Mitsushima et al., http://www.jcb.org/cgi/content/full/jcb.201007136/DC1

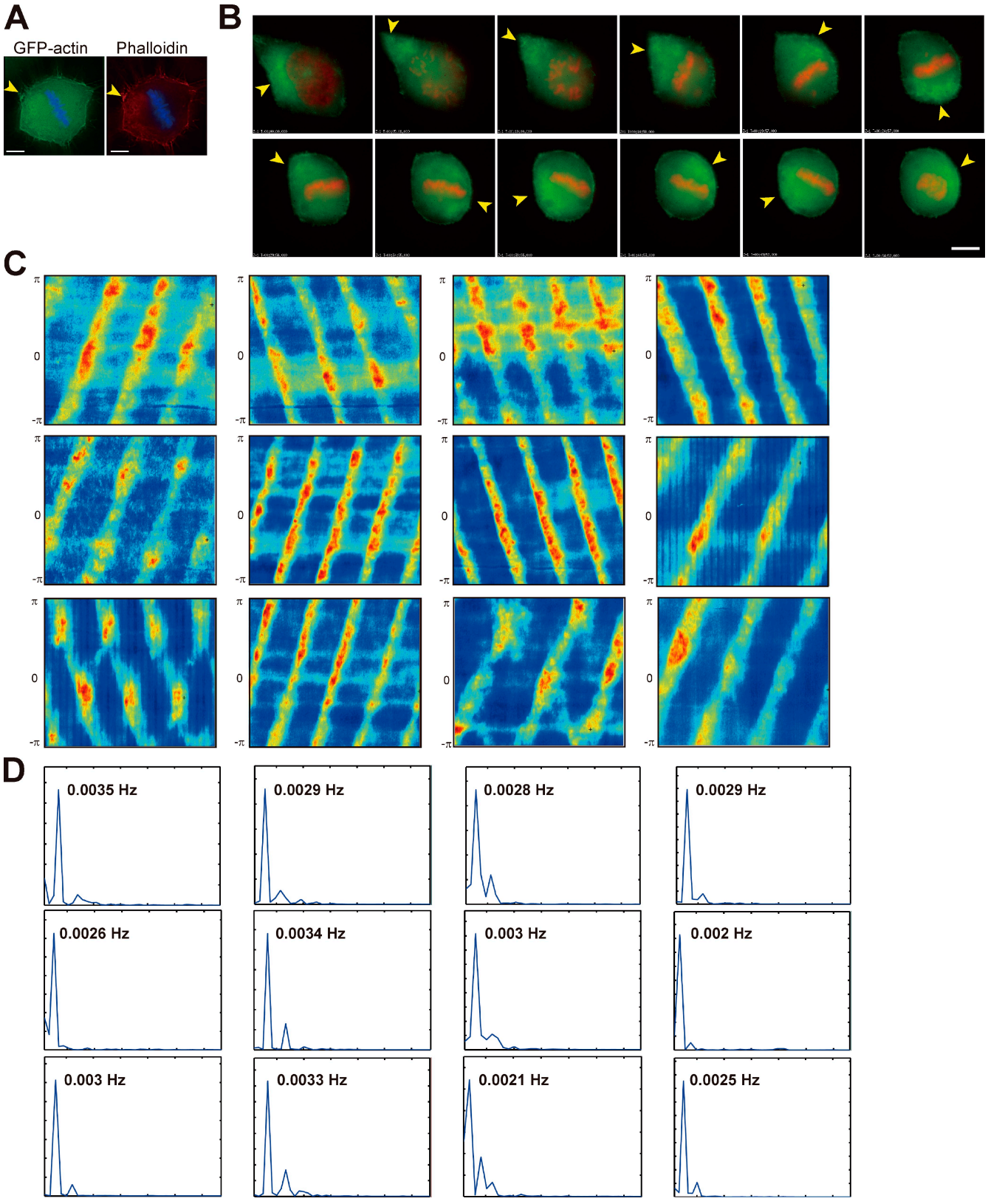


Figure S1. **Revolving movement of the amorphous actin cluster in HeLa cells.** (A) Staining with phalloidin of HeLa cells expressing GFP-actin at metaphase. Arrowheads indicate the actin cluster. Bar, 5 µm. (B) Time-lapse images of HeLa cells expressing GFP-actin and DsRed-histone H1 during M phase. GFP-actin and DsRed-histone H1 images were taken every 3 min. Arrowheads indicate the region of an amorphous cluster of actin filaments. Bar, 10 µm. (C) Spatio-temporal representation of the amorphous cluster of actin filaments in 12 HeLa cells expressing GFP-UtrCH. Intensities of GFP-UtrCH signals in areas between the 0.70–0.75 or 0.60–0.65 radius away from the center of the cell were averaged and plotted. (D) Fourier transform of C.

