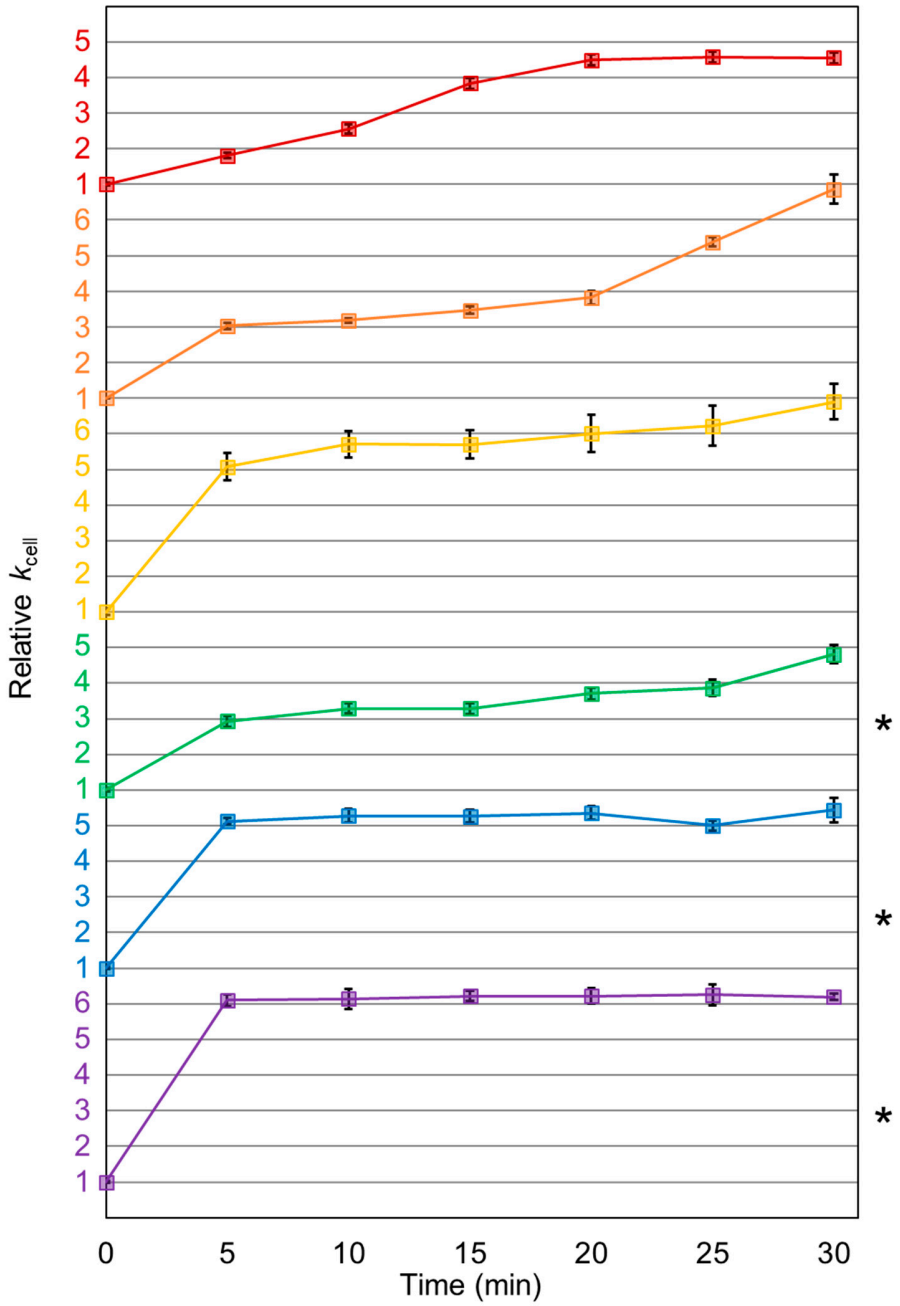

**Supplemental Figure S4:** All untreated cells show minimal variation in cell stiffness over time. Each point represents the average $k_{cell}$ obtained from 5-6 force-distance curves taken at a given time, normalized to the $k_{cell}$ at time 0 to account for changes in the starting cellular stiffness. The error bars are the standard deviation of the average. Each time course represents a single cell, starting with a normalized $k_{cell}$ of 1. These six cells are representative of the 26 untreated cells that were followed over time in near-native conditions. The asterisks indicate the cells presented in Figure 2 of the main text.



