

Supplementary information

Vector construction. Three BACs containing either chromosome 17 alphoid (α) DNA (138kb, BAC 227J24 and 57 kb, BAC 302P22) (Kim *et al.*, 1996) or chromosome 21 α DNA (67 kb, BAC pWTR11.32) (Ohzeki *et al.*, 2002) were modified prior to HSV-1 amplicon delivery. BAC pWTR11.32 contains a neomycin resistance gene (neo^r) under the control of the SV40 promoter, and was modified by the vector pHG (Wade-Martins *et al.*, 2003) containing the HSV-1 amplicon elements (ori_s and pac) and GFP by restriction enzyme digestion and ligation. Briefly, the plasmid vector pHG (originally known as pSG80A-HG, a kind gift from Yoshinaga Saeki) was linearised with Sal I generating a 6 kb Sal I fragment which was ligated to a 67 kb Sal I fragment containing the BAC pWTR11.32 generating pHSV21 α Neo (73 kb) containing the HSV-1 elements. The vector pHG was then modified to contain a neomycin resistance gene cassette (neo^r), generating pHSVGKNeo, prior to retrofitting BAC 227J24 and BAC 302P22 by Cre-*loxP* recombination (Wade-Martins *et al.*, 2001). The resulting BACs were called pHSV17 α -1Neo (146 kb) and pHSV17 α -2Neo (65 kb). To generate vector pHSV21 α HPRTNeo, a Xba I/SalI 2.7 Kb fragment, corresponding to the HPRT minigene, was cut from pBT/PGK-HPRT (Magin *et al.*, 1992) and ligated to XbaI/SalI pHG. The resulting construct (pHSVGKHPRT) was then SalI linearised and ligated to pWTR11.32 as described above generating pHSV21 α HPRTNeo.

Tissue culture. The human cell lines HT1080, 293, G16-9, MRC-5V2 and MRC5 were cultured at 37°C in 5% CO₂ (vol/vol) in D-MEM Glutamax (Invitrogen, UK) supplemented with 10% FCS (PAA Laboratories, Austria), penicillin (100 U/ml) (Biowhittaker, UK) and streptomycin (100 ug/ml) (Biowhittaker, UK). BeFA cells were cultured in Keratinocyte-SFM (Invitrogen, UK) supplemented with EGF and Bovine Pituitary Extract (according to manufacturer's instructions). The packaging cell line Vero 2-2 (a kind gift from Rozanne M. Sandri-Goldin) was cultured in G418 (Geneticin; Invitrogen, UK) 500 ug/ml. G16-9 cells, a derivative of the human glioma cell line Gli-36 (Kashima *et al.*, 1995), were cultured with 200 ug/ml hygromycin (Hygromycin Gold, Invitrogen, UK). The following cell lines were purchased from ECACC: HT1080

(85111505), 293 (85120602), MRC5 (97113601). BeFA cells were a kind gift from Alain Hovnanian and MRC5-V2 a kind gift from Elaine Levy.

Amplicon production. HSV-1 amplicons containing pHSV_{GK}Neo, pHSV17 α -1Neo, pHSV17 α -2Neo, pHSV21 α Neo and pHSV21 α HPRTNeo were generated in Vero 2-2 cells, and concentrated as described (Wade-Martins *et al.*, 2001). We estimated the titer following transduction of 2.5×10^5 G16-9 (glioma) cells by monitoring the number of GFP expressing cells in each well divided by the total number of cells. Titers of $2-4 \times 10^7$ transducing units (t.u.)/ml were usually obtained for each amplicon. Each human cell type (HT1080, MRC-5V2, G16-9, BeFA, 293, MRC5) was then infected with the HSV-1 amplicons at a multiplicity of infection (MOI) of 1, 5 or 10. As the G16-9 cells were used to estimate the number of transducing units and titer of each amplicon preparation, the GFP data related to this cell line are not shown in Table 1. Cells were split and replated in 4 wells and after 24 hours selection was applied: 15 μ g/ml for BeFA and MRC5 cells, 100 μ g/ml for 293 cells, 350 μ g/ml G418 for HT1080, MRC-5V2, and 500 μ g/ml for G16-9 cells. Typically cells died under selection within 4-8 days. Positive clones were identified 14-21 days later.

