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SUPPLEMENTARY MATERIAL

corresponding to:

Actin assembly states in *Dictyostelium discoideum* at different stages of development and during cellular stress

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Full Text for this paper and **Supplementary Movies** are available at: https://doi.org/10.1387/ijdb.190256am

Supplementary Movies 1 A,B. TIRF microscopy showing cortical and substrate-attached actin structures. *Recording by TIRF microscopy showing patterns of actin assembly visualized by expression of GFP-LimEG in the cell cortex (A), and at the substrate-attached surface (B) of a D. discoideum wild-type cell. The time series of the cell moving on a glass surface was acquired with intervals of 1 second and 100 mseconds exposure time for each image. Time is indicated in seconds. Bar, 10 μm.*

Supp. Movie 2. Actin waves in an electro-fused *D. discoideum* cell. *The cell expressed mRFP-LimEΔ as a label for filamentous actin (red) and GFP-PHcrac as a marker for PIP3 (green). The video was recorded using a confocal microscope (Zeiss LSM 780), and shows that actin waves subdivide the membrane into distinct domains: in PIP3-rich inner territories circumscribed by a wave, and in the external area depleted of PIP3. Bar, 10 μm.*

Supp. Movie 3. Uptake of fluid via macropinocytosis. *Recording of a large cell of D. discoideum produced by electric-pulse induced fusion that takes up extracellular medium by macropinocytosis. The cell expressed mRFP-LimEΔ (red) to visualize filamentous actin, and PHcrac-sfGFP (green) as a marker for PIP3, and was recorded by confocal microscopy (Zeiss LSM 780). Time is indicated in seconds. Bar, 10 μm.*

Supp. Movies 4 A-C. Cortical dynamics of *D. discoideum* cells during the slug stage recorded by two-photon microscopy. *D. discoideum cells were expressing GFP-filamin to label the cortex, and front, middle and rear areas were recorded by two-photon microscopy using a multiphoton LaVision Biotech (Bielefeld, Germany) TrimScope II system connected to an upright Olympus microscope, equipped with a Ti;Sapphire Chameleon Ultra II laser (Coherent) tunable in the range of 680 to 1080 nm and a 16 × water immersion objective (numerical aperture 0.8, Nikon). Time-lapse images were acquired with interval of 5 seconds per frame. 840 nm was used as an excitation wavelength, with 1030 × 1030 pixels. The signal was detected by Photomultipliers (G6780-20, Hamamatsu Photonics, Hamamatsu, Japan). ImSpector Pro (LaVision) was used as acquisition software.*

Supp. Movie 5. Two-photon microscopy of *D. discoideum slugs* showing collectively moving cells and sentinel cells. *D. discoideum slug stage cells expressing GFP-filamin (green) to label the cortex, showing sentinel cells visualized by the previous uptake of ethidium bromide (red). Two-photon imaging was acquired using a multiphoton LaVision BioTech (Bielefeld, Germany) TrimScope II system connected to an upright Olympus microscope, equipped with a Ti;Sapphire Chameleon Ultra II laser (Coherent) tunable in the range of 680 to 1080 nm, and a 16 × water immersion objective (numerical aperture 0.8, Nikon). Live images were acquired with 20 μm depth, with z-interval of 1 μm. 840 nm was used as an excitation wavelength, with 1030 × 1030 pixels. The signal was detected by Photomultipliers (G6780-20, Hamamatsu Photonics, Hamamatsu, Japan). ImSpector Pro (LaVision) was used as acquisition software.*

Supp. Movie 6. Germination of a *D. discoideum* amoeba. *Germination of spores prepared from cells expressing GFP-cofilin was recorded by confocal microscopy (Zeiss LSM 510).*

Supp. Movie 7. Image reconstruction of nuclear rods. *D. discoideum cells expressing both GFP-cofilin (green) and mRFP-actin (red) were treated with 10% DMSO to induce nuclear rods, and were fixed with methanol at -20°C for 15 min, and labeled with DAPI (blue). 3D confocal images acquired with a Zeiss LSM 880 microscope were reconstructed by using the Imaris software package (Bitplane, Zurich, Switzerland) with 3D volume rendering mode.*