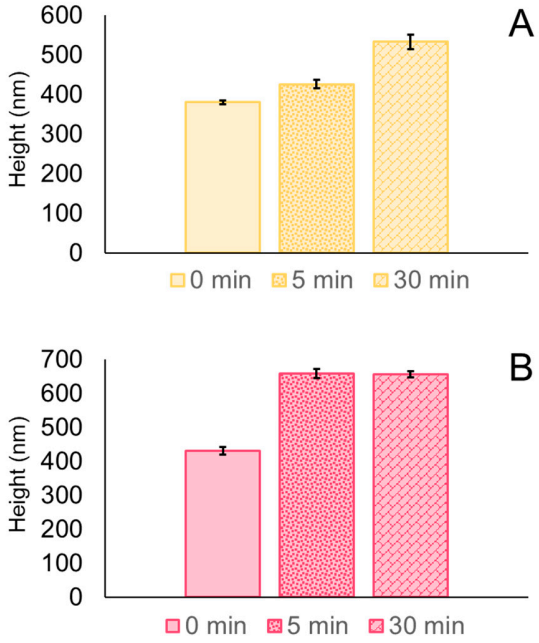

**Supplemental Figure S5:** All 31cells treated with 25 μM MAG2 show substantial decreases in cell stiffness. Each point represents the average $k_{cell}$ obtained from 5-6 force-distance curves taken at a given time, normalized to the $k_{cell}$ at time 0 to account for changes in the starting cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized $k_{cell}$ of 1. The asterisks indicate the cells presented in Figure 2 of the main text.



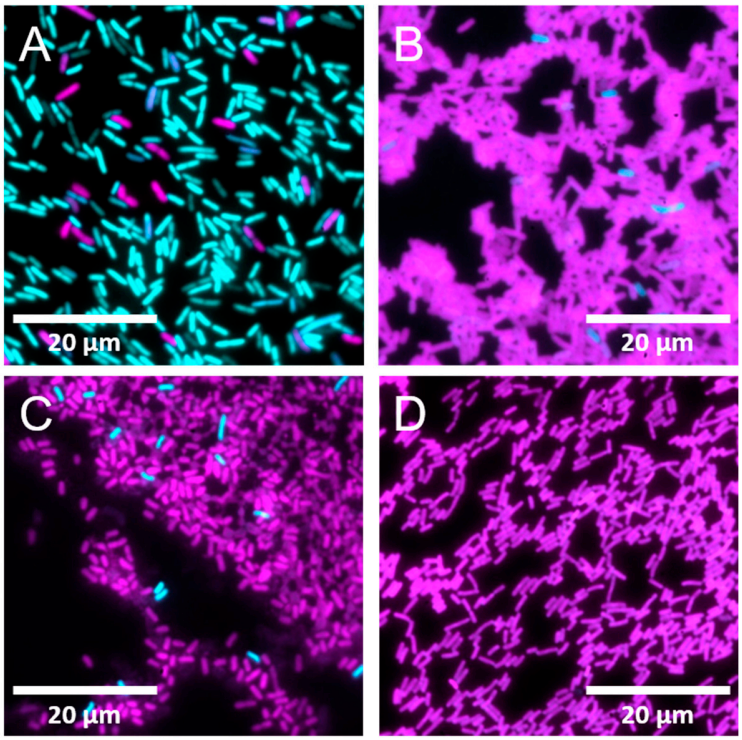

**Supplemental Figure S6:** All 40 cells treated with 125 µM MAG2 show substantial increases in cell stiffness. Each point represents the average $k_{cell}$ obtained from 5-6 force-distance curves taken at a given time, normalized to the $k_{cell}$ at time 0 to account for changes in the starting cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized $k_{cell}$ of 1. The asterisks indicate the cells presented in Figure 2 of the main text.



