

## Description of Additional Supplementary Files

**File Name:** Supplementary Movie 1

**Description:** Time-lapse movie of cluster formation and the periodic actomyosin wave in a control experiment. The height of the chamber was 100  $\mu\text{m}$ . Actin polymerization was initiated at 0 s by the elevation of temperature. Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 2

**Description:** Time-lapse movie of the periodic actomyosin wave observed by a confocal microscope, showing persistent wave generation for more than 90 min. The height of the chamber was 60  $\mu\text{m}$ . Scale bar, 50  $\mu\text{m}$ .

**File Name:** Supplementary Movie 3

**Description:** Time-lapse movie of the cluster formation and periodic wave generation in mm-scale systems. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 4

**Description:** Time-lapse movie of the actomyosin wave generation before and after the laser cut observed by a confocal microscope. The height of the chamber was 60  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 5

**Description:** Time-lapse movie of the actomyosin wave generation before and after the laser cut observed by a confocal microscope. The timing of the laser ablation was indicated in the movie. The height of the chamber was 60  $\mu\text{m}$ . Scale bar, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 6

**Description:** Time-lapse movie of the droplet for control experiments (Supplementary Figure 3a), 10 mM CK666 (Supplementary Figure 3b), 300 nM calyculin A (Supplementary Figure 3c), 100  $\mu\text{M}$  Y27632 (Supplementary Figure 3d), and 1 mM Y27632 (Supplementary Figure 3e). The cluster was assembled at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 7

**Description:** Time-lapse movie of the droplet containing 200  $\mu\text{M}$  cytochalasin D

(Supplementary Figure 5a). The cluster was not assembled, and the wave did not occur, indicating that actin polymerization is essential for cluster formation and actomyosin wave generation. Actin polymerization was initiated at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 8

**Description:** Time-lapse movie of the droplet containing 10  $\mu\text{M}$  phalloidin

(Supplementary Figure 5b). The cluster was formed and actomyosin waves were generated several times, followed by cessation of wave generation, indicating that actin depolymerization is essential to maintain periodic actomyosin waves. Actin polymerization was initiated at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 9

**Description:** Time-lapse movie of the side view of the droplet. The images were

reconstructed from Z-stack confocal images. Scale bars, 50  $\mu\text{m}$ .

**File Name:** Supplementary Movie 10

**Description:** Time-lapse movie of cluster movement from the droplet boundary to the

center. The cluster started to move at 0 s. The height of the chamber was 60  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 11

**Description:** Time-lapse movie of cluster movement from the droplet center to the

edge. The cluster was started to move at 0 s. The height of the chamber was 60  $\mu\text{m}$ . Scale bars, 50  $\mu\text{m}$ .

**File Name:** Supplementary Movie 12

**Description:** Time-lapse movie of the cluster oscillation. The coexistence of

actomyosin waves and radial actomyosin bridges indicates that cluster oscillation is driven by these two distinct actomyosin dynamics. The movie was recorded approximately 5 min after the cluster formation. The height of the chamber was 60  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 13

**Description:** Time-lapse movie of the symmetry breaking of cluster positioning

observed by a confocal microscope. The radial actin bridges appear to be connected between the cluster and the droplet boundary. Rapid movement of the cluster toward the droplet boundary indicates that the off-centered cluster positioning is driven by the contraction of radial actin bridges. Movement was initiated from ~1 min after encapsulation. The height of the chamber was 30  $\mu\text{m}$ . Scale bar, 50  $\mu\text{m}$ .

**File Name:** Supplementary Movie 14

**Description:** Time-lapse movie of the motion of the cluster after cutting the

actomyosin bridges observed by a confocal microscope. The height of the chamber was 30  $\mu\text{m}$ . Scale bar, 50  $\mu\text{m}$ .

**File Name:** Supplementary Movie 15

**Description:** Time-lapse movie of the droplet containing 250 nM  $\alpha$ -actinin. The

cluster was assembled at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 16

**Description:** Time-lapse movie of the droplet containing 300 nM gelsolin. The cluster

was assembled at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 17

**Description:** Time-lapse movie of the droplet containing 30 nM mDia2. The cluster

was assembled at 0 s. The height of the chamber was 100  $\mu\text{m}$ . Scale bars, 100  $\mu\text{m}$ .

**File Name:** Supplementary Movie 18

**Description:** Numerical simulation of cluster positioning dynamics, with parameters:

$D = 120 \mu\text{m}$ ,  $L = 8 \mu\text{m}$ ,  $\tau = 1 \text{ s}$ . The yellow cross mark represents the bulk actin network being crosslinked. Peripheral contraction of the cluster was initiated, when all of the crosslinking-sites in bulk space  $N = R/L = D/(2L)$  were occupied by crosslinkers. The cluster was initially in the center and was finally entrapped at the edge.

**File Name:** Supplementary Movie 19

**Description:** Numerical simulation of cluster positioning dynamics, with the following parameters:  $D = 300 \mu\text{m}$ ,  $L = 8 \mu\text{m}$ ,  $\tau = 1 \text{ s}$ . The yellow cross symbol represents the bulk actin network being crosslinked. The cluster is initially in the edge, and finally entrapped at the center.

**File Name:** Supplementary Movie 20

**Description:** Actomyosin waves in non-circular geometry chambers. The height of the chamber was  $60 \mu\text{m}$ . Scale bars,  $100 \mu\text{m}$ .

**File Name:** Supplementary Movie 21

**Description:** Time-lapse movie of the migrating droplet containing  $10 \text{ nM}$  His-VCA emulsified with  $10\%$  DGS-NTA(Ni) in eggPC. The height of the chamber was  $100 \mu\text{m}$ . Scale bar,  $100 \mu\text{m}$ .