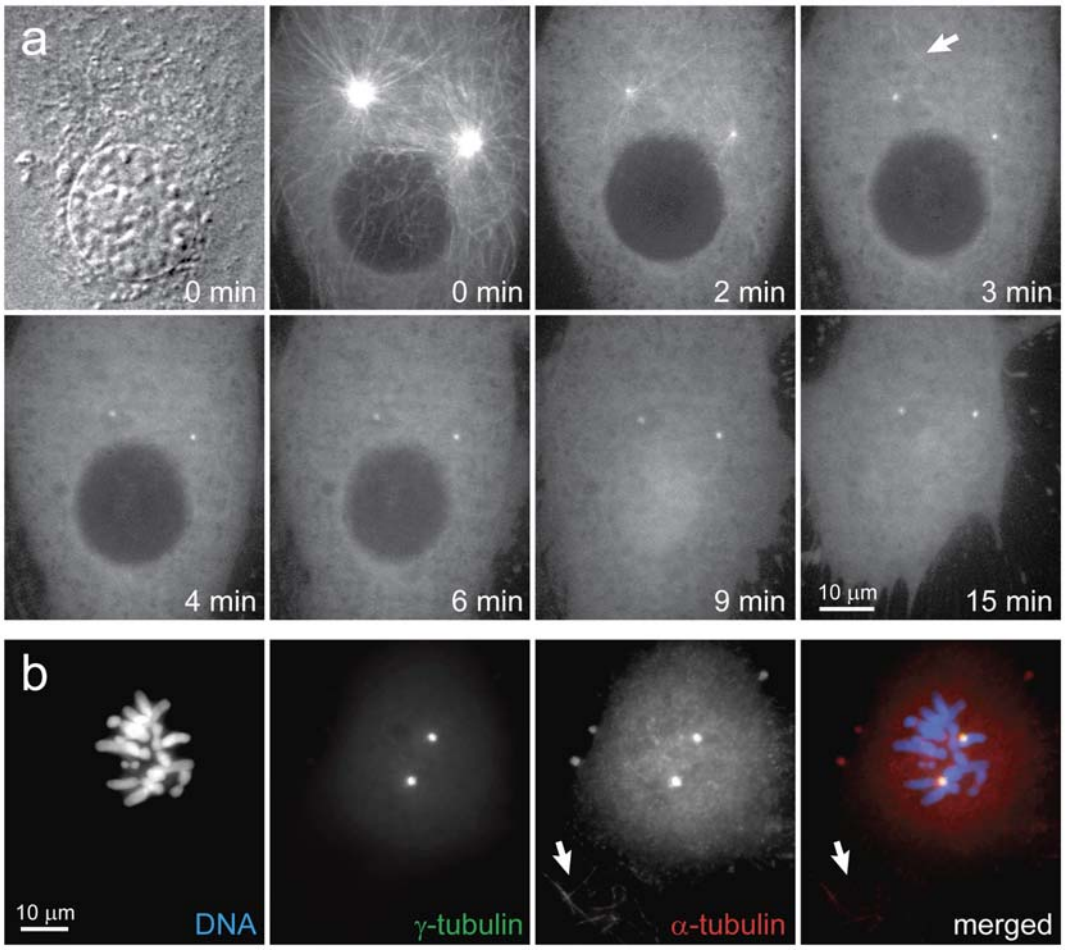


Figure S1. Time-course of microtubule depolymerization in mitotic PtK cells upon treatment with 5-μM nocodazole. (A) Selected frames from a time-lapse movie (1-min intervals) of a prophase cell (see DIC image in the first frame) that expresses α-tubulin-GFP. 5-μM nocodazole was perfused into the chamber at time "0-min". The last remaining microtubules can be detected 3 min after perfusion of nocodazole (arrow in "3 min"). Nuclear envelope breaks down ~7 min after nocodazole perfusion (cf. 6 and 9 min frames) and the cell enters mitosis in complete absence of microtubules. Each fluorescence image is maximal-intensity projection of a complete Z-series through the entire volume of the cell. **(B)** Similar to (A) except this experiment was conducted in PtK cells that expresses γ-tubulin-GFP. The cell was fixed ~15 min after addition of nocodazole and stained with anti-α-tubulin antibody. Immunostaining revealed complete absence of microtubules in the mitotic cell although some of interphase cells may still contain individual stable microtubules at this point (arrows in "α-tubulin" and "merged" frames). All images are maximal-intensity projections spanning the entire volume of the cell.

