Full Methods

Live cell microscopy and laser ablations. PtKG-23 (a clone of PtK_1 line, constitutively expressing γ-tubulin/GFP[1]) and PtKαT (a clone of PtK_2 line, constitutively expressing α-tubulin/GFP[2]) cells were maintained in Ham's F12 medium (Invitrogen, Carlsbad, CA) supplemented with 10% foetal calf serum at 37°C, 5% CO_2. For laser microsurgery cells grown on 24x24-mm^2 coverslips were mounted in Rose chambers. Temperature of the chambers on the microscope stage was maintained between 35 and 37°C by a custom-built electronically-controlled stage-heater

Monastrol, synthesized as described earlier[3] was kept as 100-mM stock in DMSO and used at 100 μM. Nocodazole (Sigma, Saint-Louise, MO) was kept as 10-mM stock in DMSO and used at 5 μM. For drug washout top coverslip of the Rose chamber was removed and cells (on the bottom coverslip) were washed with 5-7 changes of full growth medium. To unambiguously identify the cell of interest its position was marked using a diamond objective-scribe (Zeiss, Thornwood, NY).

Laser ablation of centrosomes was performed as described elsewhere[4, 5]. Note that after laser ablation and drug washout time-lapse sequences were recorded not on the laser-microsurgery microscope but on a different similarly equipped Nikon Eclipse TE2000 or TE300 microscope. This was necessary to make the laser-microsurgery workstation available for other experiments during rather lengthy periods of time-lapse recordings. Time-lapse image sequences were recorded at 30-s or 60-sec intervals.

Immunofluorescence. For microtubule and NuMA staining cells were fixed with 1% glutaraldehyde in PEM buffer (100-mM PIPES, 3-mM $MgCl_2$, 5 mM EGTA, pH 6.9), permeabilized with 1% Triton X-100, and reduced with 1 mg/ml $NaBH_4$. All reagents were purchased from Sigma. For CREST and Mad2 staining cells were fixed with 3.8% paraformaldehyde in PBS, and then permeabilized and reduced exactly as for

microtubule/NuMA staining. The following primary antibodies were used: mouse anti-α-tubulin (cloneDM1α, Sigma-Aldrich); rabbit anti-NuMA (kind gift of Dr. Duane Compton, Dartmouth Medical School, Hanover, NH); human serum CREST SH (kind gift of Dr. Bill Brinkley (Baylor College, Houston, TX); and rabbit anti-Mad2 (BAbCO, Richmond, CA).

Fluorescence images were recorded with a 60x1.4 PlanApo lens as Z-series at 200-nm steps (detailed in reference[5]). They were deconvolved using SoftWorx deconvolution software (Applied Precision, Issaquah, WA) and presented as maximal intensity projections. Inter-kinetochore distances were measured in 3-D deconvolved datasets using SoftWorx.

Electron Microscopy. Serial-section EM analysis was conducted and surface-rendered 3-D models constructed as described in reference[6].

References For Full Methods

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