Full Methods

Cell Culture

Cell lines were maintained at 37°C with 5% CO_2 atmosphere in DMEM (U20S, hTERT-BJ, MCF-7, N1E-115, CFPAC-1), McCoy's (Caco2, HT-29, MDA-231), DMEM:F12 (hTERT-RPE1), MEM (UPCI:SCC114), or RPMI (BT549**)** containing 10% FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin. Murine p53 $^{-/-}$ tetraploid cells from tumors (tMMECs) were grown in DMEM:F12 supplemented with 2% FBS, 2 mg/ml insulin, 25 μg/ml EGF, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml fungizone. The tetracycline-inducible U2OS cell line expressing Myc-Plk4 (a kind gift from E. Nigg) was grown in DMEM supplemented with 10% of tetracycline-free FBS, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml hygromycin and 1 mg/ml G418. Myc-Plk4 expression was induced by addition of 1 μg/ml of doxycyclin for 15 hours.

To generate tetraploid cells with extra centrosomes, hTERT BJ and RPE-1 cells were transfected with 50 nmol p53 siRNA (SmartPool, Dharmacon) using Lipofectamine RNAi Max (Invitrogen). Approximately 6 hours later, fresh medium containing 0.2 μm cytochlasin D was added for an additional 16 hours. Cells were then washed 6 times for 5 minutes each to remove cytochalasin D and allowed to recover for ~6-24 hours.

Indirect Immunofluorescence Microscopy

All cells stained for centrioles were washed in PBS and then fixed in ice-cold methanol for 10 min. Following fixation, cells were rehydrated in PBS for 5 min., extracted in PBS-0.5% Triton X-100 for 5 min., blocked in TBS-BSA (10 mM Tris, pH 7.5, 150 mM

NaCl, 5% BSA, 0.1% Tween) for 30 min., and then incubated with primary antibodies in TBS-BSA for 30-60 min. Antibodies included mouse anti-tubulin (DM1α, 1:500; Sigma-Aldrich), human anti-centromere ACA (1:500; Antibodies, Inc), and rabbit anti-centrin2 (1:200; Santa Cruz).

To visualize merotelic kinetochores, cells were permeabilized in Ca^{2+} buffer (100 mM PIPES, pH 6.8, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 0.5% Triton X-100) for 5-10 min at room temperature, followed by fixation in 1% glutaraldehyde in PBS for 10 min., and two 12 min. washes in freshly prepared 0.5 mg/ml $NaBH_4$. After a 60 min. block in TBS-BSA, cells were incubated with primary antibodies in TBS-BSA for 2-4 hours. Antibodies included rat anti-tubulin (YL 1/2, 1:1000; Novus), rabbit anti-pericentrin (1:1000; AbCam), and mouse anti-Hec1 (1:200, Novus). All primary antibodies were detected using species-specific fluorescent secondary antibodies (Molecular Probes) and DNA was detected with 0.2 μg/ml DAPI (Sigma-Aldrich). Coverslips were mounted with ProLong Antifade mounting medium (Molecular Probes).

Confocal immunofluorescence images were collected at 405, 488, 561, and 640 nm with a Yokogawa CSU-X1 spinning disk confocal mounted on a Nikon Ti-E inverted microscope (Nikon Instruments). A series of 0.2 μm optical sections were acquired using a 100x 1.4 NA Plan Apo objective lens with an Orca ER CCD camera (Hamamatsu Photonics). Acquisition parameters, shutters, filter positions and focus were controlled by Andor iQ software. Images presented in figures are maximum intensity projections of entire z-stacks, except for high magnification images of merotelic attachments, which are

comprised of only enough z-layers to visualize entire kinetochores. Cells were classified as having >2 centrosomes if more than two distinct pairs of centrioles were observed during mitosis. Lagging chromosomes were scored as centromere-positive chromosomes that were completely separated from the two main masses of chromosomes during mid-anaphase (see Supp. Fig. 7). Kinetochores were scored as being merotelically attached if they were clearly attached to two visible kinetochore fibres emanating from opposite poles.

Long-term live-cell imaging

H2B-GFP was cloned into the pLenti6/V5 lentiviral vector (Invitrogen). Cells infected with lentivirus encoding H2B-GFP were FACS sorted by GFP fluorescence. GFP-positive cells were grown on glass-bottom 12-well tissue culture dishes (Mattek) and imaged on a TE2000-E2 inverted Nikon microscope equipped with a cooled CCD camera (Orca ER, Hamamatsu) and the Nikon Perfect Focus system. The microscope was enclosed within a temperature and CO_2-controlled environment that maintained an atmosphere of 37°C and 3-5% humidified CO_2. GFP and/or DIC images were captured at multiple points every 10 minutes for 3-5 days with a 20X 0.5 NA Plan Fluor objective. All captured images were analyzed using NIS-Elements software.