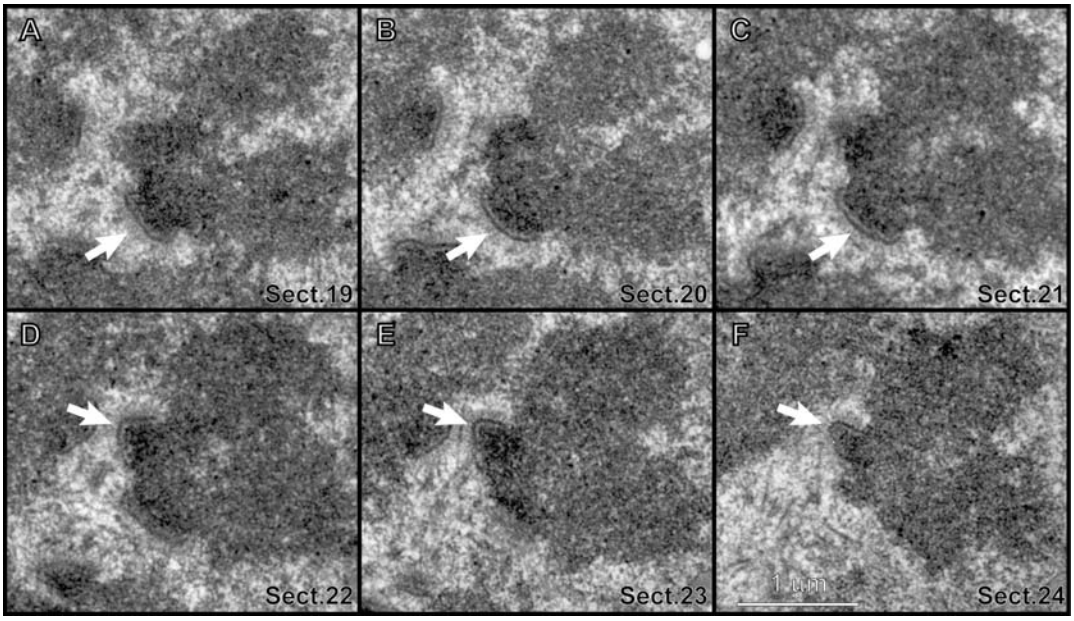


Figure S2. **Juxtaposed sister kinetochores on syntelic chromosomes do not return to the opposite sides of the centromere in the absence of microtubules. (A-F)** Selected 80-nm EM sections through the centromere of a chromosome in cell double-treated with monastrol and then nocodazole. Both sister kinetochores (arrows) reside on the same side of the centromere next to one another. Juxtaposed sister kinetochores were found on at least 3-4 chromosomes (of total 11-13) in each of the 3 serially-reconstructed cells. Surface-rendered reconstruction of this chromosome is presented in Fig.1.