Lipofection of Cultured Cells. HAC vector DNA embedded in agarose was condensed with PEI and introduced into human HT1080 cells by lipofection as described (Mejía *et al.*, 2002). After 48 hrs, G418 350 μ g/ml was applied to the media, and positive clones were isolated 10-14 days later.

Generation of subclones. Some clones that contained HACs were also subcloned to obtain a higher percentage of HAC-containing cells. Cells were harvested from subconfluent dishes by trypsinisation, counted using a hemocytometer, and diluted to a single cell per well. Cells were seeded in 96-well plates and wells containing single cells were identified. Clones were allowed to form and then cells were expanded for further analysis.

FISH analysis. The clones derived from HAC amplicon infection were expanded, and metaphase spreads were prepared from cells onto glass slides. Chromosomes were harvested using standard techniques. FISH probes were labeled with biotin-16-dUTP or digoxigenin-11-dUTP (Roche, UK) by nick translation using a commercial kit (Invitrogen, UK) following manufacturer instructions. Hybridization was carried out at

42°C overnight, followed by 3 post-hybridization washes in 0.1xSSC at 60°C. Biotin labeled probes were detected using Avidin FITC (Molecular Probes, Invitrogen, UK), followed by biotinylated anti-avidin antibody (Vector Laboratories, UK), and a second layer of Avidin FITC. Digoxigenin probes were detected with rhodamine conjugated sheep anti-digoxigenin antibody (Roche, UK), followed by rhodamine anti-sheep antibody (Chemicon Europe, UK). Chromosomes were counterstained with DAPI and slides mounted in DABCO (Sigma, UK) antifade. Slides were analyzed with an Olympus BX60 microscope for epifluorescence equipped with a Sensys CCD camera (Photometrics, USA). Images were collected and pseudocoloured using MacProbe 4.3 software.

ACA and CENP-A detection. The capacity of HACs to bind centromeric proteins was shown by immunofluorescence on metaphase chromosome spreads with anti-centromere autoimmune serum (ACA; a kind gift from William Earnshaw) which reacts with all of the major centromere proteins at the centromere, and anti-human CENP-A antibody which recognizes CENP-A, a centromere specific histone-like protein. For ACA detection, 50 ul of chromosome suspension fixed in methanol:acetic acid (3:1), were dropped onto clean slides and briefly allowed to air dry. After a 5 min incubation in PBS, slides were washed in TEEN buffer (1mM Triethanolamine:HCl pH 8.5/ 0.2 mM EDTA/ 25 mM NaCl), containing 0.1% Triton X-100, and 0.1% BSA, and incubated 30 minutes at 37°C in ACA serum (a kind gift from William Earnshaw) diluted 1:5000 in the same buffer. After 3 washes in 10 mM trisHCl, pH 8.0/ 0.1% BSA, FITC-anti-human secondary antibody (10 ng/ml, Sigma, UK) were applied to the slides. After 3 further washes in 10 mM trisHCl, pH 8.0/ 0.1% BSA, cells were fixed in 4% paraformaldehyde (Science Services, UK) diluted in PBS, for 15 min. Slides were washed in 2xSSC for 5 min, and then 10-15 ul of FISH probe was applied under a coverslip. The probe and the slide DNA were simultaneously denatured at 80°C for 5 min on a PCR block. FISH was then carried out as above.

For CENP-A detection, a subconfluent layer of cells was incubated for 3 hours in 0.03 ug/ml Colcemid (Karyomax, Invitrogen, UK). Cells were harvested and resuspended in cold KCl 56 mM at 7×10^5 /ml. Approximately 200 ul of cell suspension were usually spun onto poly-lysine coated slides (BDH, UK) at 800 rpm for 4 min, using a Shandon

cytospin machine. The cells were then permeabilised in KCM buffer (Mejía *et al.*, 2002) for 10 min and incubated 30 minutes at 37°C in 2 ng/ml anti-human CENP-A antibody (murine; Upstate, UK) diluted in PBS and 1% BSA. Slides were washed 3 times for 5 min in KCM, and then 10 ng/ml FITC conjugated anti-mouse secondary antibody (Chemicon, UK) was applied to the slides. Slides were washed again 3 times for 5 min in KCM buffer, and then the preparations were fixed in 2% paraformaldehyde (Science Services, UK) diluted in KCM for 15 min. Slides were washed in 2xSSC for 5 min, and then 10-15 ul of FISH probe was applied under a coverslip. The probe and the slide DNA were simultaneously denatured at 80°C for 5 min on a PCR block. FISH and image processing was then carried out as above.