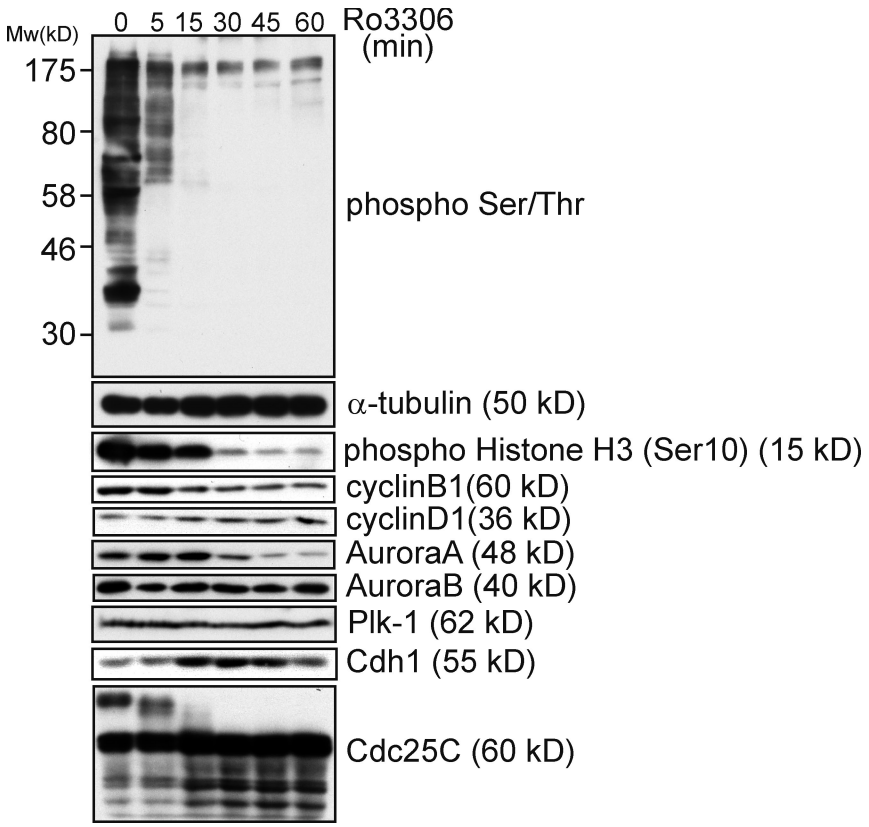


Figure S2. **The effects of the Cdk1 inhibitor Ro3306 on M phase events.** HeLa cells were synchronized at S phase by a double thymidine block. 9 h after the release from the block, cells were treated with 200 ng/ml nocodazole to arrest cells in prometaphase, then cells were treated with 20 μM Ro3306 for the indicated times. Cell lysates were subjected to immunoblotting with the indicated antibodies. Phospho Ser/Thr indicates immunoblotting with anti–phospho Ser/anti–phospho Thr-Pro antibody.

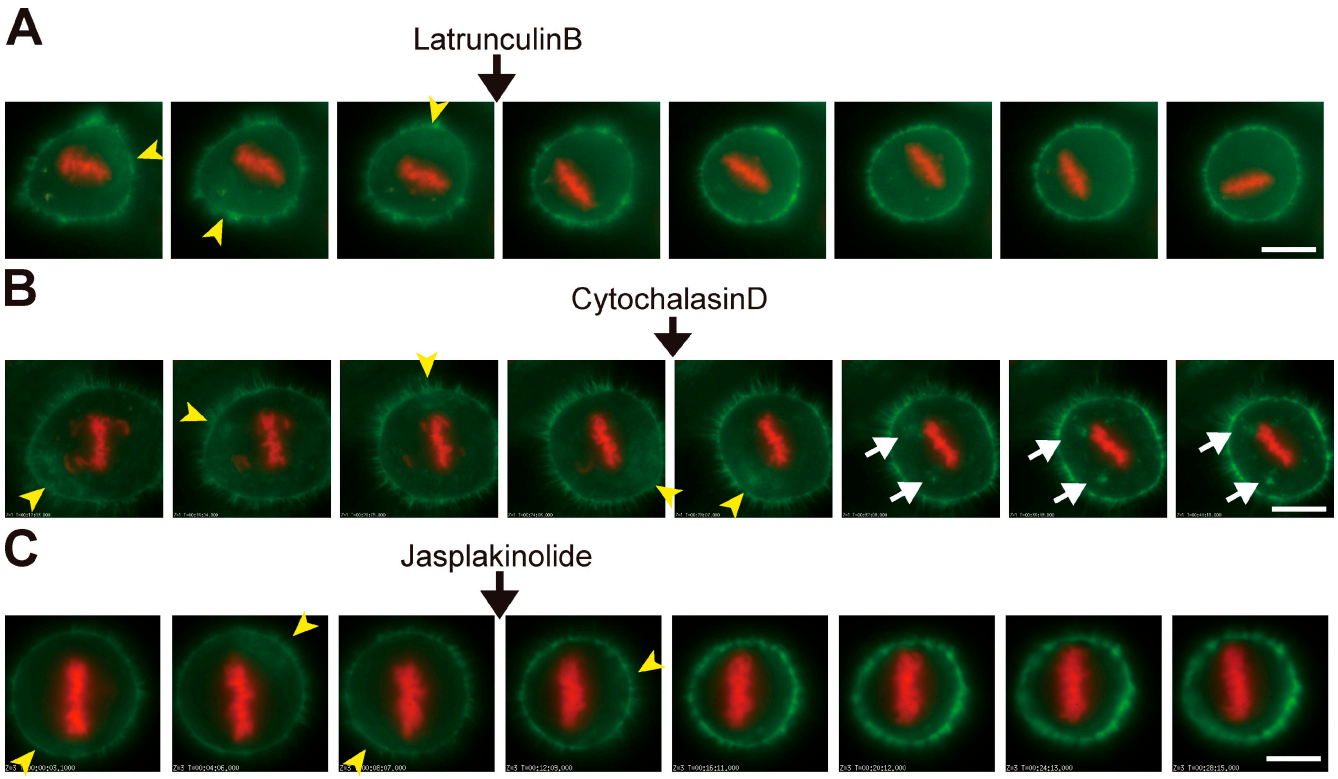
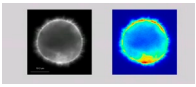
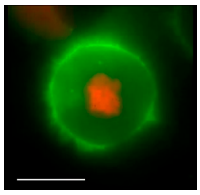


