Supplemental Material:

Non-perturbative Fluorescence Imaging of Nucleoid Morphology in Live Bacterial Cells

S. Bakshi, *et al.*

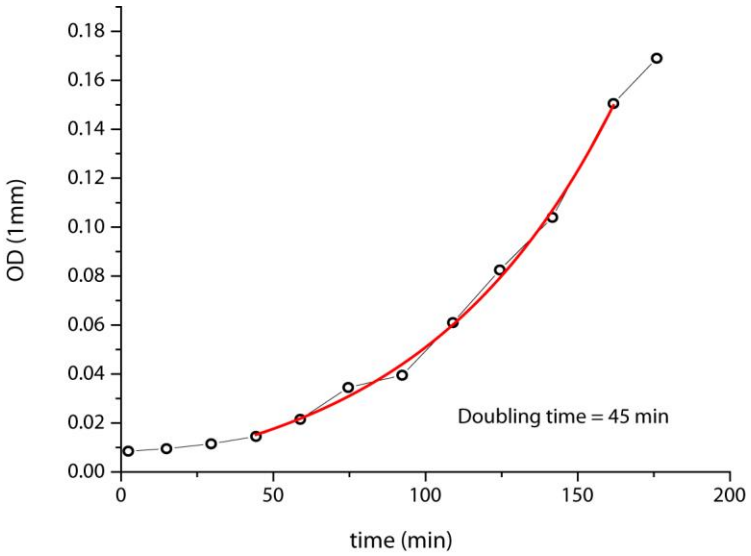

Figure S1. Growth curve at 30ºC for the VH1000 in bulk culture. Measurements were carried out in a cuvette of 1 mm path length. The relationship to the more usual OD(1 cm) measurements is: 10 OD(1 mm) = OD(1 cm). Red line is an exponential fit to the OD vs time plot. Doubling time is 45 min.

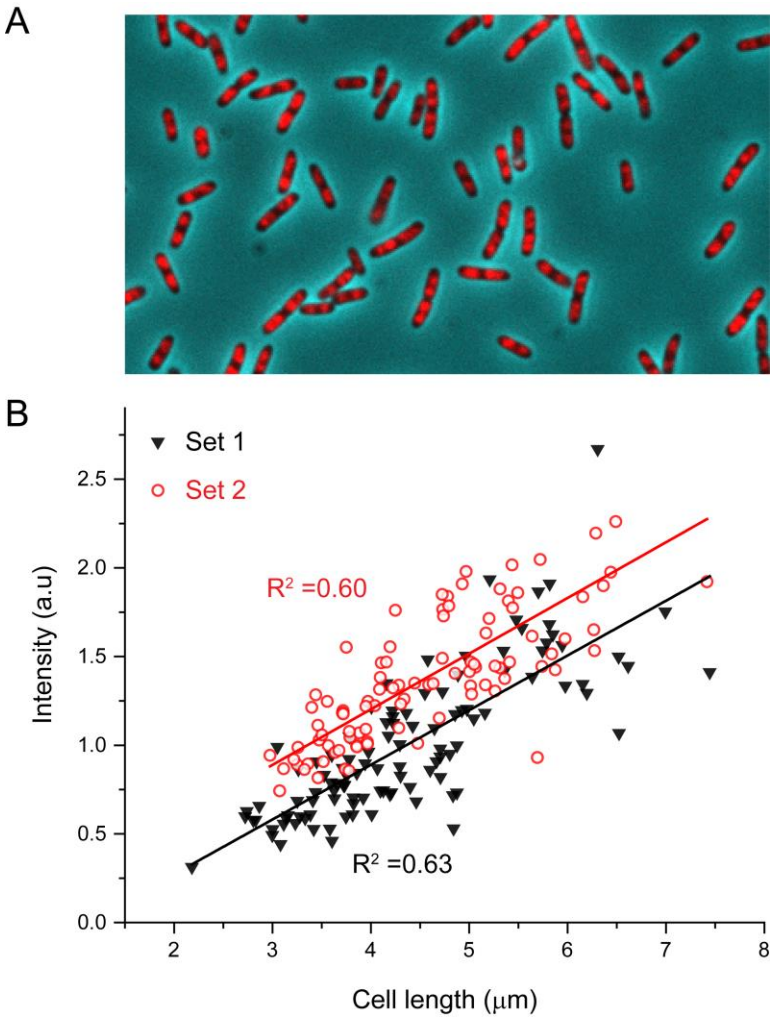

Figure S2. Correlation of total Sytox Orange intensity with cell length. (A) Large field of view of Sytox Orange stained VH1000 cells. Cell width of 1 μm sets scale. (B) Total intensity of each cell is plotted vs cell length for two batches of stained cells. Set 1 (black triangles) contains 108 cells and Set 2 (red circles) contains 96 cells.

