

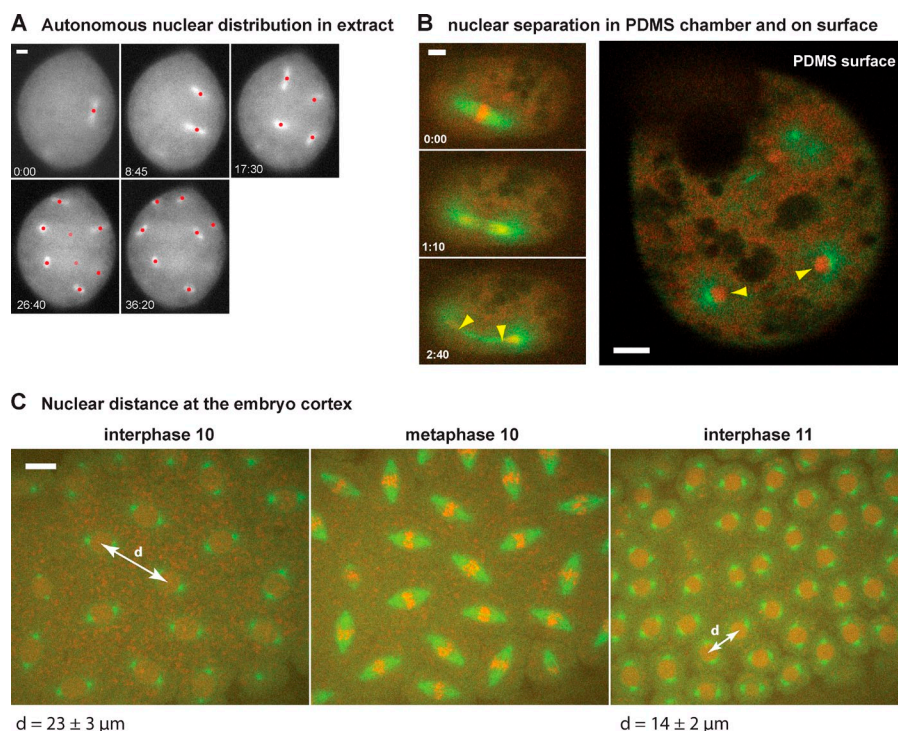
Telley et al., <http://www.jcb.org/cgi/content/full/jcb.201204019/DC1>

Figure S1. **Nuclear distribution in preblastoderm extract under confinement and at the embryo cortex.** (A) Epifluorescence microscopy time-lapse image sequence showing an extract droplet with initially one spindle (visualized by the microtubule-binding protein Jupiter-GFP, marked with a red dot) undergoing multiple rounds of nuclear divisions of cycles 6–10. Starting initially on one side of the droplet, the newly forming spindles distribute over the entire droplet volume while dividing every 9–10 min. Time is shown in minutes/seconds. Bar, 10  $\mu\text{m}$ . (B, left) Time-lapse confocal microscopy image sequences of nuclear divisions in a PDMS microchamber generating spatial confinement. Spindles deform (bending indicated by arrowheads) during nuclear separation in telophase. Bar, 5  $\mu\text{m}$ . (right) Nuclear division (arrowheads) is normal in unconfined extract droplets on the PDMS surface. DNA is in red, and microtubules are in green. Bar, 10  $\mu\text{m}$ . (C) Confocal microscopy images of nuclear divisions at the cortex of a syncytial blastoderm embryo during cycle 10 (left, middle image) and the subsequent cycle 11 (right). Nuclei are anchored at the cortex and form a monolayer, and, during duplication, this limited space has to be shared by twice as many nuclei. This leads to dense packing and a drastic reduction in internuclei distance ( $d$ ) after division. DNA is in red, and microtubules are in green. Bar, 10  $\mu\text{m}$ .

