Supplementary Material for "Smectic-like bundle formation of planktonic bacteria upon nutrient starvation"

Takuro Shimaya,[1] Fumiaki Yokoyama,[1] and Kazumasa A. Takeuchi[1,2]

[1] *Department of Physics, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[2] *Institute for Physics of Intelligence, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*

SUPPLEMENTARY FIGURES

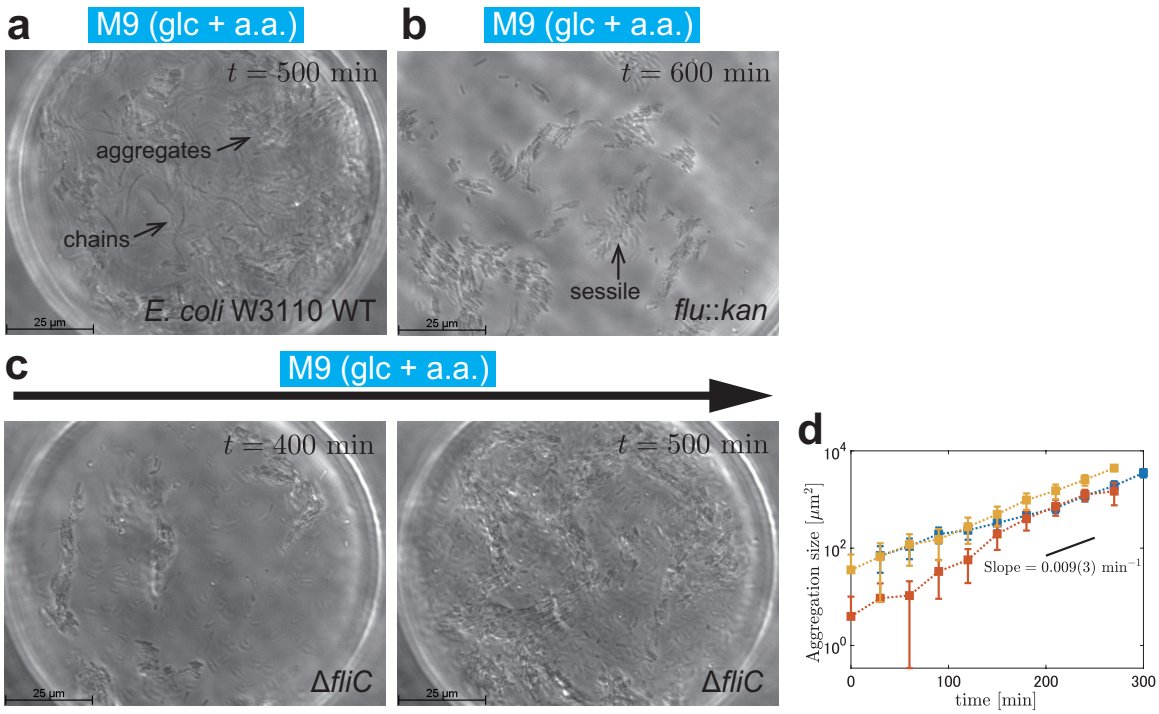

FIG. S1. Aggregation processes of planktonic *E. coli* cells in steady growth environments. (a) Populations of the wild-type W3110 (WT) in a well (diameter $\phi = 110\,\mu$m) cultured with M9(glc+a.a.) for 500 min since the observation was started. Initially there were 5 cells in the well. The appearance of chains and aggregates was confirmed in all 30 wells of various diameters recorded in a single experiment (WT growth in Table II). See also Movie 1. (b) Aggregates of the *flu*::*kan* mutant (without Ag43 expression), in a well ($\phi = 150\,\mu$m) cultured with M9(glc+a.a.) for 600 min since the observation was started. Initially there were 10 isolated cells in the well. No chain formation was observed in 23 out of 28 wells with various diameters recorded in a single experiment (*flu*::*kan* growth in Table II). In the other 5 wells, chain formation was observed, which may be due to flagella as mentioned below. In this image, cells adhering to the substrate (slightly out of the focus) can be seen. See also Movie 2. (c) An aggregation process of the Δ*fliC* mutant (without flagella) in a well ($\phi = 110\,\mu$m) cultured with M9(glc+a.a.). $t = 0$ is the time at which the observation was started. Initially there were 8 cells in the well. No chain formation was observed in 28 out of 30 wells with various diameters recorded in a single experiment (Δ*fliC* growth in Table II). In the other 2 wells, chain formation was observed. This suggests that both flagella and Ag43 are important for chain formation, though they can sometimes complement each other. While the adhesion of cells to the substrate was found in 27 out of 30 wells for the WT and in 23 out of 28 wells for the *flu*::*kan* mutant, this happened only in 8 out of 30 wells for the Δ*fliC* mutant. See also Movie 3. (d) Time evolution of the aggregate size of the Δ*fliC* mutant. Each time series represents the data in a separate well ($\phi = 110\,\mu$m). The error was estimated to be the perimeter × 5 pixels ($\sim 0.85\,\mu$m), which we considered to be the range of uncertainty in manual detection of the edge of the regions. From the slope of the semi-log plot, we evaluated the growth rate at $0.009(3)\,\mathrm{min}^{-1}$.