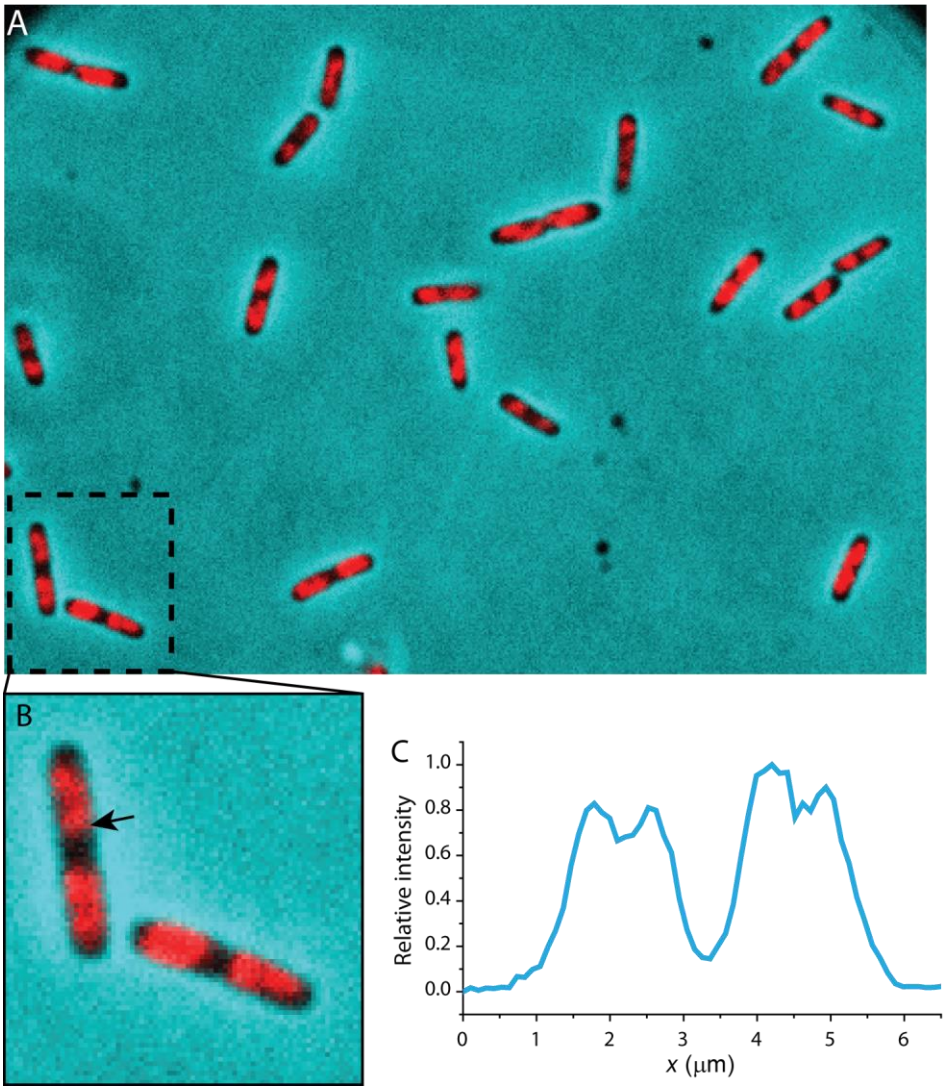

Figure S3. Widefield fluorescence images of HU-YFP distribution in live *E. coli*. HU-YFP expressed from a plasmid. (A) A field of view with 21 cells. Cell width of 1 μm sets scale. (B) Zoomed view of the dashed box in (A). (C) Axial line scan of HU-YFP signal from the cell denoted by the black arrow. The two pairs of nucleoid sub-lobes are well separated. Compare with SYTOX Orange images in Figs. 1 and 2 of main text.