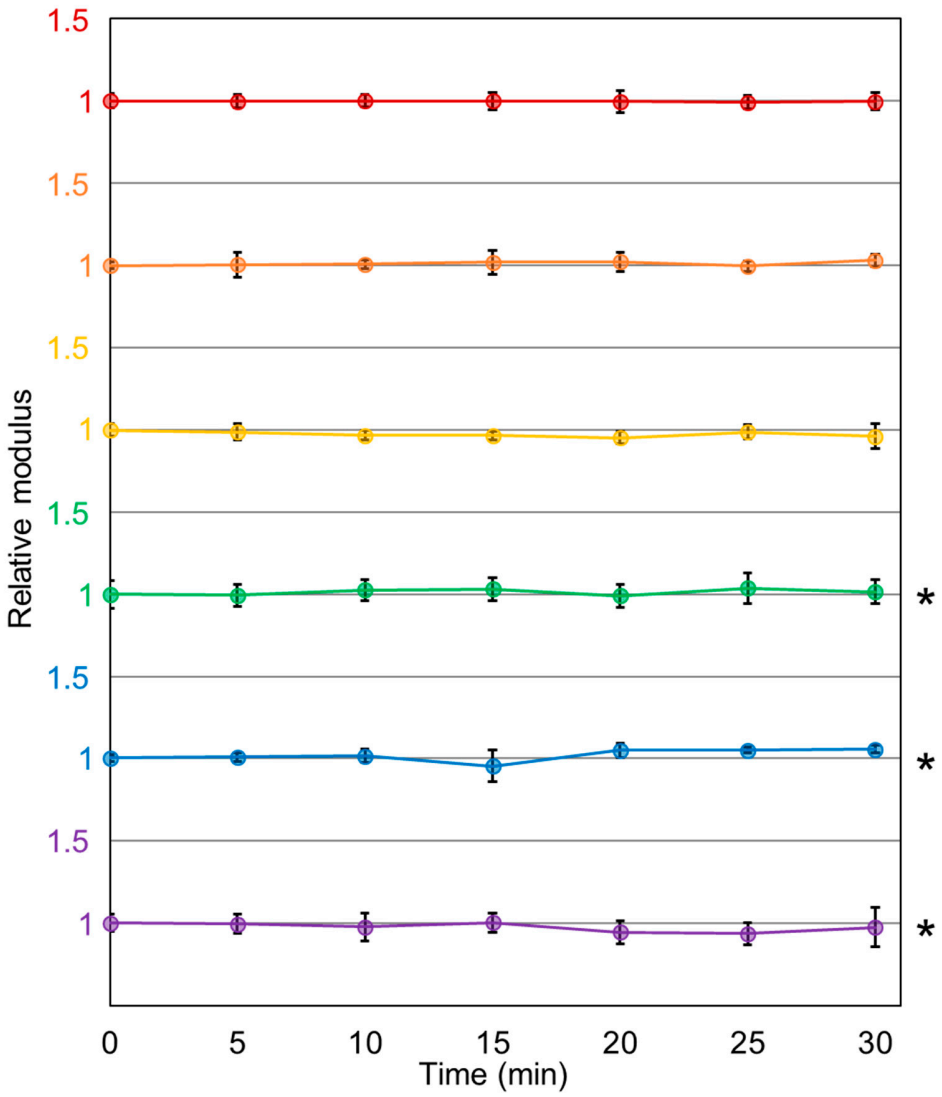

**Supplemental Figure S7:** All 46 cells treated with 500 µM MAG2 show substantial increases in cell stiffness. Each point represents the average $k_{cell}$ obtained from 5-6 force-distance curves taken at a given time, normalized to the $k_{cell}$ at time 0 to account for changes in the starting cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized $k_{cell}$ of 1. The asterisks indicate the cells presented in Figure 2 of the main text.