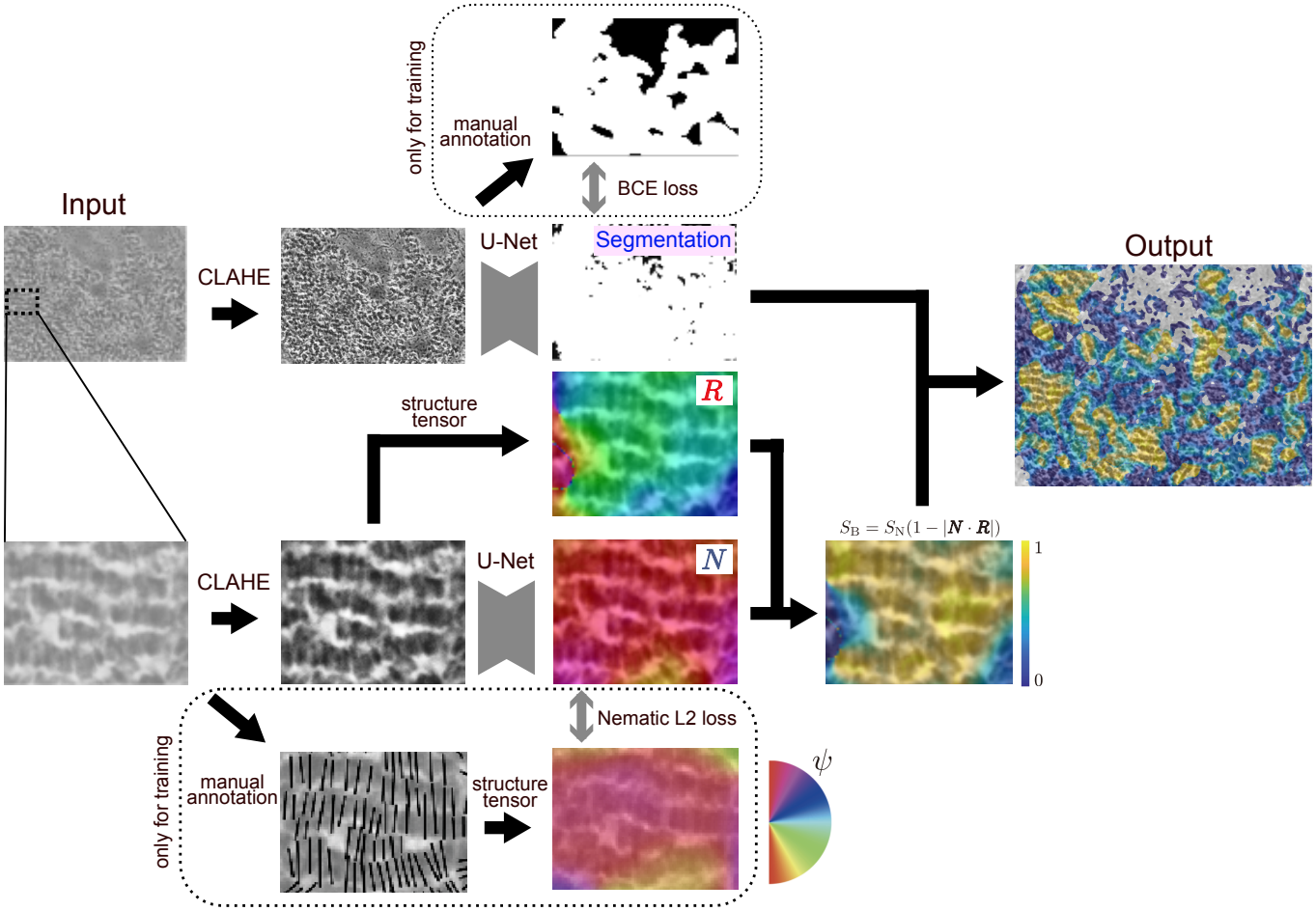

FIG. S2. The entire pipeline of the image analysis. A deep neural network, namely the U-Net, was employed in two places, specifically for the segmentation of the regions with planktonic aggregates and for the estimation of the cell orientation $\boldsymbol{N}$. Note that entire images were given as the input of the U-Net for estimating $\boldsymbol{N}$ while the figure shows a small part of the images for visibility. See also Materials and Methods in the main text for details.