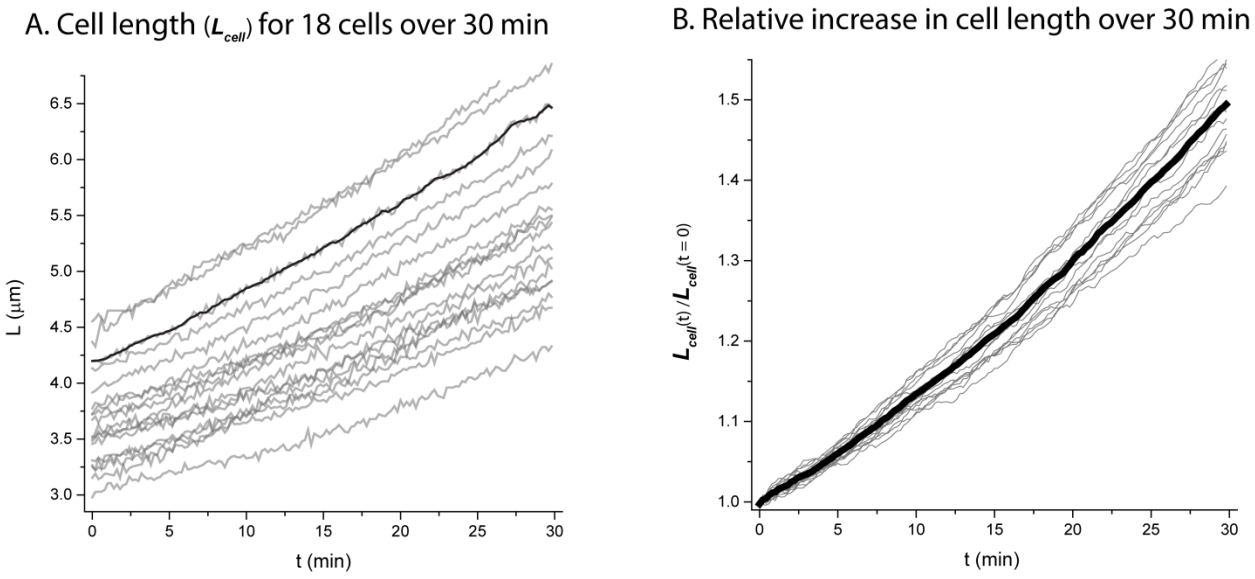

Figure S4. (A) Time course of tip-to-tip cell length L_{cell} calculated from phase contrast images by MicrobeTracker for 18 *E. coli* cells growing in the microfluidics device at 30ºC. $L_{cell}(t)$ increases roughly exponentially for each cell over 30 min. (B) Relative change in cell length vs time is calculated by smoothing the $L_{cell}(t)$ data using adjacent averaging. An example of a smooted curve is shown by the black line in (A). Each smoothed curve is then scaled to the value at $t = 0$ to yield the relative length vs time. The mean of the relative cell length curves $\langle L_{cell}(t)/ L_{cell}(t = 0)\rangle$, shown as the heavy line in panel (B), follows an exponential function with $1/e$ time constant of $\tau = 68.5$ min, which corresponds to a doubling time $t_{1/2}$ of 47.5 min. For comparison, the doubling time in bulk medium estimated from OD measurements is 45 min. See supporting information of Bakshi *et al.* (1) for details.