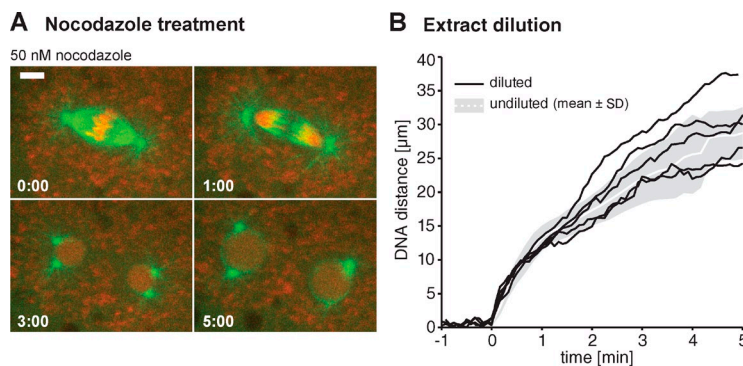
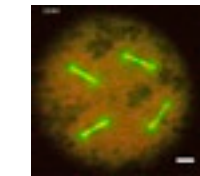
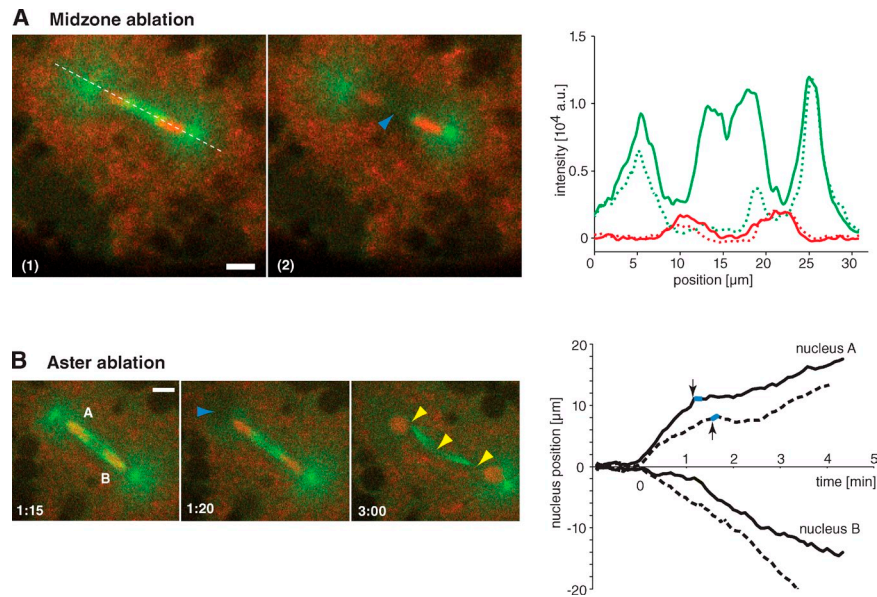
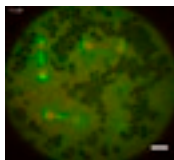


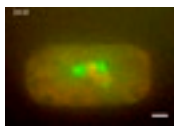
Figure S2. **Small doses of nocodazole reduce the nuclear separation, and simple extract dilution is tolerated.** (A) Time-lapse image sequence of a nuclear division in extract treated with a low dose (50 nM) of nocodazole, an alternative microtubule-depolymerizing drug. The aster size is slightly reduced, and the final distance between nuclei was only 18  $\mu\text{m}$ . DNA is in red, and microtubules are in green. Time is shown in minutes/seconds. Bar, 5  $\mu\text{m}$ . (B) Dilution of extract with buffer (see Materials and methods) does not significantly alter the separation dynamics during nuclear division, as shown in the distance–time plot of the separating DNA masses in diluted extract (black lines) versus undiluted extract (white line on gray background; mean  $\pm$  SD; from Fig. 1 D). Each distance–time curve represents an independent experiment.



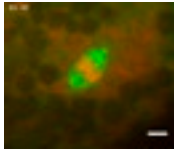
**Video 1. Single-*Drosophila* embryo extract recapitulates repeated nuclear divisions.** Confocal fluorescence microscopy time-lapse video of an extract droplet with initially one nucleus undergoing five consecutive divisions. Nuclei distribute evenly within the droplet and do not fuse or aggregate. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 20 $\times$  UPlanApo 0.8-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 20 s. The video was accelerated to 10 frames per second. Bar, 10  $\mu\text{m}$ .



