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Supporting Information

Protein Patterns and Oscillations on Lipid Monolayers and in Microdroplets

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Supplementary information

Methods

Proteins: MinC**,** MinD, MinE, eGFP-MinC and eGFP-MinD were purified as His-tagged proteins following a protocol described previously[1]. MinE was fluorescently labeled with ATTO655 maleimide (ATTO-TEC, Siegen Germany), following the manual of the manufacturer. FtsZ-mts is an FtsZ fusion in which the amino acids 1-366 of *E. coli* FtsZ are fused to YFP-Venus and an amphipathic helix. Its purification has been described previously.[2]

Droplet assay: 15% Cardiolipin and 85% DOPC in Chloroform (Avanti Polar Lipids, Alabaster, AL, USA) are mixed in a glass vial. The lipids are dried under a gentle nitrogen stream and subsequently mixed with mineral oil (Sigma Aldrich, St. Louis, MO) at a concentration of 2.5mg/ml. 100µl of the oil/lipid mixture are pipetted in a plastic tube and 5ul of a protein/buffer master mix is added. The master mix comprised defined concentrations of Min proteins in buffer (25 mM Tris-HCl, pH 7.5, 150 mM KCl, 5 mM $MgCl_2$). The buffer was supplemented with ATP, when Min proteins were reconstituted and with GTP when FtsZ-mts was reconstituted. For the reconstitution of Min patterns the following concentrations were used: 1µM MinD supplemented with 10% eGFP-MinD and 1µM MinE. For the reconstitution of FtsZ-mts networks: 0.5µM FtsZ-mts. For the co-reconstitution of Min proteins and FtsZ-mts the same concentration of MinD, MinE and FtsZ were used and supplemented with MinC at 0.1 µM. The aqueous buffer does not mix with the oil phase and thus, the 5ul buffer/protein droplet sinks to the bottom of the plastic tube. To break the 5ul-droplet into smaller droplets the droplet-oil solution was manually pipetted up-and-down several times.

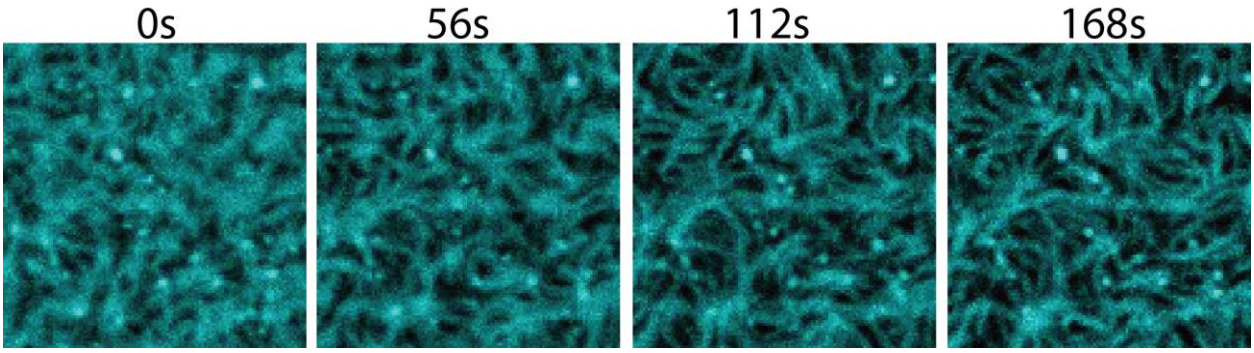
To prevent rupture of droplets during imaging, a hydrophobic support surface was generated by coating a glass cover slip with polydimethylsiloxane (Sylgard 184 Silicone Elastomere Kit, Dow Corning, Midland, MI). The droplet-oil mixture was pipetted on the polydimethylsiloxane-coated cover slip and imaged with an inverted confocal microscope (LSM 780, Zeiss, Germany).