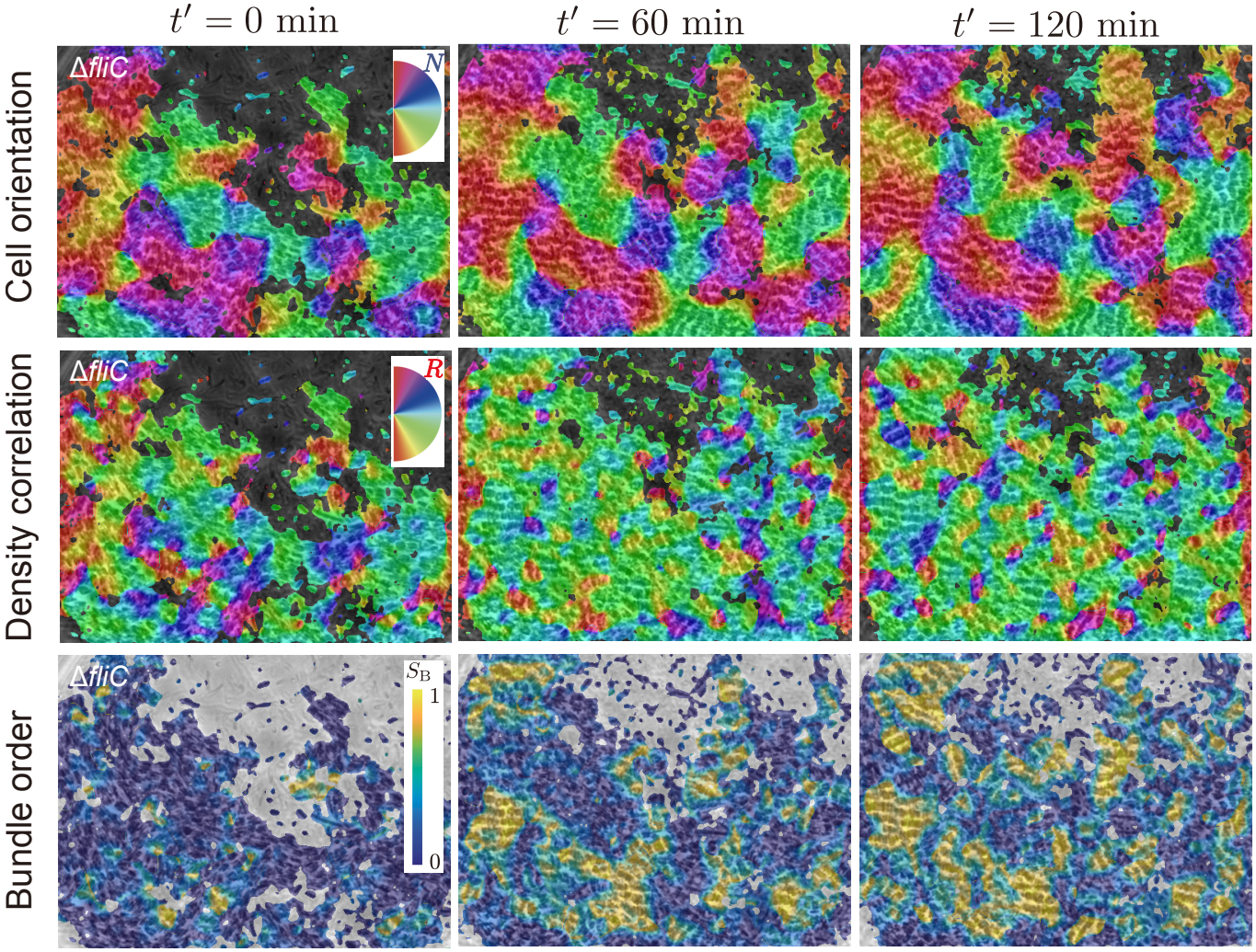

FIG. S3. Cell orientation $\boldsymbol{N}$, density correlation director $\boldsymbol{R}$, and the bundle order parameter S_B, overlaid on the microscope images. These are the results for the Δ*fliC* mutant grown in M9(glc+a.a.) and starved in PBS (Δ*fliC* starvation in Table II). $\boldsymbol{N}$ and $\boldsymbol{R}$ are perpendicular if bundles are formed. The spatially-averaged bundle order parameter is shown in Fig. 3d by the purple curve. See also Movies 5 and 6.

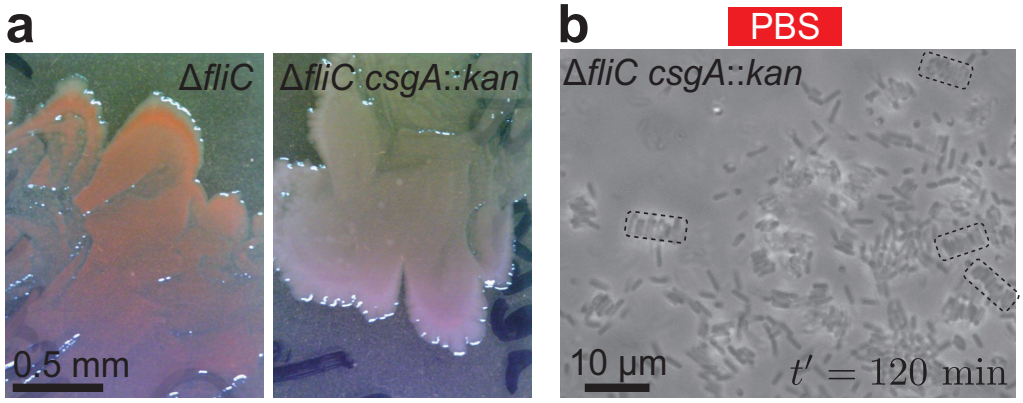

FIG. S4. Investigation of the relevance of curli to the bundle formation. (a) Confirmation of the loss of curli in the Δ*fliC csgA*::*kan* mutant, by congo red. This dye selectively stains amyloid fibers including curli, as confirmed for the Δ*fliC* strain (left). No signal was detected for the Δ*fliC csgA*::*kan* strain (right). (b) Starvation response of the Δ*fliC csgA*::*kan* mutant grown in M9(glc+a.a.) and starved in PBS. Bundle clusters are indicated by the dashed rectangles. The well diameter ϕ is 230 μm. $t' = 0$ is the time at which the environment was switched. The bundle formation was observed in 15 out of 19 wells with various diameters recorded in a single experiment (Δ*fliC csgA*::*kan* starvation in Table II). See also Movie 11.