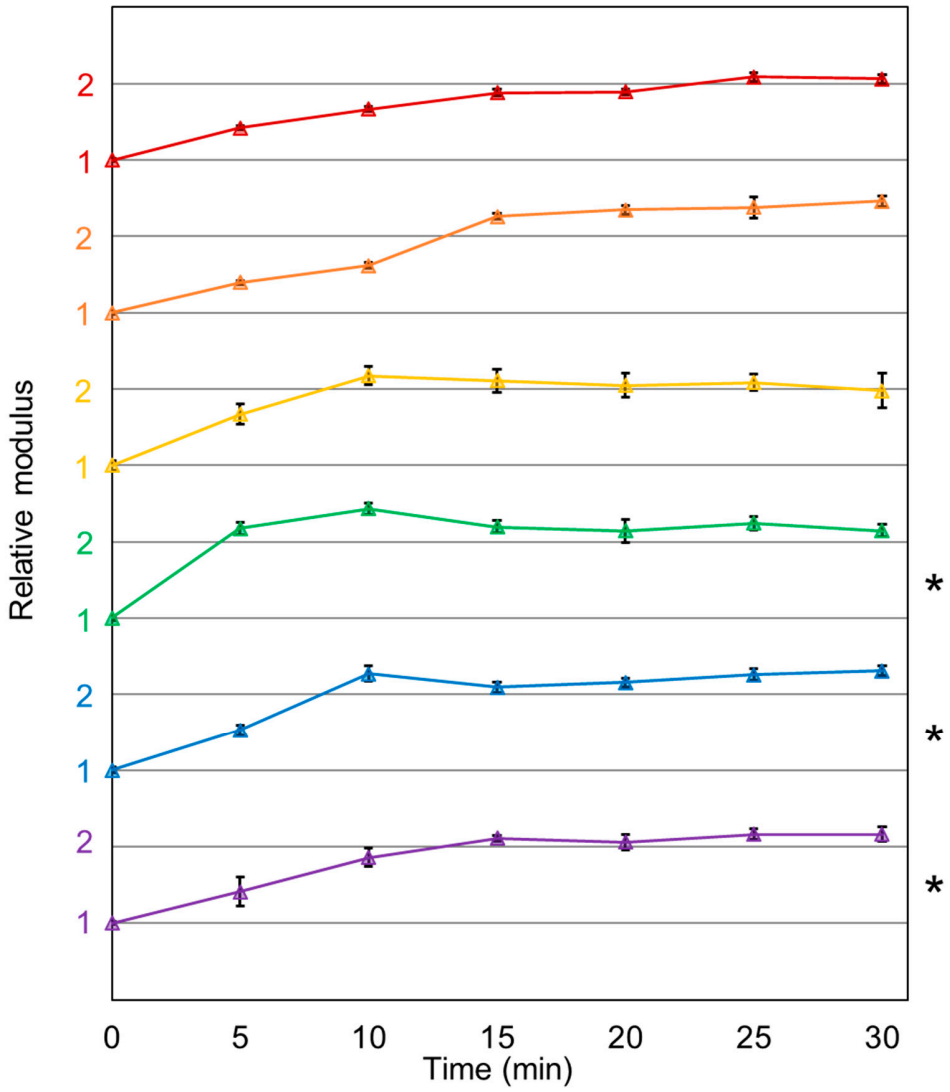

**Supplemental Figure S8:** Cell height increases gradually over time in cells treated with 125 µM MAG2 (A, n = 20), but increases rapidly in cells treated with 500 µM MAG2 (B, n = 40). Cells were imaged in fluid before and after MAG2 application. Cell height was measured at the center of the longitudinal axis, and the average and standard error were calculated for each population.



