Supplementary Movies

Movie S1

Walk-through 3-D tomography volume (corresponds to sections shown in Fig. 2X-Z')

Tomography was employed in our study to reveal fine morphological details of specific centrioles. In this approach we first reconstructed the overall organization of each centrosome from serial 100-nm thin sections. Then, double-tilt (±60^{o}) tomography series were collected for selected sections that appeared most interesting in the initial analyses. The movie presents a walk-through the 3-D volume at 2.6-nm steps (38 slices). It reveals details that are not detectable in conventional EM sections (e.g., discontinuity in some of the microtubule blades, see Fig. 2Y).

Movie S2

Development of threads in i-mCherry-CPAP cells (corresponds to Fig. 3E)

Imaging was started when the cell had two centrosomes that were normal in appearance. As the cell progressed towards mitosis, CPAP-containing threads developed in both centrosomes during G2 (6 h 15m – 7h 30m). The cell then entered mitosis and formed a bipolar spindle (7h 45m – 8h 00m). Cytokinesis was initiated normally (8h 15m) but ultimately failed (cf. 9h 30m and 9h 45m) resulting in the formation of a single binucleated progeny. Time "0" corresponds to ~12 hours after induction of CPAP expression with doxycycline. Top portion of each frame is a single DIC plane, bottom a maximal intensity projection of 25 focal planes (0.7 μm Z-steps). Note that spindle poles are often out of focus during mitosis due to the cell rounding up. The same comment holds for Movies SS3 and S5.

Movie S3

Development of threads in i-mCherry-CPAP cells (corresponds to Fig. 3F)

Similar to Movie S2, except in this cell no thread development was detected during G2. The cell formed a bipolar spindle (2h 15m) and successfully completed cytokinesis. During early G1 prominent mCherry-CPAP threads develop in both daughter cells (e.g., 4h 30m). Top portion of each frame is a single DIC plane, bottom a maximal intensity projection of 25 focal planes (0.7 μm Z-steps).

Movie S4

Lack of thread development in S-arrested i-mCherry-CPAP cells (corresponds to Fig. 3G)

The culture was treated with 2-mM hydroxyurea (HU) for 24 hr and then with HU and doxycycline for an additional 24 hr. Although fluorescence intensity of the centrosomes gradually increased no thread formation was observed for more than 60 h. Top portion of each frame is a single DIC plane, bottom a maximal intensity projection of 25 focal planes (0.7 μm Z-steps).

Movie S5

Progression through the cell cycle and mitosis in i-mCherry-CPAP cells (corresponds to Fig. 4I)

This cell was met approximately 24-hr after doxycycline induction when both centrosomes have developed complex threads (0m). In spite of the abnormal centrosome structure the cell assembled a bipolar spindle when it entered mitosis (4h 00m). During the ensuing G1, individual units separate from the single centrosome inherited by each daughter and moved individually in the cytoplasm. This type of behavior has been shown to be characteristic for individual centrioles in cycling cells. During the next mitosis, both sister cells assembled multipolar spindles. As a result of partial cytokinesis failure often seen in multipolar mitoses, one cell gave birth to a mononucleated and a trinucleated cells (27h 15m). Mitosis in the other cell resulted in the formation of four mononucleated cells. Subsequently, several of these granddaughter cells

died; however, two were able to progress through at least one more round of multipolar mitosis (not shown).