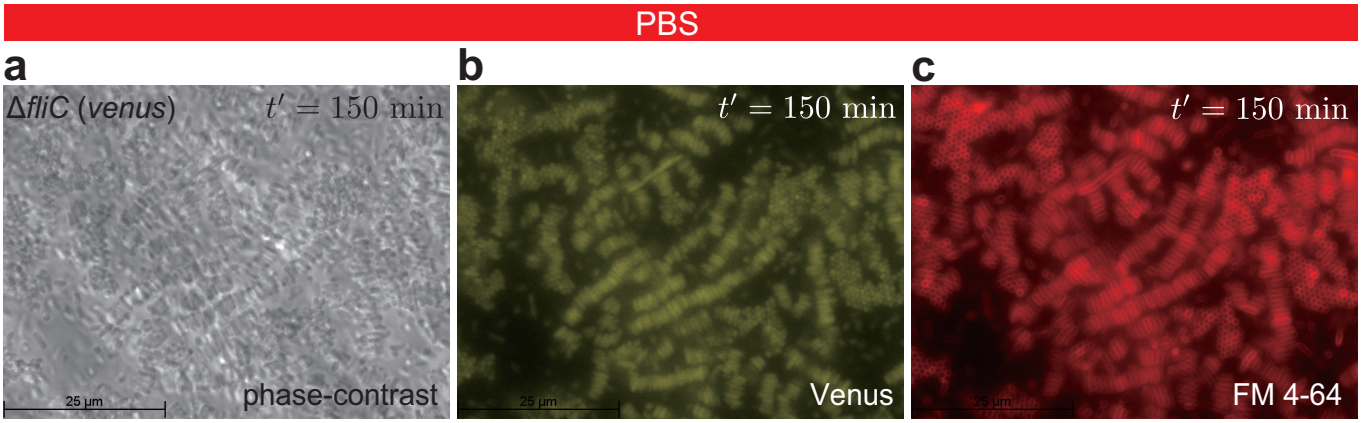

FIG. S5. Multi-channel observation of the bundle formation of the Δ*fliC* (*venus*) mutant, 150 min after the environment was switched from M9(glc+a.a.) to PBS. (a) Phase-contrast image. (b) Venus fluorescence image, which visualizes the entire cell bodies. (c) FM 4-64 fluorescence image, which visualizes the cell membranes and OMVs.

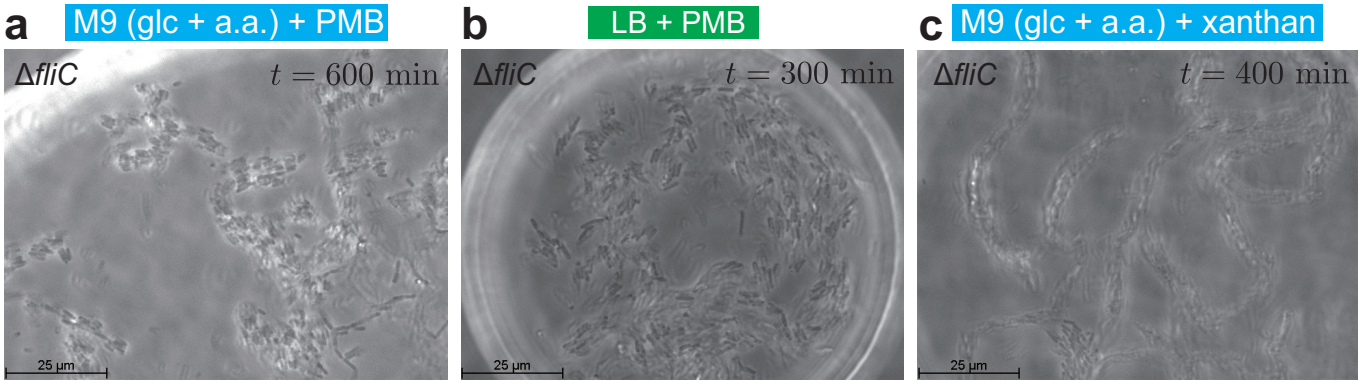

FIG. S6. Steady growth measurements intended to test the relevance of depletion attraction. $t = 0$ is the time at which the observation was started. (a) Observation of the Δ*fliC* mutant grown in M9(glc+a.a.) with 250 ng/ml polymyxin B. The well diameter ϕ is 230 μm. Bundle clusters were not observed in all 6 wells with various diameters recorded in a single experiment (Δ*fliC* starvation (PMB) in Table II). See also Movie 12. (b) Observation of the Δ*fliC* mutant grown in LB with 250 ng/ml polymyxin B. The well diameter ϕ is 110 μm. Bundle clusters were not observed in all 15 wells with various diameters recorded in a single experiment (Δ*fliC* starvation (LB, PMB) in Table II). See also Movie 13. (c) Observation of the Δ*fliC* mutant grown in M9(glc+a.a.) with 0.03 wt% xanthan gum. The well diameter ϕ is 150 μm. Bundle clusters were not observed in all 6 wells with various diameters recorded in a single experiment (Δ*fliC* starvation (xanthan) in Table II). See also Movie 14.

