Supplemental Information

Cell Boundary Confinement Sets the Size and Position of the *E. coli* Chromosome

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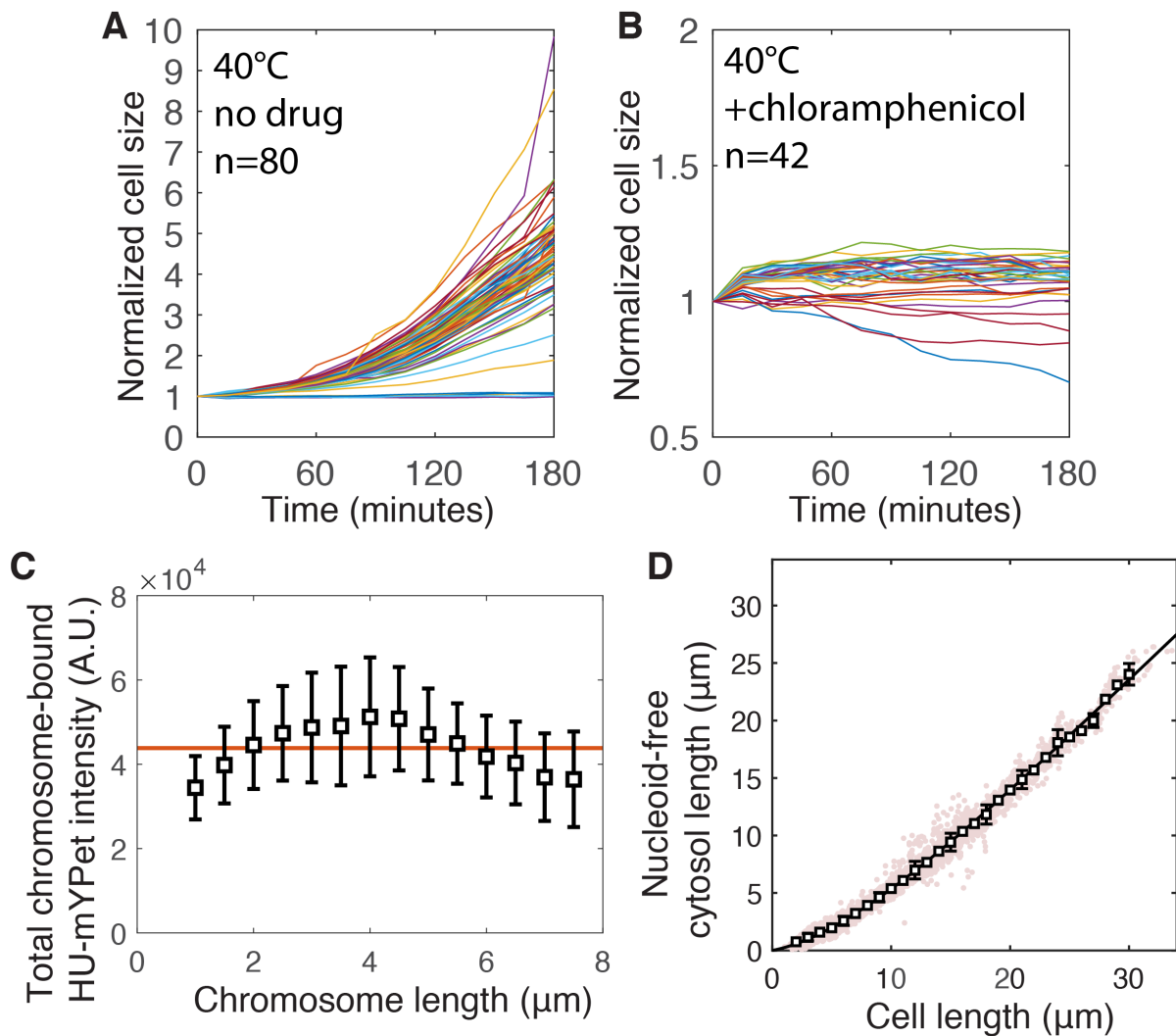
Figure S1. Cell growth with a single nucleoid requires protein synthesis and maintains a nucleoid-bound HU-mYPet level. Related to Figure 1.