Lipid monolayers: Lipid Monolayers were generated with *E. coli* polar lipids (Avanti Polar Lipids, Alabaster, AL). The assay for generating monolayers has been described previously.[3] In short, a buffer sample within an open reservoir on top of a glass cover slip is covered with lipid monolayer at the air/buffer interface. To do this, lipids dissolved in chloroform are carefully pipetted on the surface of the buffer. The lipids self-assemble into a monolayer and the chloroform evaporates. Subsequently the buffer volume is reduced by pipetting to lower the monolayer to the working distance of the objective. The glass surface of the cover slip was either with BSA or a supported lipid membrane before generating the monolayer. For BSA passivation the surface of the sample chambers was incubated for 30 min with 1mg/ml BSA, then rinsed with water and subsequently dried with nitrogen.

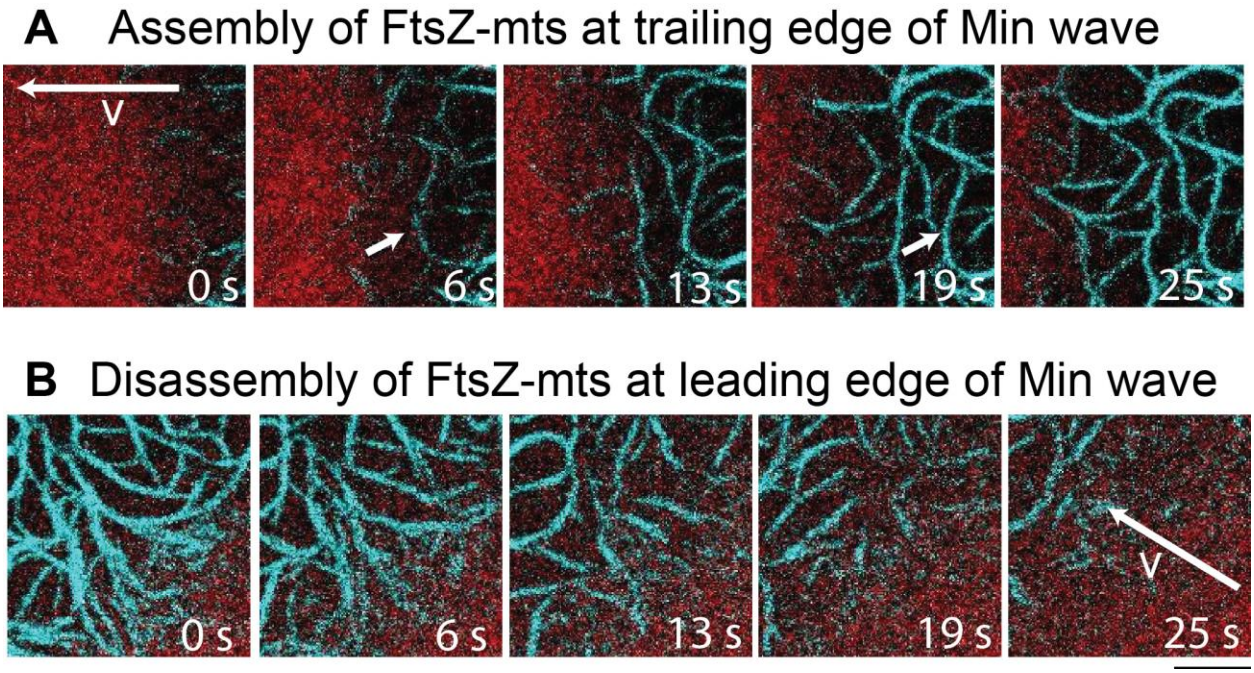
Images: Microscopic data were acquired using a confocal microscope (Confocor 2 and LSM780, Zeiss, Germany), equipped with the following objectives: C-Apochromat 40x/1.20 (water) or 20x/ 0.75 (air)(Zeiss, Germany)

Drawings in Figure 1A and 2A,B were prepared using Blender 3D software version 2.73.

