SUPPORTING MATERIAL

3D-SIM Super-Resolution of FtsZ and its Membrane Tethers in *Escherichia coli* cells

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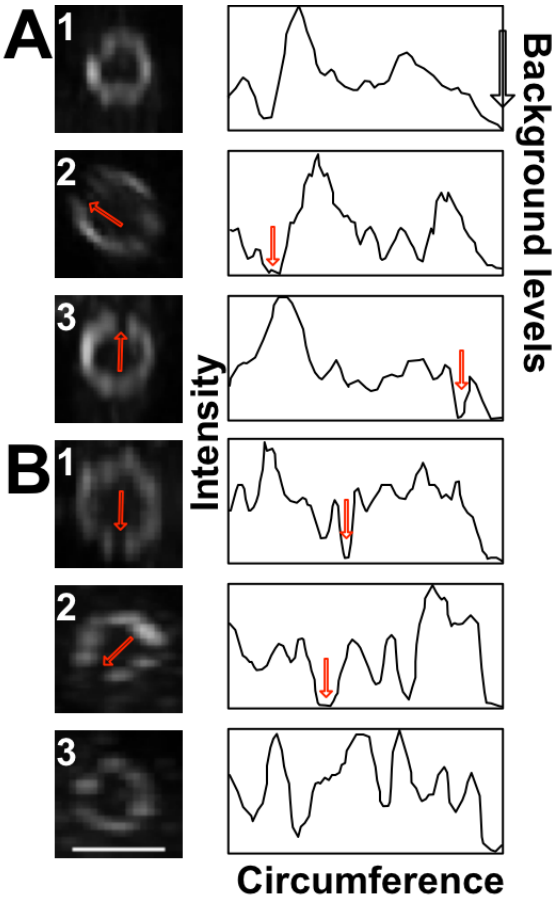

FIGURE S1 Quantitation of fluorescence intensities around the circumference of Z rings. (A) Three representative examples of FtsZ-GFP ring cross-sections and their fluorescence intensities. The plots on the right represent fluorescence intensities along a line originating at the top of the ring drawn counterclockwise around the entire circumference of the Z ring, with a final extension of the line into the lumen of the ring to define the background fluorescence intensity, shown at the far right of the plots (denoted by large arrow). Red arrows indicate areas of fluorescence intensity that are at or below background levels; areas of the ring corresponding to these areas are shown with arrows in the images on the left. (B) Three representative examples of the intensity of Z rings detected by immunofluorescence. Plots and arrows are as described above. Intensity measurements were generated using ImageJ (1) and traced in Pixelmator. Scale bar, 1 µm.

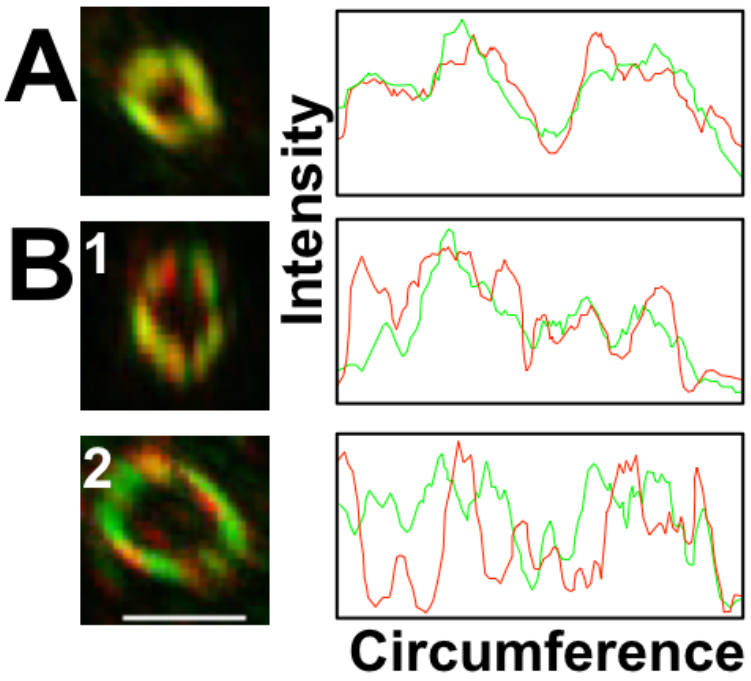

FIGURE S2 Quantitation of FtsA and ZipA colocalization. WM4769 cells producing FtsA-GFP were fixed and stained to detect ZipA localization using a red secondary antibody. (A) An example of FtsA and ZipA colocalization (another is shown in Fig. 3E-F), measured by plotting RGB intensities along a line originating at the top of the ring drawn counterclockwise around the entire circumference of the ring, with a final extension of the line into the lumen of the ring to define background intensity (far right of plot). The green line represents FtsA localization and the red line represents ZipA localization. (B) Two representative examples of colocalization patterns that have one (B1) or more (B2) significant differences in localization. RGB plots are as described above. RGB plots were generated using ImageJ (1) and traced in Pixelmator. Scale bar, 1 µm.

SUPPORTING METHODS

Preparation of samples for 3D-SIM

Cells producing FtsZ tagged with green fluorescent protein (GFP) in strain WM2026 were grown to mid-logarithmic phase, fixed using glutaraldehyde and paraformaldehyde (2), adhered to poly-lysine coated coverslips and inverted onto a drop of either ProLong Gold (Life Technologies) or Vectashield mounting medium (Vector Laboratories). Strain WM2026 harbors the native *ftsZ* gene along with a *ftsZ-gfp* gene fusion located elsewhere in the chromosome under control of an isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible *trc* promoter. To produce FtsZ-GFP as a dilute label less than the level of native FtsZ, 30 μM IPTG was added to cultures 3 h prior to fixation.

Wild-type cells (WM1074) were grown and fixed as described above, then treated with lysozyme followed by washes and incubation with affinity-purified anti-FtsZ as described (2), with the following modifications. Fixed cells were adhered to poly-lysine coated coverslips instead of a 15-well slide. The coverslips were washed by immersion in 1X phosphate-buffered saline (PBS) followed by soaking for 10 min in 1X PBS in a small container. Blocking buffer and diluted antibodies were spotted onto parafilm and coverslips were inverted onto the spots.

SUPPORTING REFERENCES

1. Schneider, C.A., W.S. Rasband, and K.W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Meth*. 9: 671-675.
2. Levin, P.A. 2002. Light microscopy techniques for bacterial cell biology. *In* Methods in Microbiology, Vol. 31: *Molecular Cellular Microbiology*. P. Sansonetti and A. Zychlinsky, editors. Academic Press Ltd., London, United Kingdom. 115-132.