## Supplemental Experimental Procedures

### Three Dimensional Analysis

Background subtraction was performed on image stacks before image quantification because a typical nuclear volume contained tens of thousands of voxels. The background fluorescence contributed ~20% of the total integrated fluorescence intensity. To measure background, off-cell pixel intensity was measured in two areas for each section, and the mean pixel intensity value was subtracted.

Nuclei were isolated for analysis because Imaris software would frequently join individual nuclei when using the entire imaging field. Also, it is much easier to recognize patterns of chromatin organization while working with isolated nuclei. Image stacks of isolated nuclei were extracted from the full image stack using the subset processing function in Zen Black software. Since we have yet to find a nuclear lamin antibody that works well with array tomography (AT), DAPI was used to determine the location of the nuclear membrane with confirmation using Concanavalin A (ConA). To create a surface surrounding the entire nucleus, a threshold was established based on laminar DAPI intensity, and a binary image for the nuclear area was created for each image section. A nuclear surface was created from the binary image stack using Imaris software (Bitplane , Switzerland). Once the nuclear surface was created, this object could be used to extract intensity information for every fluorescence channel acquired for the experiment.

The heterochromatin threshold was determined by sampling the DAPI pixel intensity surrounding pericentromeric heterochromatin, and this region was also confirmed to contain a high density of the repressive histone modifications used in this study (H3K9me3, H4K20me3, H3K27me3). With DAPI fluorescence filling the dynamic

range of the camera, this threshold typically was ~50 on an 8 bit scale. Imaris software was used to generate a surface around the heterochromatin (see Figure 1E), and H3K27me3 immunostaining was used to split the surface containing Xi heterochromatin from attached pericentromeric heterochromatin. The heterochromatin threshold includes constitutive, major satellite-rich heterochromatin as well as areas of densely packed facultative heterochromatin (for example Xi). In general, most neurons have highly decondensed chromatin, and laminar globules of chromatin (~200 nm diameter) fell below the heterochromatin threshold. For quantification of pixel intensity within the major satellite territory, a similar strategy was used to create a threshold surrounding the major satellite FISH signal.

**Confocal Laser Scanning Microscopy**

Mice were anaesthetized by intraperitoneal injection of Avertin (2,2,2-tribromoethanol) and perfused transcardially with 4% paraformaldehyde in PBS with 4% sucrose. Brains were cryoprotected overnight in 30% sucrose in PBS. Cryostat sections (20 μm) underwent antigen retrieval with cold (-20°C) acetone for 8 minutes. Sections were washed with water and PBS for 5 minutes before treatment with boiling citrate buffer for 8 minutes. Sections were cooled to RT, followed by incubation in blocking solution containing PBST (0.01% Triton X-100 in PBS) and 10% normal donkey serum for 1 hour at RT. Sections were incubated overnight at 4°C with rabbit anti-MeCP2 (Cell Signaling, 1:500) and mouse anti-H4K20me3 (Abcam, 1:500) in blocking solution. Sections were washed and incubated for 1 hour at RT with Alexa secondary antibodies (donkey anti-rabbit 568 and donkey anti-mouse 488) in blocking solution. Sections were

mounted using Prolong Gold plus DAPI. All images were acquired using a Zeiss LSM710 system with a 63X objective.