A and B. Cell area measurement of *dnaC2(ts)* allel growing at non-permissive temperature without and with chloramphenicol treatment

C. Total chromosome-bound HU-mYPet intensity in *dnaC2(ts)* cells growing into different sizes.

D. Cell length unoccupied by the single nucleoids in cylindrical *dnaC2(ts)* cells growing into different sizes. Error bars represent standard deviations.
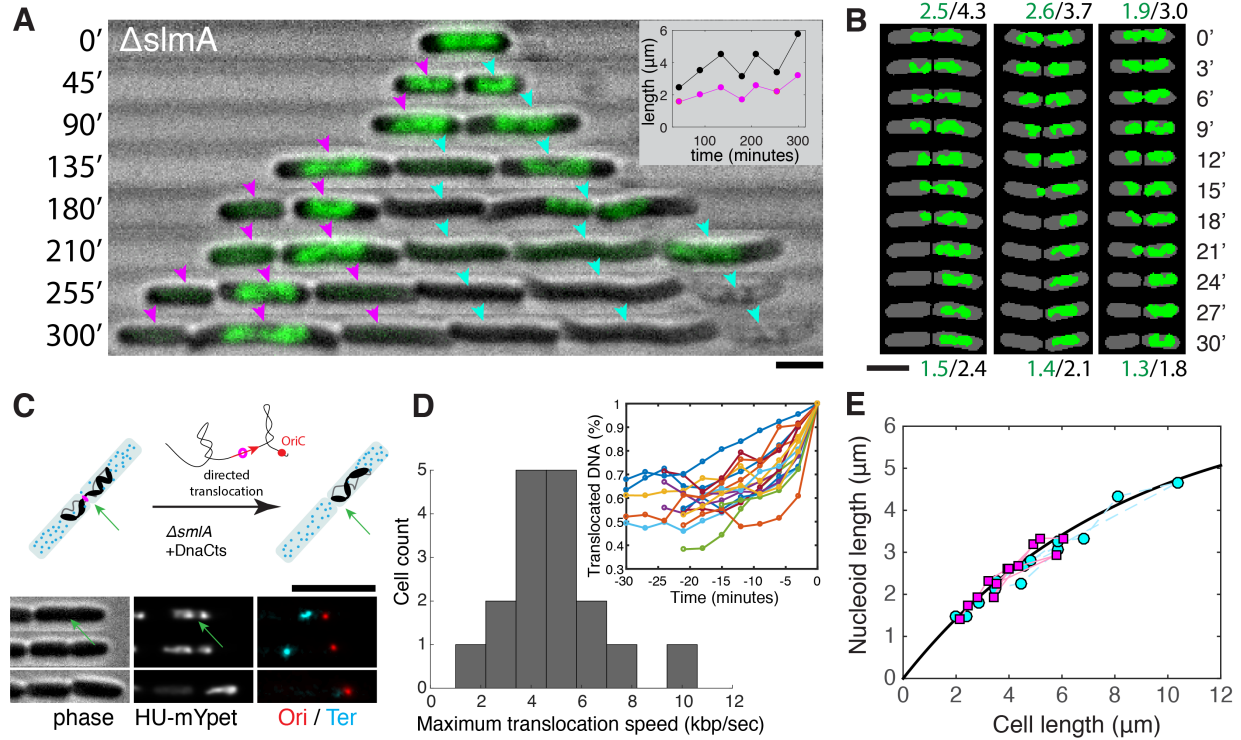
Figure S2. Cell division and chromosome contraction of *ΔslmA/dnaC2* mutant. Related to Figure 2.
A, time-lapse images nucleoid dynamics during the growth and division of *ΔslmA/dnaC2* cells growing at non-permissive temperature. Phase contrast in grey scale, and HU-mYPet in green. Time is labeled in minutes. Magenta and cyan arrows traces two cell lineages. Scale bars, 2 μm. Inset, cell size (black) and nucleoid size (magenta) change over time measured from the *nucleoid-containing* cell lineage at the left.