Short-term Live-Cell Imaging

GFP-centrin RPE-1 cells (a kind gift from A. Khodjakov) were infected with retrovirus encoding mRFP-H2B and sorted by RFP fluorescence. For imaging, cells were grown on glass-bottom 12-well tissue culture dishes (Mattek) overlayed with mineral oil and

maintained at 37°C. Confocal immunofluorescence images were collected at 488 and 561 nm with a Yokogawa CSU-X1 spinning disk confocal mounted on a Nikon Ti-E inverted microscope (Nikon Instruments). A series of 1 µm optical sections were acquired every 2 minutes using a 60x 1.4 NA Plan Apo objective lens with an Orca ER CCD camera (Hamamatsu Photonics). Images presented in figures are maximum intensity projections of entire z-stacks.

Generation of tetraploid cells with 2 centrosomes

Human hTERT RPE-1 and hTERT BJ cells were treated with 0.2 µm cytochalasin D for ~16hrs, washed every 5 minutes for 30 minutes, and then FACS sorted by DNA content using Hoechst at 1:2500 (Molecular Probes). Cells with a DNA content of 8c were isolated and cultured for ~1 week before a second FACS sorting to re-isolate 8c cells. A significant portion (~50%) of tetraploid cells displayed two centrosomes after only 2 sorts, and these cells were used to measure lagging chromosomes. By sort 4, nearly 100% of tetraploid cells had two centrosomes, and these were used for FISH analysis. We verified p53 function was intact in these late-passage tetraploid BJ and RPE-1 cells by western blotting for phospho-p53 (Ser15) after 5 hr. treatment with 200 ng/ml doxorubicin.

Chromosome Spreads

Diploid and late passage tetraploid cells were treated with colcemid (50 ng/ml) for 4 hrs, trypsinized, and resuspended in 0.56% KCl for 30 min. at 37°C. Cells were then fixed with 3:1 ice-cold methanol:acetic acid, pelleted, and then washed twice more with

methanol:acetic acid before being dropped on a pre-cleaned glass side from a height of ~12 inches. Cells were allowed to dry on the slide and were then stained for 3 min. with Giemsa stain in 1X Gurr's buffer (Gibco). Following a wash in Gurr's buffer, coverslips were added to slides and sealed with Permount (Fisher). Images of spreads were taken with a 100X objective on a Zeiss upright microscope and chromosomes were counted using PhotoShop.

FISH

Diploid and late passage hTERT BJ and RPE-1 cells were transfected with 50 nmol p53 siRNA (SmartPool, Dharmacon) using Lipofectamine RNAi Max (Invitrogen). Approximately 6 hours later, fresh medium containing 0.2 μm cytochlasin D was added for an additional 16 hours. Cells were then washed 6 times for 5 minutes each to remove cytochalasin D and allowed to recover for ~6-10 hours. Mitotic shake-off was then used to isolate dividing cells at a low density on fresh coverslips. Once cells were reattached, 0.56% KCl was added to the coverslips for 30 minutes followed by fixation in 3:1 methanol acetic acid. After two additional methanol:acetic acid washes, coverslips were dried completely and aged for two days. Coverslips were then washed in 2X SSC + 0.5% NP-40 for 30 minutes at 37°C and then dehydrated by sequential 2 min. washes in 70%, 85%, and 100% ethanol. Fluorescently-labeled centromeric FISH probes specific for chromosomes 6, 8, 7, or 11 (Cytocell) were diluted 1:10 into hybridization buffer (Cytocell) and added to coverlips. Coverlips were affixed to a pre-cleaned slide with rubber cement and then placed in a hybridization oven at 75°C for 5 min., followed by an overnight incubation at 37°C in a humidified chamber. The following day, cells were

washed in 1X PBD (0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, 0.1% NP-40) for 2 minutes to remove the rubber cement followed by a wash in 1X wash buffer (0.5X SSC + 0.1% SDS) for 5 minutes at 65°C. Coverslips were then incubated with 1X PBD + 0.2 μg/ml DAPI for 20 minutes before being sealed on a slide with mounting medium. FISH signals were only counted in daughter cells that possessed a single nucleus, eliminating the possibility that the cells had undergone a multipolar mitosis followed by cytokinesis failure. FISH signals in micronuclei were ignored since it could not be definitively determined which of the two daughter cells the micronuclei were within. Thus, our rates of chromosome missegregation in tetraploid cells with extra centrosomes are likely to be underestimates. The reported rates of chromosome missegregation are a sum of each of the 4 individual probes we used (6, 8, 7, 11).