SUPPLEMENTAL METHODS

Determination of centromere location using CENPA IF-FISH and ChIP-PCR

For each cell line and HSA17 that had not been previously studied, the location of the functional centromere was defined using metaphase chromosome CENPA immunostaining and FISH with probes specific to D17Z1 and D17Z1-B as previously described (Maloney et al. 2012). Chromatin fiber CENPA IF-FISH was used in some instances in which it was difficult to separate D17Z1 and D17Z1-B on metaphase chromosomes (Lam et al. 2006; Sullivan et al. 2011). All immunofluorescence experiments were visualized using the DeltaVision Elite imaging system (Applied Precision, Inc./GE Healthcare) running the SoftWoRx software. CENPA ChIP-PCR was used to confirm centromere location using custom human CENPA polyclonal antibodies (AP3497, Quality Controlled Biochemicals, QCB, Hopkinton, MA) and magnetic ChIP followed by quantitative PCR as previously described (Sullivan et al. 2011; Maloney et al. 2012).

FISH on Fixed Interphase Cells

Exponentially growing cultures were arrested by treating with 10 ug/mL colcemid (Invitrogen) for 45 min. Cells were pelleted, incubated in hypotonic (1:1:1 75mM KCl:0.8% NaCitrate:dH2O) for 8 minutes, and fixed (3:1 methanol:acetic acid). Cells were dropped on microscope slides and aged overnight at room temperature before proceeding with FISH. Chromosomes were incubated in RNase/2X SSC for at least 30 minutes at 37°C and dehydrated through 70%, 95%, and 100% ethanol for 2 minutes each. Slides were denatured in 70% formamide/2X SSC pH 7 at 75°C for 55 seconds and again dehydrated. Two slides for each single cell clone were hybridized. On one, HSA17 was detected using 150ng of a plasmid p17H8 (D17Z1) labeled with

Alexa Fluor 488-dUTP (Molecular Probes, Invitrogen). On the other, HSA17 was detected using 150ng p17H8 labeled with Alexa Fluor Texas Red-dUTP or 568-dUTP (Molecular Probes, Invitrogen). Probes were resuspended in 65% formamide hybridization mixture, and post-hybridization washes consisted of 65% formamide/2X SSC/0.1% Tween. For detection of control mouse chromosomes, BAC probes RP23-433d12 and RP24-75b21 and mouse Cot-1 DNA were denatured in 50% formamide hybridization mixture and pre-annealed at 37°C prior to addition to denatured slides. Post-hybridization washes contained 50% formamide. At least 100 cells were analyzed per slide. Images were collected using the 100X objective on either a Zeiss AxioCam microscope managed by AxioVision software or the DeltaVision Elite. Images were exported to Adobe Photoshop CS5.1 or CC 2015.5 for visualization.

Alpha satellite array sizing using DNA Fibers

Proliferating cells were pelleted, washed with 1X PBS, resuspended in hypotonic (1:1:1 75 mM KCl:0.8% NaCitrate:dH2O) to a concentration of 1 x 10^4 cells/mL, then centrifuged onto charged glass microscope slides (Superfrost plus) for 4 minutes at 800 rpm (high acceleration) using a Shandon Cytospin 4. Using double cytofunnels, cells were deposited into two 12x12mm circles on the slides. The slide was placed between two microscope (boundary) slides that had been glued onto a flat plastic surface. Two 1mm coverslips had been glued to the top of each boundary slide. Ten microliters of lysis buffer (0.5% SDS, 5mM EDTA, 100mM Tris-HCl) was applied to each cell sample area and incubated for 5 minutes at room temperature. A fresh cover glass was used to manually drag the liquid down the length of the slide. The slides were air dried for 5 minutes followed by immersion in 3:1 methanol:acetic acid fixative for 5 minutes

at room temperature. Slides were aged overnight at room temperature before proceeding with FISH with plasmid probes specific for D17Z1 (p17H8) and D17Z1-B (p2.5-3) (generous gifts of H.F. Willard). To prevent cross-hybridization, high stringency (68% formamide) hybridization and washes were used. Images were captured using an Olympus IX71 inverted microscope connected to the DeltaVision Elite deconvolution imaging system (Applied Precision) and processed with the SoftWoRx Resolve3D program. Since fibers spanned multiple fields of view, images were collected using the Collect Panels option of SoftWoRx. Images were deconvolved using the conservative algorithm with 10 iterations and viewed for presentation and analysis using the quick projection option. Fibers extending through multiple fields of view were merged into a single image using the Stitch function. Array sizes were calculated in micrometers using the Measure Distances tool and selecting the Multiple Segment option. At least 20 fibers were analyzed for each cell line, and for consistency, only fibers greater than 15 micrometers were measured. The ratio of D17Z1:D17Z1-B sizes was calculated by dividing the D17Z1 array size by the D17Z1-B array size. The ratios were averaged for each cell line analyzed.

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