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Supplementary Materials and Methods

Cell culture, synchronization, and reagents. HeLa cells, HeLa-S3 cells, NIH 3T3 cells, and NRK cells were cultured in DMEM with 10 % bovine calf serum or fatal bovine calf serum. To synchronize cells in M phase, HeLa cells and NIH 3T3 cells were arrested at G1/S boundary by a double-thymidine block, released from the arrest by washing with fresh medium, and incubated for 10 h. Lat B (10 μM, Calbiochem), Y-27632 (50 μM, Calbiochem), and blebbistatin (100 μM, CosmoBio) were added into the medium 8 h after the release and incubated for 2 h. MG132 (50 μM, Calbiochem) was added into the medium 9 h after the release and incubated for 40 min, followed by the additional incubation with Lat B for 20 min. For the release from nocodazole arrest, HeLa cells were arrested in pro-metaphase by the incubation with nocodazole (80 ng/ml, Sigma) for 15 h, released from the arrest by washing with fresh medium, and incubated for 1 h. To synchronize NRK cells in M phase, cells were arrested at G1/S boundary by a 16-h treatment with aphidicolin (2.5 μg/ml, Sigma), released from the arrest by washing with fresh medium, and incubated for 8h. Nocodazole (10 ng/ml) and Lat B were added into the medium 6 h and 7h, respectively, after the release. GRADSP peptide (400 μM, Calbiochem), GRGDNP peptide (400 μM, Bachem), mouse IgG (5 μg/ml, Zymed), and anti-β1 integrin, β3 integrin, and αVβ6 integrin antibodies (5 μg/ml, Chemicon, clone 6S6, 25E11, and 10D5, respectively) were added into the medium at the time of plating of the cells on a coverslip. In all experiments, cells were plated on the fibronectin-, collagen-, or poly-L-lysine-coated coverslips. Collagen- and poly-L-lysine-coated coverslips were purchased from Asahi Techno Glass. Fibronectin-coated coverslips were were purchased from BD Biosciences.

Cell culture in three dimensions. GFP-H2B expressing HeLa cells, synchronized by a double-thymidine block, were trypsinized immediately after the release from a double-thymidine block. Cells (5,000 cells) were suspended in 60 μl of Matrigel Basement Membrane Matrix (BD Biosciences), and spread evenly in the well of a glass-bottom dish. The dish was placed in a cell culture incubator for 30 min to allow the basement membrane to solidify, then culture medium was added over the basement membrane. After 10 h incubation, cells were analyzed by the time-lapse observation.

Cell staining and image analysis. For double-staining of α-tubulin and β-tubulin, cells were fixed for 5 min with 3.7 % formaldehyde at 37 ℃, followed by incubation for 20 min with ice-cold methanol. Cells were washed twice with PBS and blocked for 1 h with 3% BSA in PBS. Cells were incubated for overnight with primary antibodies (mouse anti-α-tubulin and rabbit anti-β-tubulin antibodies, Sigma), and then incubated for 1 h with secondary antibodies (AlexaFluor 594-goat anti-mouse and 488-goat anti-rabbit IgG antibodies, Molecular Probes). For detection of astral microtubules and actin cytoskeleton, cells were fixed for 15 min with 4 % paraformaldehyde in PBS at 37 ℃ and permeabilized for 10 min with 0.5 % Triton-X100 in PBS. For actin staining, cells were incubated for 1 h with rhodamine phalloidin (2U/ml, Sigma) at 37 ℃. For membrane staining, cells were fixed for 5 min with 3.7 % formaldehyde and incubated for 30 sec with DHCC (2.5 μg/ml, Sigma). The cell height in Figure. 4A and Figure. 5A was measured by taking Z-stack images from 0.5 μm-thick sections of a metaphase cell, that were stained with DHCC. Images were acquired using a DeltaVision opitical sectioning systems with softWoRx software. Stacks of x-y images were taken with 0.5 or 1 or 2 μm step size. Deconvolving images and viewing the image data in 3-D (x-z projections) were performed by using softWoRx. During the acquisition of the time-lapse images, cells were grown in the medium with 20 mM Hepes (pH7.3) in glass-bottom chambers set on a temperature-controlled stage.