MMCT. MRC5-V2 cells were transformed with a blasticidin resistant cassette (pUBBsds, Invitrogen). Stable clones were obtained after selection in 6 ug/ml blasticidin (Invivogen), and 2 clones were expanded and used as recipient cells in the MMCT experiment. The donor parental cells, AG6-1 (Mejía *et al.*, 2001) were incubated 48 hours with Colcemid 7 ug/ml. The metaphases were harvested and resuspended in 50% Percoll (Sigma) in D-MEM 10% FCS, 10ug/ml Cytochalasin B (Sigma). Centrifugation was carried out at 20° C in a AVANTI J301 Beckman Coulter centrifuge, 75 minutes at 19.000 rpm, in a JA20 rotor. The fraction containing the microcells was diluted 1:2 in complete medium, centrifuged and mixed in a 3:1 ratio to the recipient cells and fused using PEG 1000 (Sigma). After 48 hours, selection was applied as follows: G418 125 ug/ml, puromycin (Sigma) 0.22 ug/ml, blasticidin 4 ug/ml. Stable clones were recovered after 14 days.

Cytokinesis block micronucleus assay. Cytochalasin B 4ug/ml was added to subconfluent dishes of HT1080, G16-9, MRC5-V2 and 293. After 24 hours the cells were harvested by trypsinization and resuspended in PBS at 10⁵ cells/ml. 100 ul of the cell suspension were spun onto poly-lysine coated slides, and subjected to CENP-A detection as described above. Binucleated cells were then scored for the following criteria: presence of bridges, and micronuclei (MNs) (either CENP-A positive or CENP-A negative). Only binucleated cells with round nuclei, of similar size and shape were considered. MN's were only scored when they appeared to be between 1/3-1/6 of the nucleus, of similar colour and density.

The remaining cells were resuspended in Carnoy's fixative containing 3.7% formaline, then washed twice in Carnoy's fixative only, and dropped onto clean slides. The cells were then hybridized as described above to BAC 227J24 and BAC pWTR11.32. To estimate the level of aneuploidy, binucleated cells were analysed for the presence of non-disjunction events (one nucleus showing one or more centromeric signal than the modal number, and the other one or more less), or chromosome loss (MN showing a centromeric signal, and corresponding nucleus lacking one or more signals). The data for the two probes were then pooled together. It must be noted that BAC pWTR11.32 detects both chromosome 21 and chromosome 13, while BAC 227J24 only recognizes chromosome 17.

Congression analysis. HT1080, G16-9, MRC-5V2 and 293 cells were grown on a 24x24 mm coverslips until they reached about 60% confluency. Keeping the slides at 37°C, the cells were then fixed in 4% paraformaldehyde in cytoskeleton buffer (Wheatley and Wang, 1996), permeabilized with 0.15% Triton X-100 in cytoskeleton buffer and incubated in mouse anti- β -tubulin antibody (Sigma) plus ACA serum.

Western blotting. For each cell line, a nuclear protein fraction was prepared from a confluent 10 cm dish, either unsynchronized or blocked in metaphase by treatment with Colcemid 1 μ g/ml for 24 hours. Briefly, the harvested cells were lysed in PBS, 0.5% Triton X-100, 1x Protease Inhibitor (Roche), for 10 minutes, then extracted overnight in 0.2M HCl. 20 μ g of each preparation were then run on polyacrilamide gels, and electroblotted onto PDVF membranes (Invitrogen). These were reacted with: anti-AIM 1 mouse antibody (BD Biosciences), anti Kin17 rabbit antibody (Abcam), anti SMC3 rabbit antibody (Abcam), anti topoisomerase I rabbit antibody (Abcam), anti Topoisomerase II α mouse antibody (Oncogene), anti HP1 α mouse antibody (Upstate), anti CENP-A mouse antibody (Abcam), anti-H3 trimethylated in lysine 9 rabbit antibody (Abcam), anti-H3-phosphorylated in serine 10 (Upstate) mouse antibody, anti-H3-phosphorylated in serine 28 (Abcam) rabbit antibody. The signals were detected with the anti rabbit/mouse HRP (Biorad) and ECL plus system (Amersham) using Biorad ChemDoc XRS software. The densitometrical analysis of each band was carried out using the software QuantityOne. Values for H3-phosphorylated in serine 10 and H3-phosphorylated in serine 28 were corrected by adjusting for the differences in mitotic

index among cell lines, which were as follows: HT1080 33.3%, G16-9 19%, MRC5-V2 26%, 293 22%, primary MRC5 52%. The cytosolic protein fractions were prepared using the Nuclear extraction kit (Panomics) according to the supplier instruction. Specific proteins were detected using anti-HPRT rabbit antibody (Abcam) and anti GAPDH rabbit antibody (Abcam). Quantification was carried out as described above.