B, three examples of chromosome translocation after cell constriction and prior to septation. Grey indicates automatically identified cell shape, and green indicates automatically identified nucleoid.
C, illustration and time-lapse images showing that the chromosome translocation is oriented towards the cell half with the Ori focus.
D, histogram showing the maximum DNA translocation speed estimated from the time-lapse fluorescent images. Inset, progression of DNA translocation in single cells over time.
E, nucleoid/cell length relation in the two nucleoid-containing lineages indicated in A measured over two cell division events. Time interval is 15 minutes. Each had 13 time points. The black smooth line shows a section of the nucleoid-boundary response curve shown in Figure 1C.
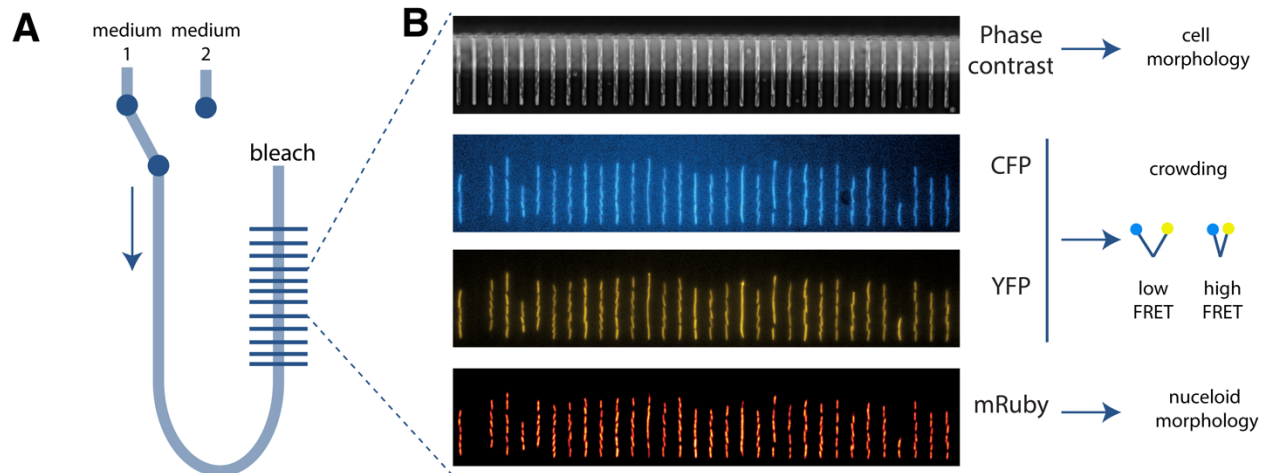
Figure S3. Mother machine setup, experimental approach, and observables in the hyperosmotic shock experiment. Related to Figure 2.