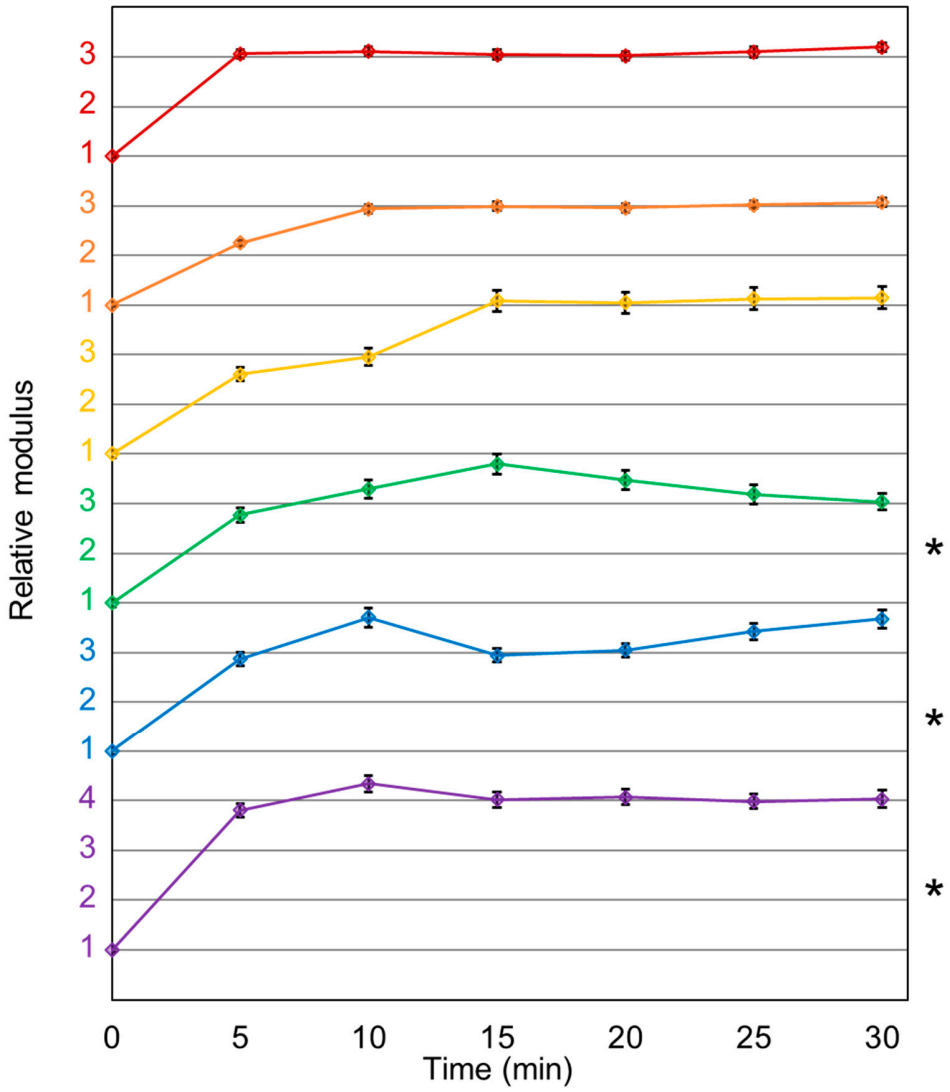

**Supplemental Figure S9:** Propidium iodide can cross the cell membrane after 30 minutes of treatment with MAG2. Representative live/dead images of untreated cells (A) and cells treated with 25 µM MAG2 (B), 125 µM MAG2 (C), and 500 µM MAG2 (D) are shown. This figure is identical to Figure 5 in the main text, but the coloration has been changed to accommodate those with color blindness. In this figure, the cells stained with SYTO 9 appear cyan, while those stained with propidium iodide appear magenta.