References

Kashima, T., Vinters, H.V. and Campagnoni, A.T. (1995) Unexpected expression of intermediate filament protein genes in human oligodendrogloma cell lines. *J. Neuropathol. Exp. Neurol.* **54**, 23-31.

Kim UJ, Birren BW, Slepak T, Mancino V, Boysen C, Kang HL, Simon MI, Shizuya H (1996) Construction and characterization of a human bacterial artificial chromosome library. *Genomics.* **34**, 213-218.

Magin, TM, McEwan, C, Milne, M, Pow, AM, Selfridge, J, and Melton DW (1992) A position and orientation dependent element in the first intron is required for expression of the mouse HPRT gene in embryonic stem cells. *Gene* **122**, 289-296

Wheatley SP, Wang YL (1996) Midzone microtubule bundles are continuously required for cytokinesis in cultured epithelial cells. *J Cell Biol* **135**, 981-989.

Legends to Figures in Supplementary Information

Figure 4.

HPRT FISH. Clone HF15.1 (HT1080, derived from pHSV21 α HPRTNeo) hybridized to PAC 71G4 (red signal), containing the whole HPRT genomic locus. The probe highlights the HAC (white arrowhead) and the endogenous HPRT gene on chromosome X (yellow arrowhead).

Figure 5

GFP gene expression in stable clones. A) G16-9 clone 17 α III.10.1 (derived from pHSV17 α 1Neo) on selection. B) After 90 days in culture in the absence of selection, the number of GFP expressing cells does not decrease. C) HT1080 clone 21 α Neo A.10.1 (derived from pHSV21 α Neo), on selection. D) After 90 days in culture in the absence of selection the number of GFP expressing cells is unchanged, but the intensity of GFP fluorescence is much lower. GFP expression in the HSV-1 amplicons is driven by the promoter of the HSV I/E genes, which is transactivated by the viral VP16 protein. This protein enters the cells at the moment of infection, but is then degraded. While G16-9 has been stably transformed with a VP16 expressing construct, HT1080 lacks this transactivating protein, and so a decrease in the overall fluorescence in these cells is expected.

Figure 4

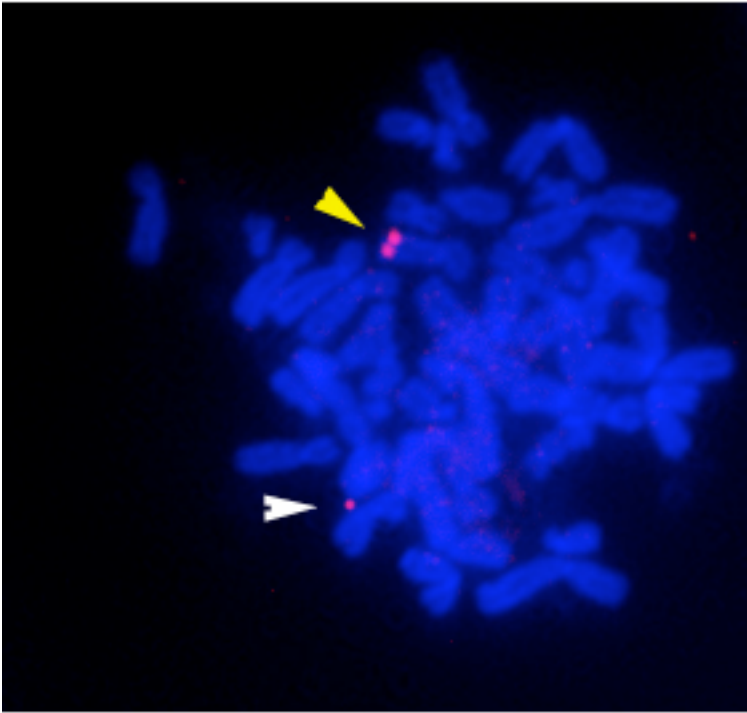


Figure 5