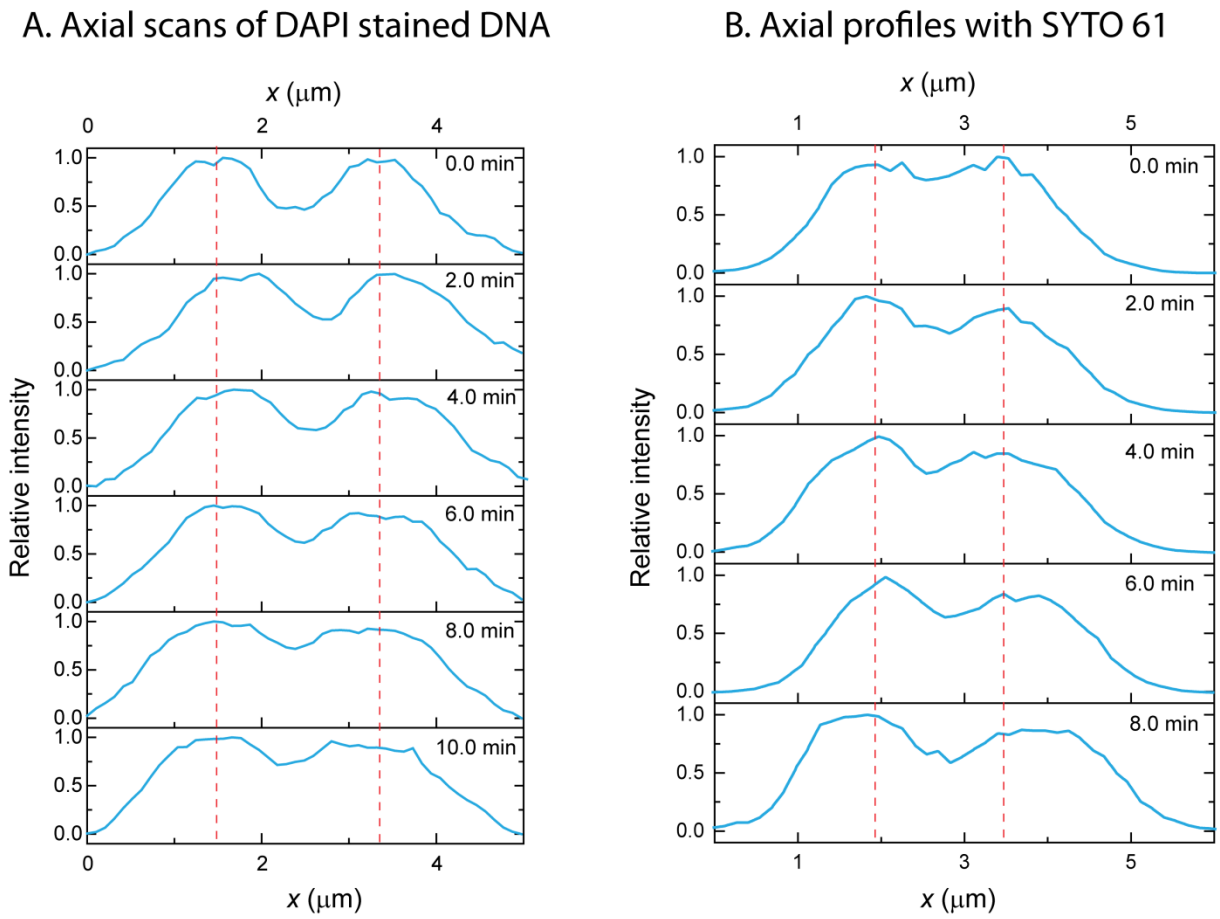

Figure S5. Time lapse imaging of DNA with DAPI and SYTO 61 staining. (A) Axial line scans of intensity distributions over time of the same DAPI-stained *E. coli* cell shown in Fig 5B. The two segregated DNA lobes merge over time. (B) Time course of axial line scans of the same SYTO 61-stained cell shown in Fig. 5C. As growth occurs, the two nucleoid lobes gradually separate, but there is less fine detail than for SYTOX Orange. Compare Figs. 1 and 2.

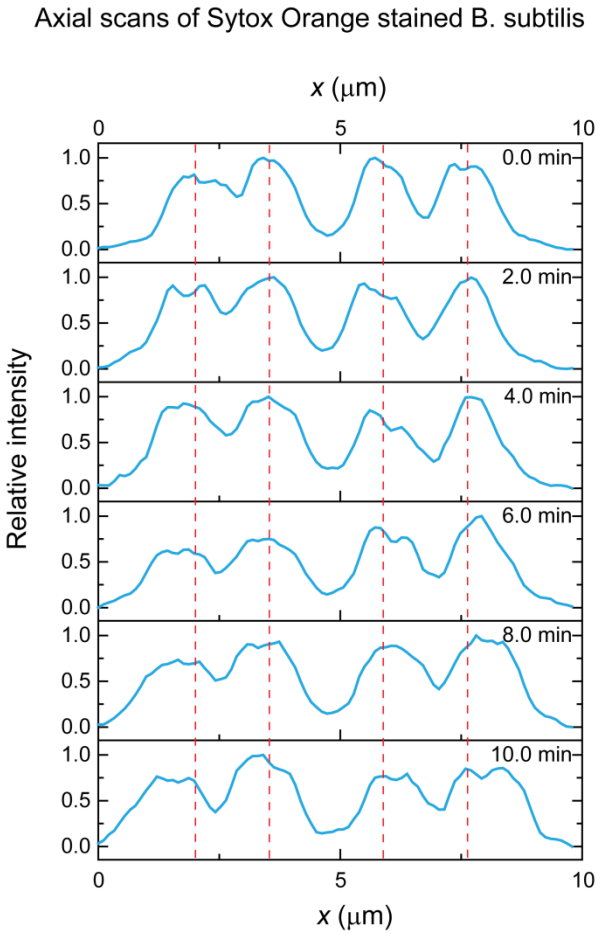

Figure S6: Axial line scans vs time of Sytox Orange-stained nucleoids of a *B. subtilis* cell.