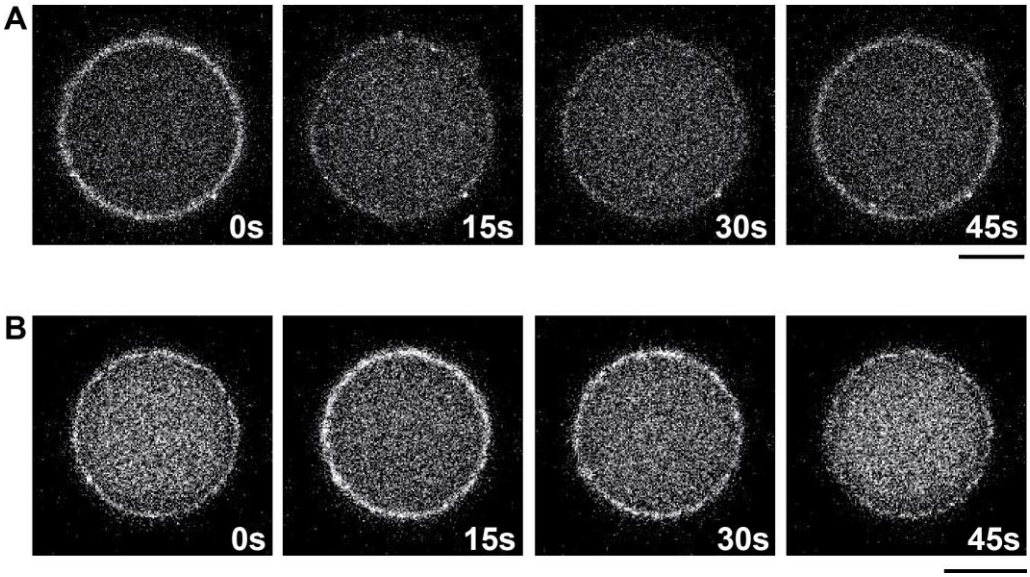
Supplementary figures



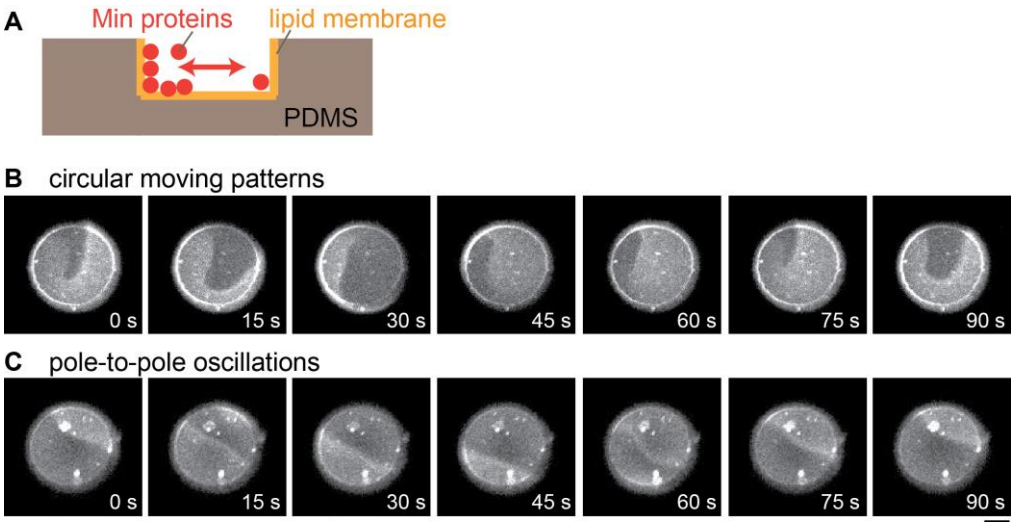
Supplementary figure 1. Network formation of FtsZ-mts on supported lipid membrane. Each frame has a size of 10 µm*10 µm