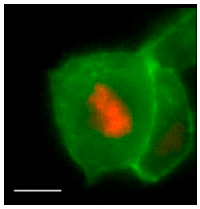
Figure S3. **Actin polymerization and depolymerization reactions are required for generating the mitotic actin cluster and movement.** (A–C) Time-lapse images of HeLa cells expressing GFP-UtrCH and DsRed-histone H1 during M phase. GFP-UtrCH and DsRed-histone H1 images were taken every 2 min, and images are shown at 4-min intervals. 1 µM LatrunculinB (A), 1 µg/ml cytochalasin D (B), or 0.1 µM Jasplakinolide (C) were added at the indicated points. Arrowheads indicate the region of an amorphous cluster of actin filaments and white arrows indicate dotlike clusters of actin filaments. Bars, 10 µm.



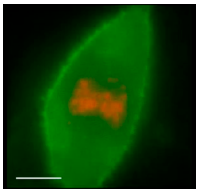
Video 1. **The revolving movement of the amorphous actin cluster in a HeLa cell expressing GFP-UtrCH during M phase.** Images were analyzed by DeltaVision optical sectioning systems (Applied Precision) equipped with an inverted microscope (IX71; Olympus). Frame interval, 3 s. Replay speed, 15 frames/s. Duration of original sequence, 930 s. Bar, 10 µm.



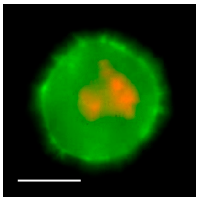
Video 2. **Cdk1 activity is required for the actin cluster formation and its revolving movement.** Nocodazole-treated, prometaphase-arrested HeLa cells expressing GFP-UtrCH and DsRed-histone H1 were treated with 20 µM Ro3306 immediately after time 0. Frame interval, 2 min. Replay speed, 6 frames/s. Duration of original sequence, 60 min. Bar, 10 µm.



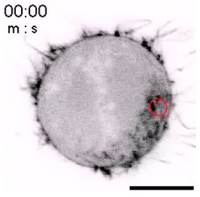
Video 3. **The revolving movement of the amorphous cluster of actin filaments in an MCF-7 cell expressing GFP-UtrCH and DsRed-histone H1 during M phase.** Frame interval, 2 min. Replay speed, 6 frames/s. Duration of original sequence, 60 min. Bar, 10 µm.



Video 4. **The formation and revolving movement of the amorphous cluster of actin filaments are incomplete in an HaCaT cell expressing GFP-UtrCH and DsRed-histone H1 during M phase.** Frame interval, 2 min. Replay speed, 6 frames/s. Duration of original sequence, 60 min. Bar, 10 μm.



Video 5. **The revolving movement of the amorphous cluster of actin filaments in a HeLa cell expressing GFP-UtrCH and DsRed-histone H1 under the 3D conditions during M phase.** Frame interval, 30 s. Replay speed, 10 frames/s. Duration of original sequence, 45 min. Bar, 10 μm.



Video 6. **The actin turnover in the amorphous cluster of actin filaments in HeLa cells expressing GFP-actin during M phase.** Images were analyzed by a confocal laser microscope FV1000-D (Olympus). At time 0, photobleaching was performed in the area, which is shown by the red circle, by high-powered laser with a wavelength of 488 nm. Frame interval, 7.3 s. Replay speed, 5 frames/s. Duration of original sequence, 2 min and 22 s. Bar, 10 μm.