# **Supporting Information**

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**Fig. S1.** Events after failed cytokinesis. (A) BrdU analysis of cells after synchronization procedure. BrDU incorporation remains low several days after removal of blebbistatin (bleb). Each day represents a 24-h treatment followed by fixation and analysis. Asynchronous cells (Asy) were used as a positive control. (*B*) A variety of events were observed by time-lapse videomicroscopy for binucleate cells after failed cytokinesis. Still images of merged phase contrast and fluorescent H2B-GFP are shown for RPE1 cells after prior failed cytokinesis. Time in hours:minutes from the Fig. 1A treatment is shown for each frame.



Fig. 52. Central-spindle markers of cytokinesis are absent in cytofission. (A) Binucleate cells imaged with time-lapse microscopy were fixed during cytofission and stained for actin, Polo-like kinase 1 (Plk1), and kinesin-like protein 1 (MKLP1) in RPE1 cells. (*Insets*) Indirect immunofluorescence signals for Plk1, MKLP1, and actin. Graphs are line-scans demonstrating intensity of signal across cytoplasmic bridge during cytofission and cytokinesis. Intensity is displayed in arbitrary units (A.U.). (B) Two control cells undergoing cytokinesis were fixed and processed in same manner as cells undergoing cytofission. (C) A similar analysis with centromere-associated protein E (CENP-E) and inner centromere protein (INCENP) during cytofission. (D) Localization of CENP-E and INCENP during cytokinesis.



**Fig. S3.** Cytofission occurs in the presence of depolymerized microtubules. (*A*) Fixed-cell analysis of microtubules confirms marked depolymerization in RPE1 interphase cells with as little as 0.2 μg/mL nocodazole. (*B*) Video time-lapse images of RPE1 cell undergoing cytofission in the presence of nocodazole.



**Fig. S4.** Flow cytometric assay for cytofission. Binucleate cells or control mononucleate cells are incubated in the presence or 5  $\mu$ M aphidicolin to prevent cell-cycle progression. Euploid cells emerge without DNA replication from a population of binucleate cells that had failed cytokinesis. (A) Cell counts over time revealing increase consistent with cytofission (SD, n = 2). As expected, cell number does not increase for control cells treated with aphidicolin owing to suppression of DNA replication. (B) DNA content by flow cytometry (n = 2, representative shown) was performed on binucleate cells (4N) at timepoints shown.

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Movie \$1. Cytofission in RPE1 cell. Corresponds to Fig. 3, Top. Merge of H2BGFP and phase contrast. Time in h:min:s from blebbistatin treatment is shown.

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Movie \$2. Cytofission in RPE1 cell. Corresponds to Fig. 3, Middle. Merge of H2BGFP and phase contrast. Time in h:min:s from blebbistatin treatment is shown.



Movie S3. Cytofission in MCF10a. Corresponds to Fig. 3, Bottom. Merge of H2BGFP and phase contrast.

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Movie S4. Cell 1: cytofission in RPE1 cell labeled with both H2B-GFP (green) and mCherry-Cdt1 (red). Corresponds to cell 1 in Fig. 4B. Overlays of each fluorescent channel with phase-contrast images are shown separately. Time is shown in h:min:s.



Movie S5. Cell 2: cytofission in RPE1 cell labeled with both H2B-GFP (green) and mCherry-Cdt1 (red). Corresponds to cell 2 in Fig. 4B. Time is shown in h:min:s.



Movie S6. Cytofission in RPE1 cell in the presence of 5 µM aphidicolin. Merge of H2B-GFP and phase contrast. Corresponds to Fig. 4C. Time is shown in h:min:s.

Movie S6



Movie 57. Start of cytofission in RPE1 cell. Merge of H2B-GFP and phase contrast. Corresponds to Fig. 5A. Cell was fixed for later analysis. Time is shown in h:min:s.



Movie S8. Control mitosis-cytokinesis in RPE1 cell demonstrating localization of GFP-Plk1. Merge of GFP-Plk1 (green), H2B-mCherry (red), and phase contrast images. Corresponds to Fig. 5C, cytokinesis cell 1. Note additional GFP-positive cytokinesis at upper right.



Movie S9. Cytofission in RPE1 cell demonstrating no localization of GFP-Plk1. Merge of GFP-Plk1 (green), H2B-mCherry (red), and phase contrast images. Corresponds to Fig. 5C, cytofission. GFP acquisition and threshold parameters were identical to Movie S7. Time in h:min:s at upper right.



Movie S10. Cytofission in RPE1 cell in the presence of nocodazole 0.4 µg/mL Merge of H2B-GFP and phase contrast. Corresponds to Fig. S3. Time is shown in h:min:s.

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