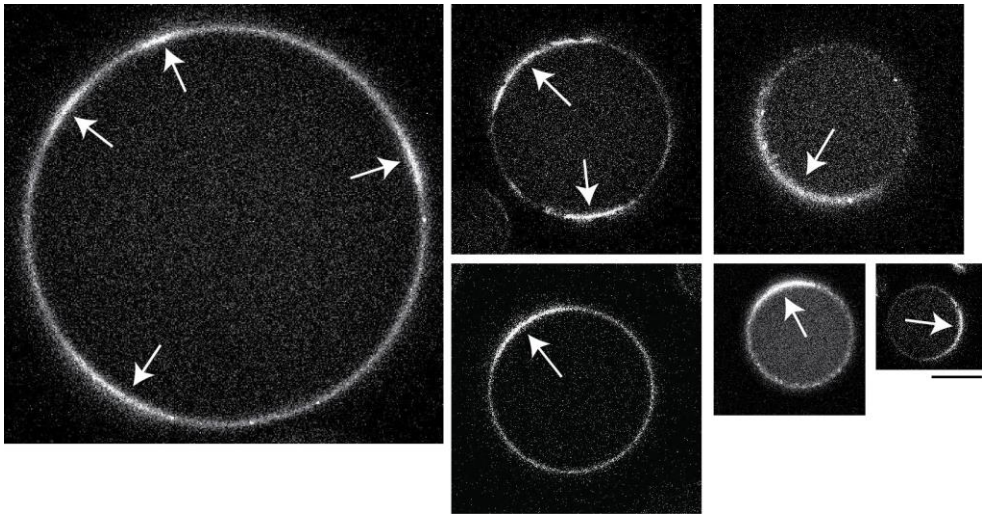
Supplementary figure 2. Co-reconstitution of Min protein waves and FtsZ-mts networks on lipid monolayers. Time-lapse confocal images of the dynamic Min-mediated (red) redistribution of the FtsZ-mts (blue) network were acquired. At the trailing (A) and leading (B) edges of Min protein waves, FtsZ bundles appeared fainter and more fragmented. A) Short, white arrows point to an FtsZ bundle with increasing intensity over time. (A, B) White, long arrows point in the travelling direction of the Min wave. Scale bar: 15 µm.



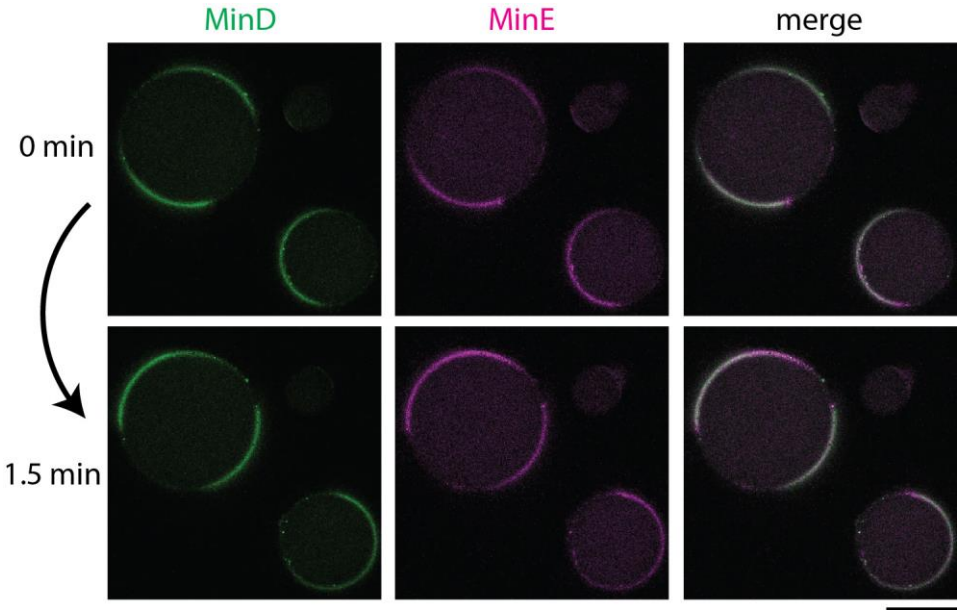
Supplementary figure 3. Pulsing Min protein patterns. A,B) Two examples of pulsing Min protein patterns in droplets. At the onset of self-organization Min protein concentrations at the droplet boundary oscillate in time. This temporal oscillation is not always coupled to a spatial inhomogeneous distribution of Min proteins at the droplet boundary. Scale bar: 20 µm.