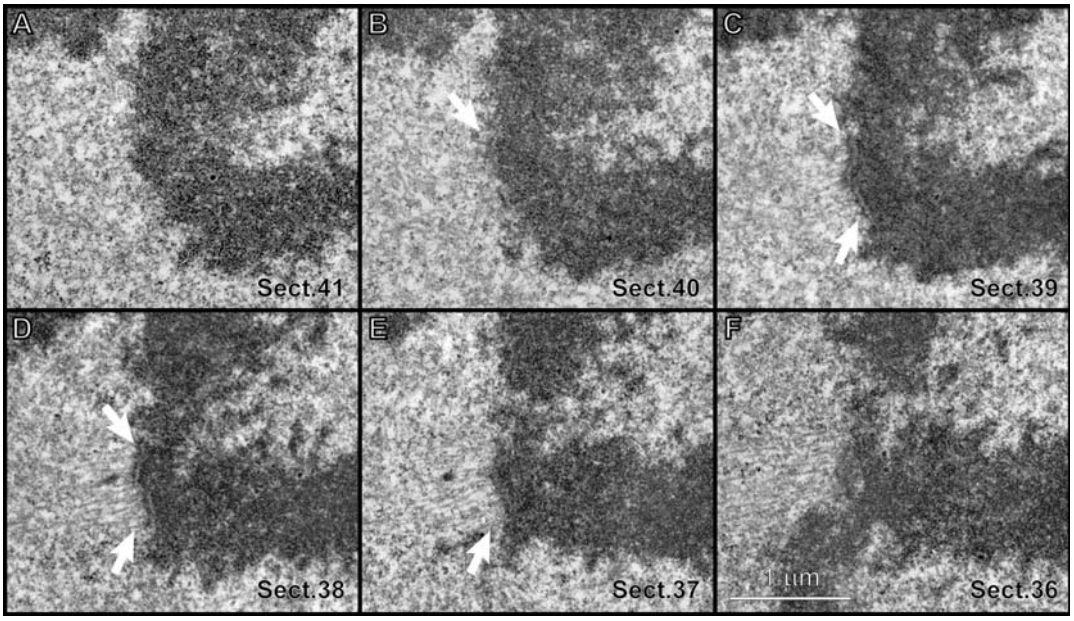


Figure S3. **Sister kinetochores remain juxtaposed upon monastrol washout in cells lacking centrosomes. (A-F)** Selected 80-nm EM sections through the centromere of a chromosome in cell that was treated with monastrol, then both centrosomes were ablated, and monastrol washed out. Both sister kinetochores (arrows) reside on the same side of the centromere next to one another. Two prominent bundles of microtubules (K-fibers) extend from the kinetochores. Surface-rendered reconstruction of this chromosome is presented in Fig.3C.

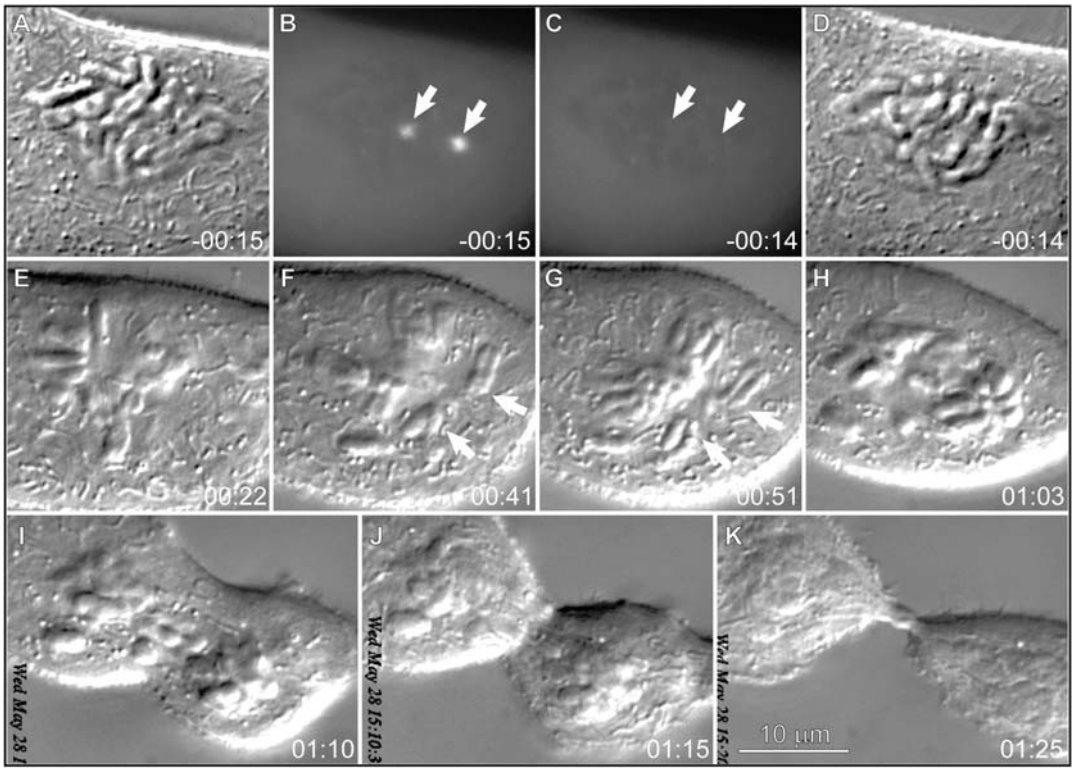
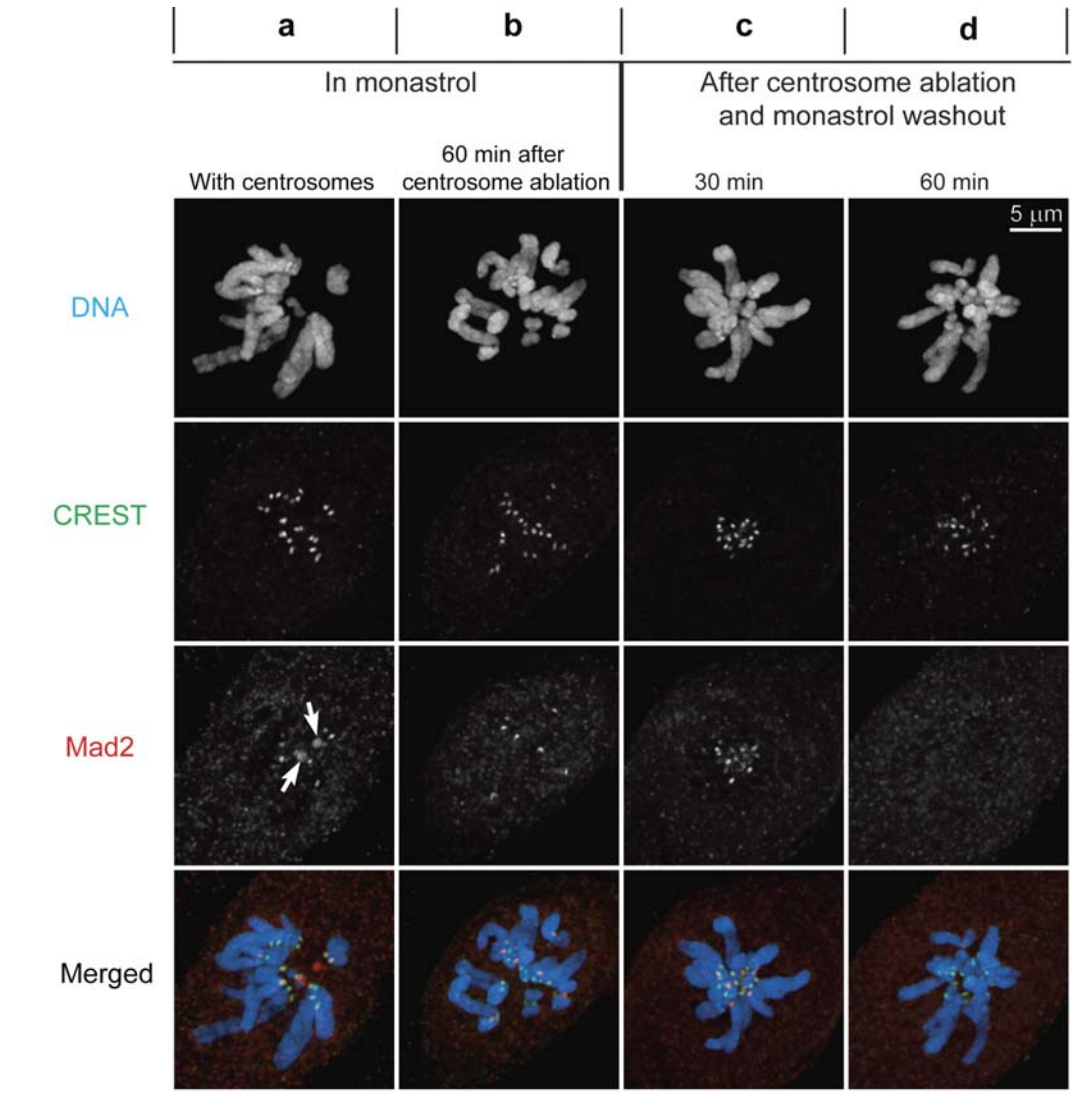