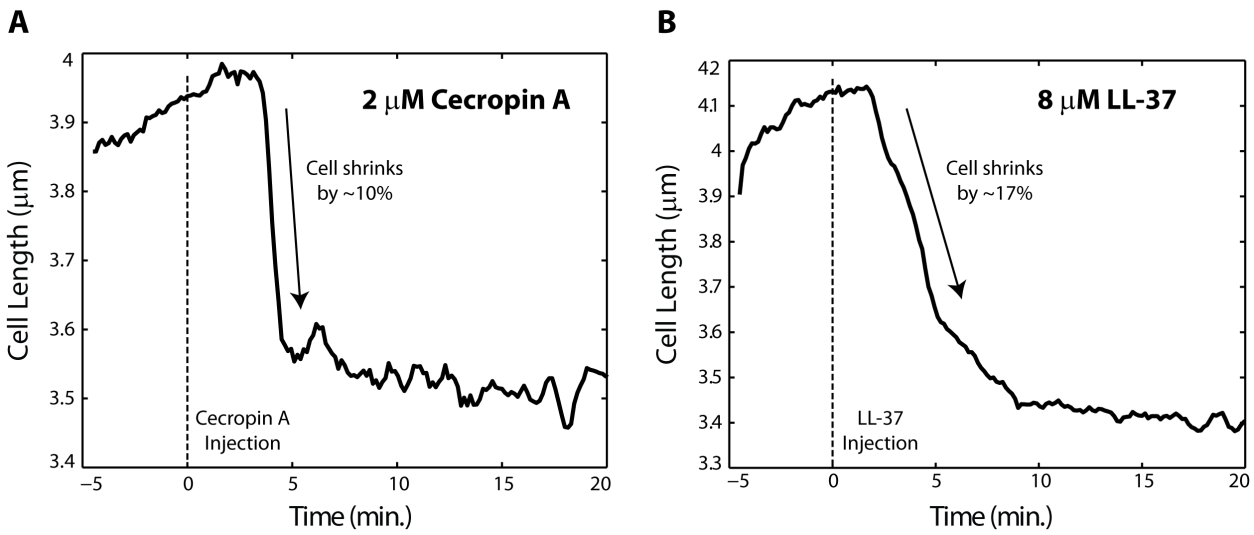

Figure S7. Plots of cell length vs time (derived from phase contrast images) for the single *E. coli* cells whose images are shown in Fig. 7 of the main text. (A) Injection of 2 μM of Cecropin A at $t = 0$ leads to abrupt cell shrinkage and halting of growth at $t = 4$ min. (B) Injection of 8 μM of LL-37 causes more gradual shrinkage beginning at $t = 2$ min.

Supplemental Movies

All movies include time stamps to mark the progression of time.

Supplemental Movie 1: Movie of growth control experiments (control experiment type 1) of SYTOX Orange-labeled cells. All the cells are labeled with SYTOX Orange. Only the cells in the top half of the field of view are illuminated with 561 nm laser. The laser is turned on 10 min after the movie starts. Emission from 561 nm illumination (red channel) is overlaid on phase contrast images of the cells (cyan channel).

Supplemental Movie 2: Movie of growth control experiments for SYTOX Orange imaging (control experiment type 2). Cells labeled with SYTOX Orange were mixed with unlabeled cells and phase contrast imaging is used to measure cell growth. Emission from 561 nm illumination (red channel) is overlaid on phase contrast images of the cells (cyan channel).

Supplemental Movie 3: Effect of DRAQ5 on cell growth and nucleoid morphology. This cells was pre-incubated with DRAQ5. Laser illumination at 633 nm begins at 5 min.

Supplemental Movie 4: Expansion of nucleoid due to UV imaging of DAPI labeled *E. coli* cells.

Supplemental Movie 5: Time lapse movie of growth of Sytox Orange-stained *B. subtilis* cells. The laser is turned on 10 min after the movie starts. Emission from 561 nm illumination (red channel) is overlaid on phase contrast images of the cells (cyan channel).

1. **Bakshi S, Siryaporn A, Goulian M, Weisshaar JC.** 2012. Superresolution imaging of ribosomes and RNA polymerase in live Escherichia coli cells. Molecular Microbiology **85:**21-38.