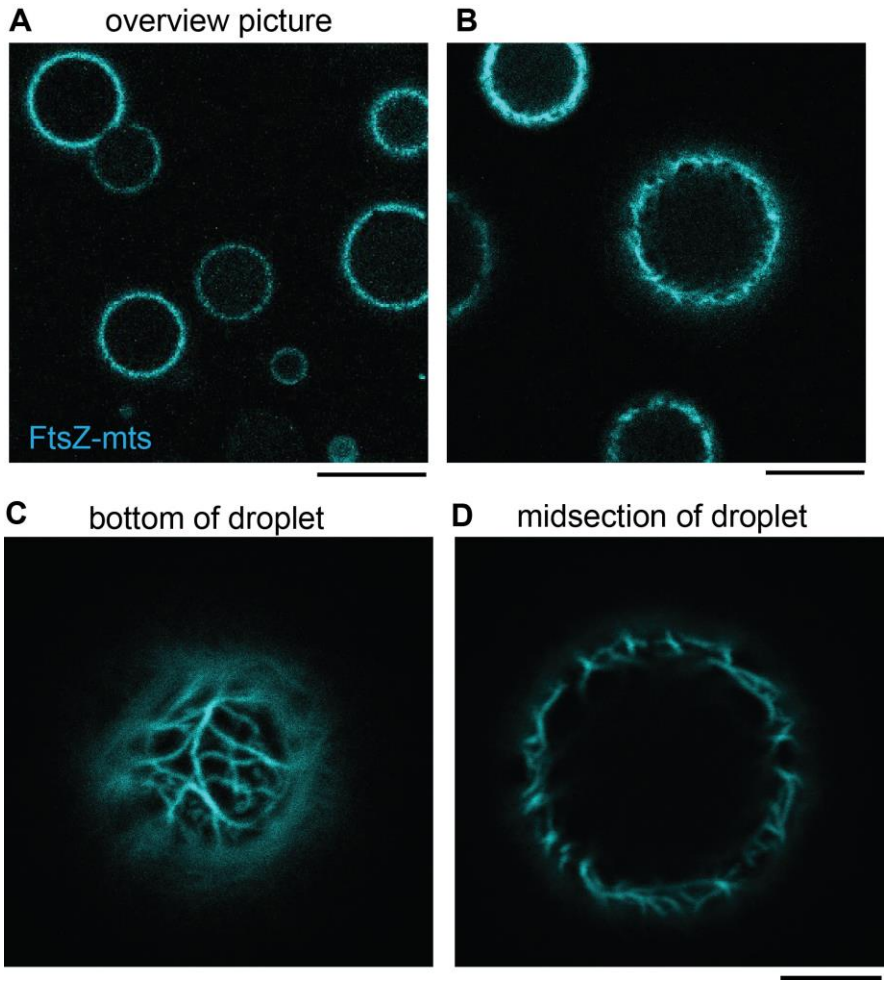
Supplementary figure 4. Min proteins in round microstructures. A) Reconstitution of Min proteins in microengineered membrane-clad compartments, an assay which we reported previously,[4] allows us to study pattern formation in compartments of defined shape. In this assay travelling Min protein patterns are pre-formed in the presence of a large buffer reservoir on top of the compartments. Subsequently the buffer amount is reduced to within the compartments which "forces" the protein patterns to adjust the patterns to the corresponding compartment geometry. B) In cylindrical compartments of identical size Min proteins self-organized into distinct oscillating or circular moving patterns. No homogeneous pulsing patterns have been observed. Scale bar: 10 µm