Figure S4. **Formation of functional mitotic spindle in a cell with opposite kinetochores in the absence of centrosomes.** Mitosis in a cell that was treated with 5-µM nocodazole, then both centrosomes were ablated (arrows in B and C), and nocodazole washed out (between D and E). The cell assembled a bipolar mitotic spindle in ~ 50 min (E-F) and subsequently divided into 2 daughter cells (G-K). However, notice that anaphase was initiated in the presence of two syntelic chromosomes (arrows in F and G).



	a	b	c	d
Average number of Mad-2-positive kinetochores	8 K/cell (12 cells)	4 K/cell (14 cells)	4 K/cell (10 cells)	0 K/cell (8 cells)
Sister-kinetochore separation (μm)	0.93±0.11	1.05±0.12	1.11±0.18	1.15±0.14

Figure S5. **The spindle assembly checkpoint (SAC) is satisfied approximately 1 hr after monastrol washout in cells lacking centrosomes.** **(A)** Monopolar mitoses induced by monastrol are arrested due to the SAC. Several kinetochores in every cell are Mad2-positive. Additionally, Mad2 is concentrated in the centrosomes (arrows). **(B)** Ablation of centrosomes does not abrogate the arrest. 60 minutes after centrosome ablation cells still contain several Mad2-positive kinetochores. **(C-D)** After ablation of both centrosomes and monastrol washout the number of Mad2-positive kinetochores gradually decreases so that 60 min after centrosome ablation and monastrol washout all kinetochores are Mad2-negative (D) and shortly after this time cells exit mitosis.