A. Schematic of the "mother machine" PDMS device used in this study. The fluidic switch valve at the inlet is used to rapidly exchange the medium to apply osmotic shocks and other perturbations to the cellular physiology.

B. Representative images of one field of view within the mother machine device, in the four imaging channels used for parallel monitoring of three observables: (i) phase contrast channel for cell morphology, (ii) FRET donor (CFP) and acceptor (YFP) fluorescence channels for intracellular crowding, and (iii) red (mRuby2) fluorescence channel for the nucleoid morphology.
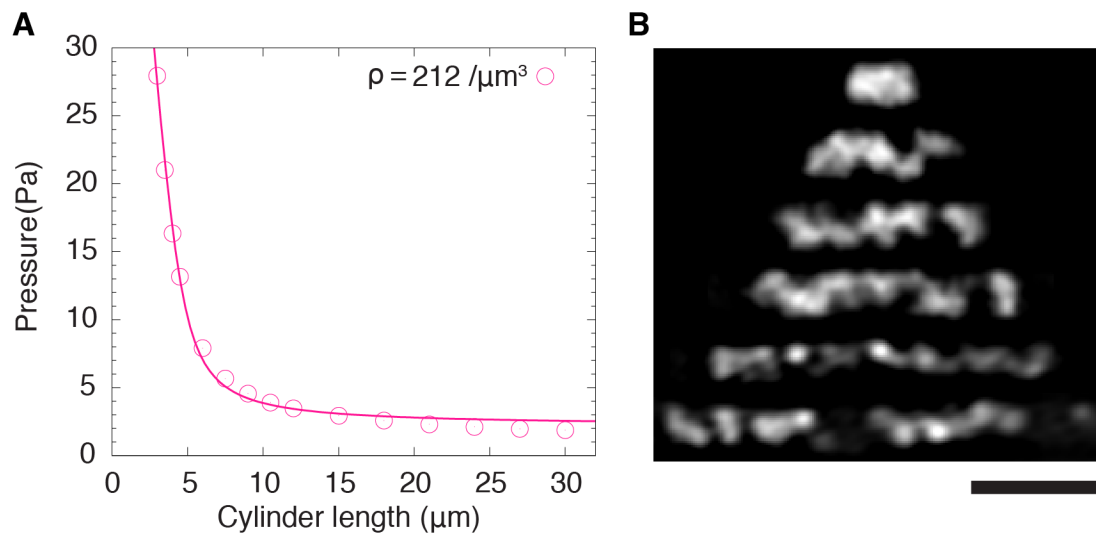
Figure S4. Comparisons between analytical, numerical, and experimental data. Related to Figure 3.
A. Pressure estimated from the numerical simulations with crowders (circle) and the piston model (solid line).
B. Structured Illumination Microscopy images of nucleoids of different lengths at their central focal planes.
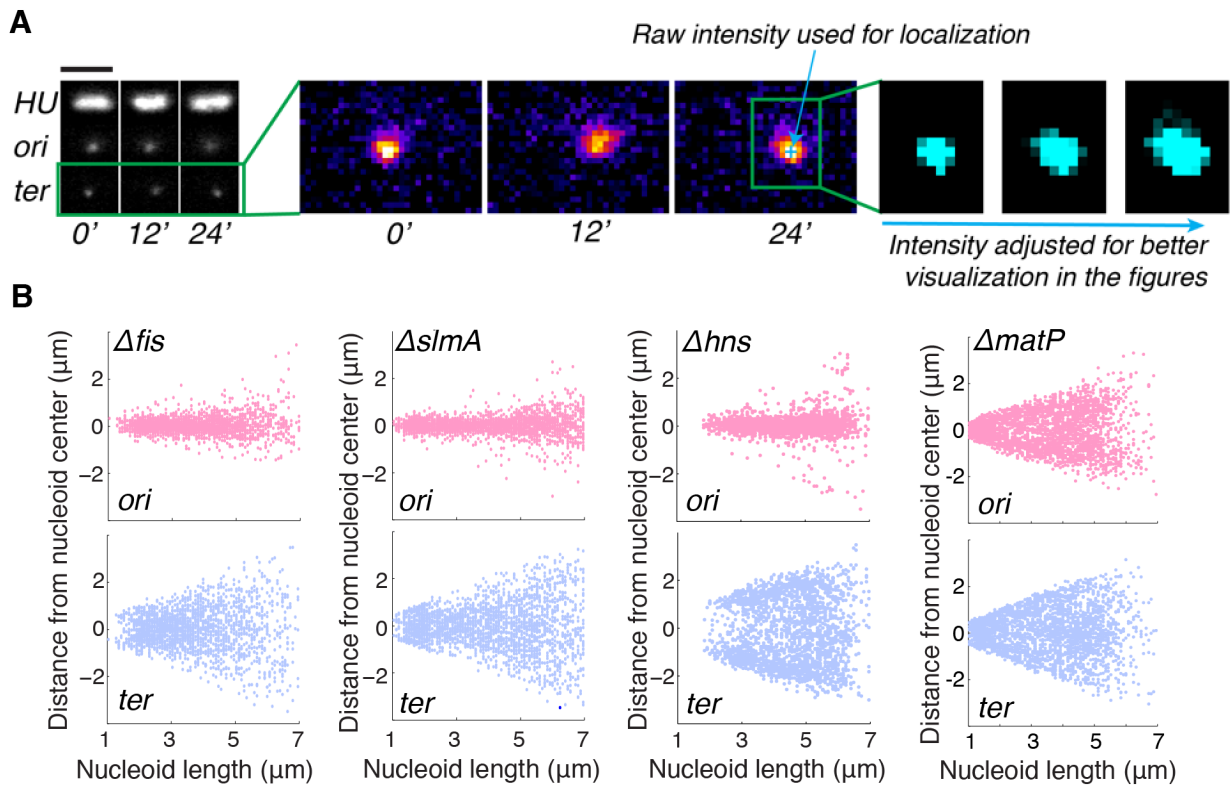Scale bar, 2 μm.

Figure S5. Ori/Ter foci positioning inside nucleoids of different lengths in different strains. Related to Figure 5.

A, examples of the grayscale images showing the localizations and morphologies of the diffraction-limited fluorescence foci in the *NAPs+* strain (Left, scale bar 2 μm). We automated the thresholding of the images for convenience of visualization. As shown in the right, the morphology of the foci displayed in the figures depend on the threshold.