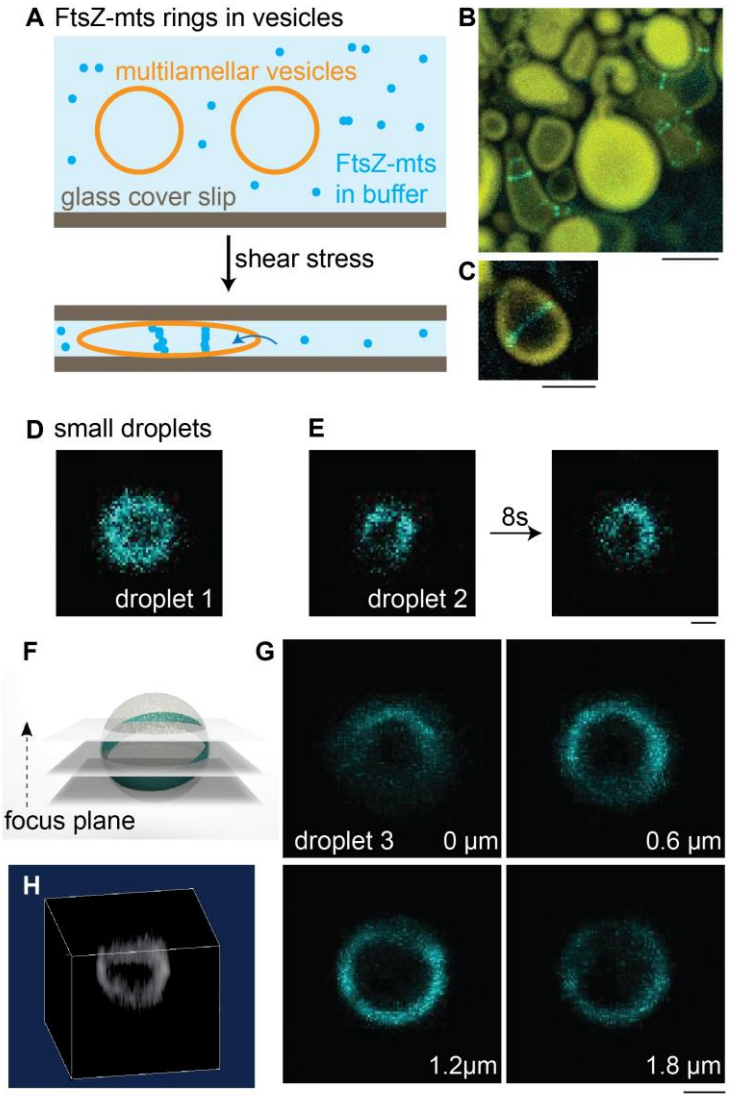
Supplementary figure 5. Large droplets have multiple polarization zones compared to smaller droplets. Scale bar: 20 µm



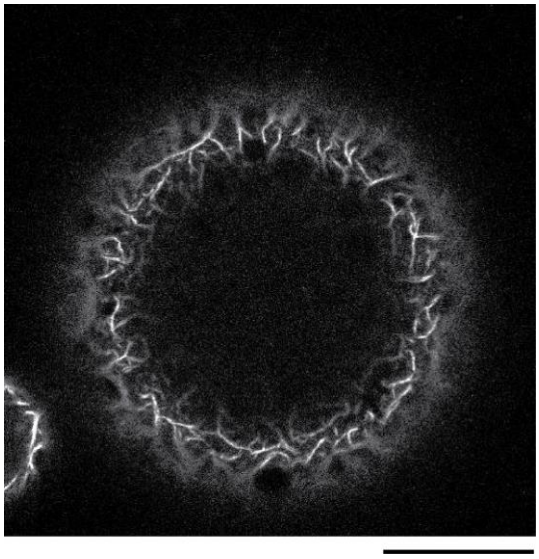
Supplementary figure 6. Min protein pattern formation in droplets. The smaller droplet has one polarization domain. The larger droplet has a more complicated pattern with two patches of Min proteins. Scale bar: 50µm