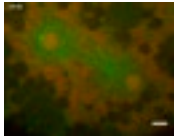
**Video 2. In extract droplets, daughter nuclei displace further after anaphase and separate even in the absence of a spindle.** Confocal fluorescence microscopy time-lapse video of an extract droplet demonstrating the large displacement of daughter nuclei after division in the absence of a dense central spindle. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40 $\times$  UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 10 s. The video was accelerated to 10 frames per second. Bar, 10  $\mu\text{m}$ .



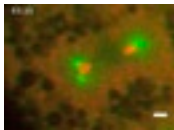
**Video 3. Space confinement leads to spindle distortion and pole fusion during nuclear division.** Confocal fluorescence microscopy time-lapse video of a (preblastoderm) nuclear division in a microchamber. The spatial confinement does not affect the mitotic program but causes spindle deformation and spindle pole fusion in the subsequent division cycle. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40 $\times$  UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 10 s. The video was accelerated to 10 frames per second. Bar, 5  $\mu\text{m}$ .



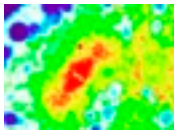
**Video 4. Microtubule-depolymerizing drugs reduce the astral microtubule density and affect nuclear separation after anaphase.** Confocal fluorescence microscopy time-lapse video of a nuclear division in extract treated with  $\sim 0.5 \mu\text{M}$  colcemid showing strongly reduced astral microtubule density and only a small nuclear separation after anaphase but an apparent rotation of daughter nuclei. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40x UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 10 s. The video was accelerated to 10 frames per second. Bar, 5  $\mu\text{m}$ .



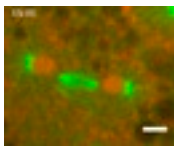
**Video 5. Daughter nuclei move further after irradiating the central spindle.** Confocal fluorescence microscopy time-lapse video of a nuclear division in extract in which the spindle midzone is irradiated using UV laser pulses. Nevertheless, daughter nuclei further move apart with the aster leading. During ablation, the reduced magnification as a result of the removal of the optovar has been compensated by rescaling the images. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40x UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 5 s. The video was accelerated to 5 frames per second. Bar, 5  $\mu\text{m}$ .



**Video 6. Repeated irradiation of midzone microtubules does not prevent nuclear separation.** Confocal fluorescence microscopy time-lapse video of a nuclear division in extract in which the spindle midzone is irradiated repeatedly using UV laser pulses. Nevertheless, daughter nuclei further move apart with the aster leading. The change in magnification was a result of the removal of the optovar for ablation. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40x UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 5 s. The video was accelerated to 10 frames per second. Bar, 10  $\mu\text{m}$ .



**Video 7. Polymerized actin colocalizes with the central spindle and astral microtubules during anaphase and telophase.** Confocal fluorescence microscopy time-lapse video of a nuclear division in extract in which actin and microtubules are fluorescently labeled. (left) Actin-RFP is in red, and microtubules are in green (Jupiter-GFP). (middle) Grayscale image of actin-RFP alone. (right) Heat map of the actin-RFP signal, with red denoting high levels and blue denoting low values. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40x UPlanFL 1.3-NA oil objective. Images were recorded every 10 s. The video was accelerated to 10 frames per second. Bar, 5  $\mu\text{m}$ .



**Video 8. Drug-induced actin depolymerization causes reduced nuclear separation after anaphase.** Confocal fluorescence microscopy time-lapse video of a nuclear division in extract treated with  $\sim 5 \mu\text{M}$  latrunculin A showing reduced separation of daughter nuclei after anaphase and no movement after central spindle microtubules disappeared. Images were acquired with a spinning-disk confocal unit (CSU-X1), an electron-multiplying charge-coupled device camera (iXon, DU-885), and a 40x UPlanFL 1.3-NA oil objective. Microtubules are in green (Jupiter-GFP), and DNA is in red (H2Av-RFP). Images were recorded every 10 s. The video was accelerated to 10 frames per second. Bar 5,  $\mu\text{m}$ .