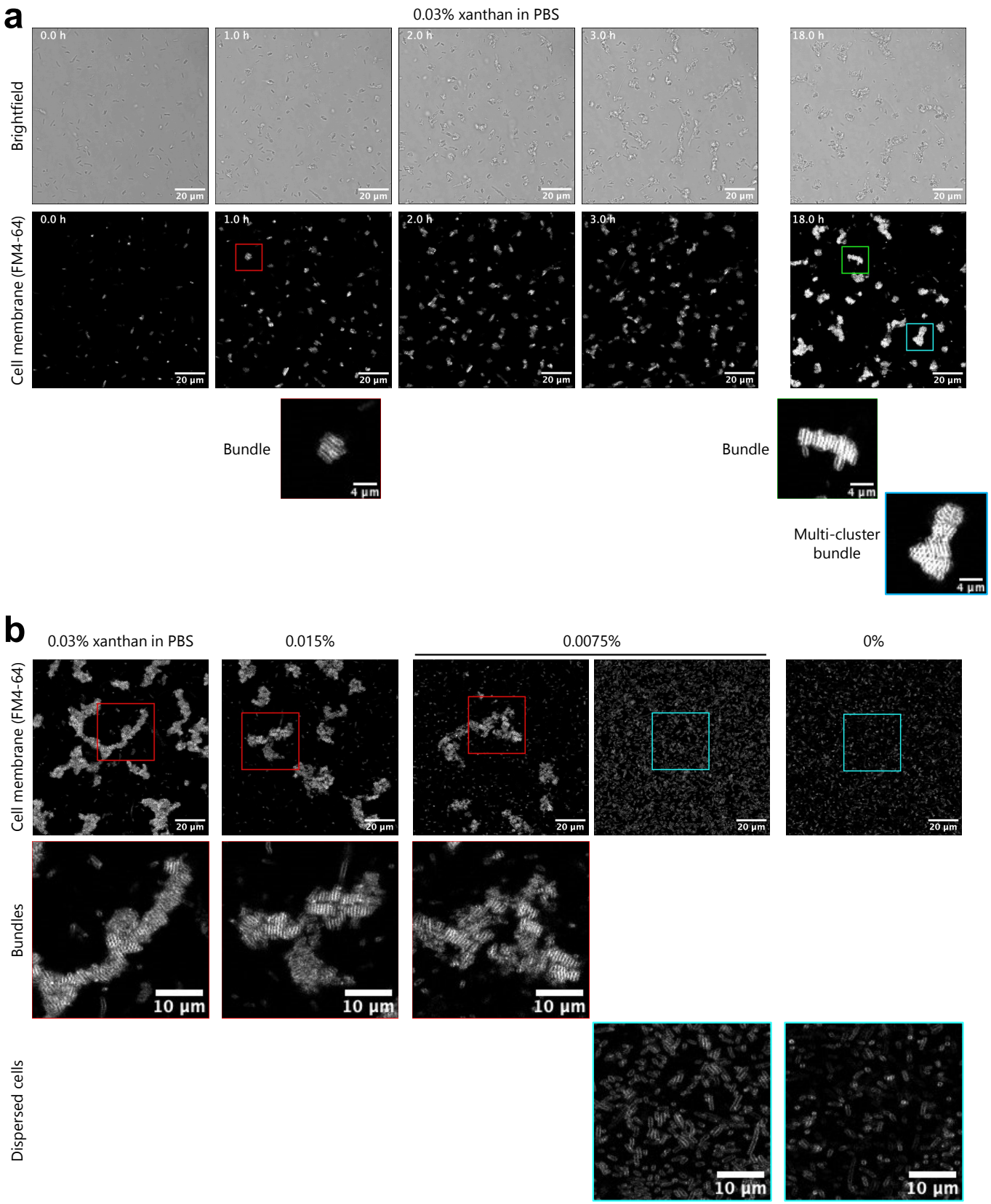

FIG. S7. Observations of aggregates with externally added polymers to investigate the relevance of the depletion effect quantitatively. (a) Time-lapse observations of the Δ*fliC* mutant, suspended in PBS with 0.03 wt% xanthan gum, 2 μM BisBAL, and 5 μg/mL FM4-64. See also Movies 16 and 17. (b) Fixed time-point observations 18 h after the incubation was started. The Δ*fliC* mutant was suspended in PBS with varying concentrations of xanthan gum (0–0.03 wt%), 2 μM BisBAL, and 5 μg/mL FM4-64. The images for 0.03 wt% xanthan gum were taken from the time-lapse observation, while the time evolution was not monitored for the other samples. See Table II (Δ*fliC* starvation-dish) for the fraction of the dishes where the bundle formation was observed.