Supplementary figure 7. FtsZ-mts (blue) assembles into higher-order networks within droplets. (A) Confocal overview image of droplets with FtsZ-mts. Scale bar: 50 µm (B) When the zoom is increased compared to (A) (at constant pixel dwell times of the laser and constant number of pixel) network-like FtsZ-mts structures at the boundary of the droplets become visible. Scale bar: 10 µm (C,D) Network of FtsZ-mts at the bottom (C) and the midsection (D) of a droplet. Compared to image (B) the twell time of the laser/per area is incresed 4-fold. Scale bar: 5 µm.



Supplementary figure 8. FtsZ-mts assembles into rings in small compartments. A) Schematic illustration of vesicle assay to reconstitute FtsZ-mts rings. Previously, FtsZ-mts rings have been reconstituted by generating multi-lamellar vesicles, adding FtsZ to the outside of the vesicles and applying shearing forces to the vesicles. This process appeared to result into a leakage of FtsZ-mts into the vesicle lumen and the formation of FtsZ rings.[2] B) Overview of reconstituted FtsZ-mts rings (blue) in multilamellar vesicles which were reproduced using a published protocol.[5] The membrane of the vesicle was labeled with DiI (yellow). The ring-like structures have variable diameters up to more than 2 micrometers. Scale bar: 5 µm C) Magnified image of an FtsZ-mts ring within a multilamellar vesicle. Scale bar: 2 µm D) FtsZ-mts in small droplet. In contrast to multiple bright lines that represent network-bundles in large droplets, small droplets had a lower number of bright zones. Here, a droplet with a bright zone on the left side and a darker zone on the right side are shown. E) As in large droplets, these FtsZ-mts structures are dynamic. This renders an acquisition of highly resolved three-dimensional images of these structures challenging. F) A z-stack of confocal images was acquired and indicates that, in agreement with the structures in multilamellar vesicles, the observed FtsZ structures in small droplets might be rings. The height of the focal plane with respect to the first image is indicated in the lower right corners. Scale bar: 1µm



Supplementary movie 1: Networks of FtsZ-mts are highly dynamic in droplets. Frame rate:10 s, scale bar: 20 µm

1 Loose, M., Fischer-Friedrich, E., Ries, J., Kruse, K. & Schwille, P. Spatial regulators for bacterial cell division self-organize into surface waves in vitro. *Science* **320**, 789-792, doi:10.1126/science.1154413 (2008).

2 Osawa, M., Anderson, D. E. & Erickson, H. P. Reconstitution of contractile FtsZ rings in liposomes. *Science* **320**, 792-794, doi:10.1126/science.1154520 (2008).

3 Chwastek, G. & Schwille, P. A Monolayer Assay Tailored to Investigate Lipid-Protein Systems. *Chemphyschem* **14**, 1877-1881, doi:10.1002/cphc.201300035 (2013).

4 Zieske, K. & Schwille, P. Reconstitution of pole-to-pole oscillations of min proteins in microengineered polydimethylsiloxane compartments. *Angewandte Chemie* **52**, 459-462, doi:10.1002/anie.201207078 (2013).

5 Osawa, M. & Erickson, H. P. Chapter 1 - Tubular liposomes with variable permeability for reconstitution of FtsZ rings. *Methods Enzymol* **464**, 3-17, doi:10.1016/S0076-6879(09)64001-5 (2009).