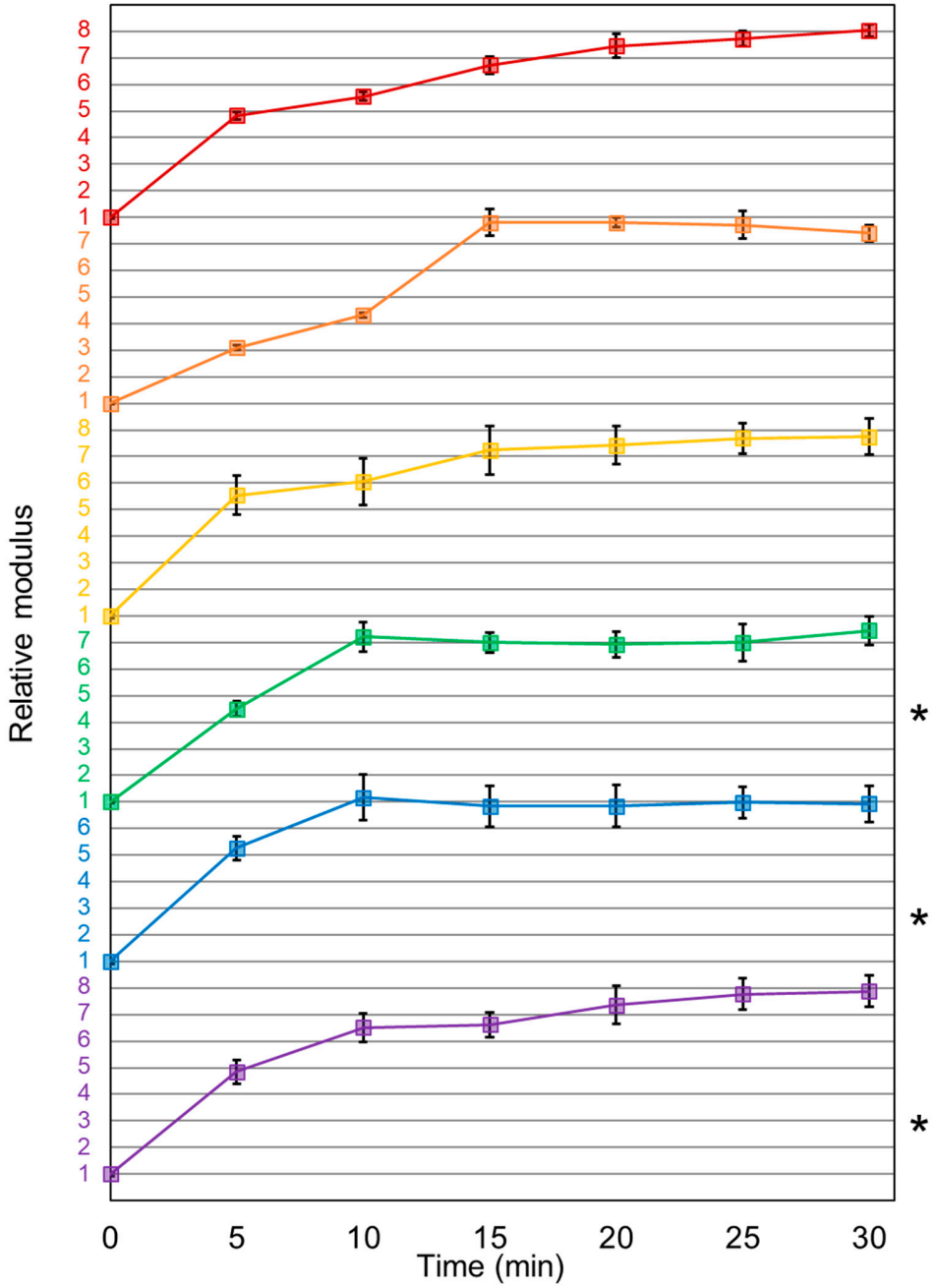

**Supplemental Figure S10:** All 26 untreated cells have only minor changes in moduli. Each point represents the average modulus from 3-5 force-distance curves collected at a given time, normalized to the modulus at time 0 to account for changes in the initial cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized modulus of 1. The asterisks indicate the cells presented in Figure 6 of the main text.




**Supplemental Figure S11:** All 26 cells treated with 25 μM MAG2 have substantial increases in moduli. Each point represents the average modulus from 3-5 force-distance curves collected at a given time, normalized to the modulus at time 0 to account for changes in the initial cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized modulus of 1. The asterisks indicate the cells presented in Figure 6 of the main text.




**Supplemental Figure S12:** All 20 cells treated with 125 µM MAG2 have substantial increases in moduli. Each point represents the average modulus from 3-5 force-distance curves collected at a given time, normalized to the modulus at time 0 to account for changes in the initial cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized modulus of 1. The asterisks indicate the cells presented in Figure X of the main text.




**Supplemental Figure S13:** All 40 cells treated with 500 µM MAG2 have substantial increases in moduli. Each point represents the average modulus from 3-5 force-distance curves collected at a given time, normalized to the modulus at time 0 to account for changes in the initial cellular stiffness. The error bars are the standard deviation of the average (some error bars are too small to distinguish from the marker). Each time course represents a single cell, starting with a normalized modulus of 1. The asterisks indicate the cells presented in Figure X of the main text.

**Supplemental Table S1**: Average and standard error of cellular spring constants ($k_{cell}$) and Young's moduli at times 0 and 30 min

| | Untreated (n = 54)[§] | 25 µM MAG2 (n = 37)[§§] | 125 µM MAG2 (n = 40)[§] | 500 µM MAG2 (n = 51)[§§] |
|---|---|---|---|---|
| Average $k_{cell}$ (N/m) at t = 0 min | 0.039 ± 0.0025 | 0.039 ± 0.0022 | 0.037 ± 0.0018 | 0.038 ± 0.0027 |
| Average $k_{cell}$ (N/m) at t = 30 min | 0.037 ± 0.0021 | 0.024 ± 0.0017 | 0.061 ± 0.0040 | 0.12 ± 0.0010 |
| Average modulus (kPa) at t = 0 min | 360 ± 15 | 370 ± 22 | 370 ± 6.8 | 320 ± 20 |
| Average modulus (kPa) at t = 30 min | 330 ± 16 | 700 ± 40 | 970 ± 45 | 2000 ± 120 |

[§] The number of cells differs from the reported number of cells used in the time course analysis because some cells did not produce usable data over the entire 30-minute period.

**Supplemental Table S2**: Average and standard error of roughness (Rq) in *E. coli* cells captured by AFM in near-native conditions after 30 min MAG2 treatment

| | Untreated (n = 20) | 25 µM MAG2[†] (n = 26) | 125 µM MAG2[§] (n = 40) | 500 µM MAG2 (n = 46) |
|---|---|---|---|---|
| Average Rq (nm) | 157 ± 8.9 | 274 ± 18 ** | 228 ± 19 ** | 259 ± 14 ** |

** Compared to untreated, $p < 0.005$ by Student's T-Test

[†] 25 µM compared to 125 µM, $p = 0.090$, compared to 500 µM, $p = 0.523$

[§] 125 µM compared to 500 µM, $p = 0.216$