B, Ori/Ter foci positioning in NAP mutants. Each panel displays the distances of Ori / Ter loci from the center of nucleoids as a function of the nucleoid length.
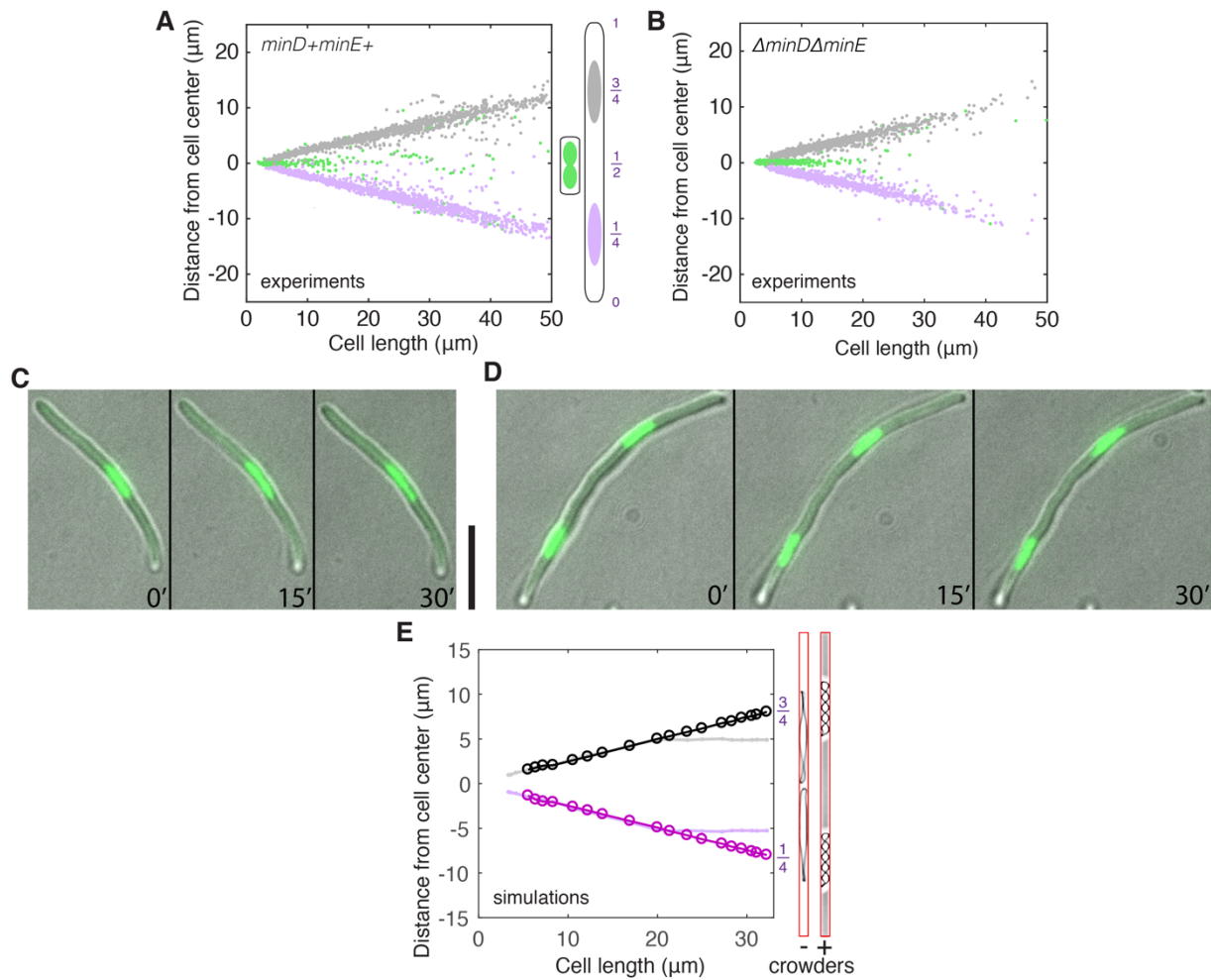
Figure S6. Sister chromosome positioning is not affected by abolishing transertion or Min proteins. Related to Figure 6.

A and B, distances of two sister chromosomes from the cell center in different cell lengths in *minDE+* and *minDE-* cells (n = 3626). Green data points represent sister chromosomes that are still connected. Grey and purple data points indicate right and left chromosomes respectively.

C and D, time-lapse images of single- or double-nucleoid cells treated by a combination of 34 μg/ml chloramphenicol and 100 μg/ml rifampicin, which were added into the agarose pad. Time 0' is 10 minutes after inoculation onto the cover glass. Scale bar, 5 μm.

E, distances of two sister chromosomes from cell center in different cell lengths obtained through simulations with (bright circles) and without (light lines) depletants.