Supplemental Protocol S2 - BAC probe preparation and 3D-FISH

**BAC probe preparation and FISH.** Bacterial artificial chromosomes (BACs) were selected and ordered from libraries available in the RP11-collection at the Sanger Institute (Cambridge, UK). For each domain, BACs were selected for a 2.2 Mb region surrounding the integration site (in the centre of the region), with an average gap size of 30 KB between BACs. BAC positions were according to UCSC genome build HG15. With BAC size averaging around 170 kb, this resulted in a homogeneously spaced set of probes with 85% coverage of the domain. BAC DNA was isolated using the REAL Prep 96 kit (Qiagen) and verified by end sequencing with SP6 (GATCCTCCGAATTCAGTGG) and T7 (TAATACGACTCACTATAGGG) primers, using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequence reactions were analyzed on an ABI 3730 Automated Sequencer (Applied Biosystems). For the preparation of domain probes, BACs of the same domain were pooled prior to DOP-PCR amplification. Four different ‘Degenerate Oligonucleotide Primer-PCR’ (DOP) PCR primers were used (Fiegler et al. 2003), each containing a different highly abundant annealing sequence: (a) 6MW (CCGACTCGAGNNNNNNATGTGG), (b) DOP1 (CCGACTCGAGNNNNNNCTAGAA), (c) DOP2 (CCGACTCGAGNNNNNNNTAGGAG) and (d) DOP3 (CCGACTCGAGNNNNNNNTTCTAG). PCR products of four independent PCRs using the different DOP-PCR primers were pooled prior to nick-translation in order to achieve a maximum DNA-probe coverage and to prevent PCR-based sequence bias. Nick-Translation was used to label the probes with FITC (Roche). All probes were tested for hybridization specificity and domain coverage on metaphase spreads.

**FISH on interphase nuclei.** Cells of HEK293 clones 1-5 were incubated with a 30 min pulse of 25 μM BrdU (Sigma-Aldrich) to label replicating DNA prior to fixation in 4% (w/v) formaldehyde, in order to detect S-phase and G2/M-phase cells, which were excluded. Denaturation was carried out at 78°C in 2x SSC, containing 50% formamide. Hybridization was allowed to proceed overnight at 37°C. Post-hybridization washes were carried out with 2x SSC/ 50% formamide at 45°C. All incubations for probe detection were done at room temperature in 4x SSC, containing 5% (w/v) non-fat dried milk. FITC-conjugated antibodies (Jackson Immunoresearch Laboratories, Inc.) were used to visualize the hybridization signals. Slides were mounted in Vectashield containing DAPI (Vecta Laboratories).

**Image processing and data evaluation.** All images were deconvoluted using Huygens Professional 2.7 software (Scientific Volume Imaging BV, Hilversum, The Netherlands), using measured point spread functions. Chromatic shifts were measured using Tetraspeck fluorescent beads (Invitrogen) and corrected by the analysis software. Image analysis was carried out with Argos software (http://homepages.cwi.nl/~wimc/argos). To identify and quantitatively analyze FISH-labeled areas, deconvolved 3D images were treated with a bandpass filter and subsequently segmented using a range of thresholds. Normalization of recorded images was carried out beforehand to scale the voxel values such that the minimum and maximum voxel intensity values were equal to the minimum and maximum values of 16 bit images (65,536 grey levels). Collections of connected voxels were regarded as regions.

**References**