MOVIE DESCRIPTIONS

Movie 1:
Growth of the wild-type W3110 (WT) in M9(glc.+a.a.). The diameter of the well is 110 μm. The movie is played 1200 times faster than the real speed.
Movie 2:
Growth of the *flu*::*kan* mutant in M9(glc.+a.a.). The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 3:
Growth of the Δ*fliC* mutant in M9(glc.+a.a.). The diameter of the well is 110 μm. The movie is played 1200 times faster than the real speed.
Movie 4:
Starvation response of the Δ*fliC* mutant. The environment was switched from M9(glc.+a.a.) to PBS at $t = 480$ min. The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 5:
The density correlation director $\boldsymbol{R}$ (top) and the cell orientation $\boldsymbol{N}$ (bottom) overlaid on Movie 4. The pseudo-color indicates the angle (see Fig. 3b, Fig. S2 or Fig. S3 for the definition). $\boldsymbol{N}$ and $\boldsymbol{R}$ are perpendicular in the regions where smectic-like bundles are formed.
Movie 6:
The bundle order parameter S_{B} overlaid on Movie 4. The pseudo-color indicates the value of the bundle order parameter. See Fig. 3c for the color bar.
Movie 7:
Starvation response of the Δ*fliC* mutant. The environment was switched from LB to PBS at $t = 225$ min. The diameter of the well is 110 μm. The movie is played 1200 times faster than the real speed.
Movie 8:
Starvation response of the wild-type W3110 (WT). The environment was switched from M9(glc.+a.a.) to PBS at $t = 480$ min. The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 9:
Starvation response of the Δ*fliC* mutant, grown in M9(glc.+a.a.) + 2 μM BisBAL and starved in PBS + 2 μM BisBAL. Bundles did not appear in this movie. The environment was switched at $t = 480$ min. The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 10:
Starvation response of the Δ*fliC* mutant), grown in M9(glc.+a.a.) + 2 μM BisBAL and starved in PBS + 2 μM BisBAL. Bundles appeared in this movie. The environment was switched at $t = 480$ min. The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 11:
Starvation response of the Δ*fliC csgA*::*kan* mutant. The environment was switched from M9(glc.+a.a.) to PBS at $t = 480$ min. The diameter of the well is 230 μm. The movie is played 1200 times faster than the real speed.
Movie 12:
Growth of the Δ*fliC* mutant in M9(glc+a.a.) + 250 ng/ml polymyxin B. The diameter of the well is 230 μm. The movie is played 1200 times faster than the real speed.
Movie 13:
Growth of the Δ*fliC* mutant in LB + 250 ng/ml polymyxin B. The diameter of the well is 230 μm. The movie is played 1200 times faster than the real speed.
Movie 14:
Growth of the Δ*fliC* mutant in M9(Glc+a.a.) + 0.03 wt% xanthan gum. The diameter of the well is 150 μm. The movie is played 1200 times faster than the real speed.
Movie 15:
Response against starvation and growth recovery of the Δ*fliC* mutant. The environment was switched from M9(glc.+a.a.) to PBS at $t = 480$ min, and from PBS to M9(glc.+a.a.) at $t = 660$ min. The diameter of the well is 230 μm. The movie is played 1200 times faster than the real speed.
Movie 16:
Brightfield images of starvation response of the Δ*fliC* mutant, pre-grown in M9(glc.+a.a.) + 2 μM BisBAL to suppress EPS production and transferred into a glass-bottom dish of PBS + 2 μM BisBAL, 0.03% xanthan gum as externally added EPS, and 5 μg/mL FM4-64 for membrane staining. The movie is played about 6000 times faster

than the real speed.

Movie 17:

Fluorescence images of starvation response of the Δ*fliC* mutant, pre-grown in M9(glc.+a.a.) + 2 μM BisBAL to suppress EPS production and transferred into a glass-bottom dish of PBS + 2 μM BisBAL, 0.03% xanthan gum as externally added EPS, and 5 μg/mL FM4-64 for membrane staining. The movie is played about 6000 times faster than the real speed.