The noncoding RNA Xist recruits silencing factors to the inactive X chromosome (Xi) and facilitates re-organization of Xi structure. Here, we examine the mouse epigenomic landscape of Xi and assess how Xist alters chromatin accessibility. Xist deletion triggers a gain of accessibility of select chromatin regions that is regulated by BRG1, an ATPase subunit of the SWI/SNF chromatin-remodeling complex. In vitro, RNA binding inhibits nucleosome-remodeling and ATPase activities of BRG1, while in cell culture Xist directly interacts with BRG1 and expels BRG1 from the Xi. Xist ablation leads to a selective return of BRG1 in cis, starting from pre-existing BRG1 sites that are free of Xist. BRG1 re-association correlates with cohesin binding and restoration of topologically associated domains (TADs) and results in the formation of de novo Xi ‘superloops’. Thus, Xist binding inhibits BRG1’s nucleosome-remodeling activity and results in expulsion of the SWI/SNF complex from the Xi.

Differential dependence of Xi regions on Xist RNA. To investigate how Xist impacts Xi chromatin accessibility, we performed an assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-Seq assay) in female mouse fibroblasts harboring an Xi on which Xist was conditionally deleted after XCI establishment (XaWT XiΔXist). These cells are hybrid and display an Xa of Mus castaneus (cas) origin and an Xi of Mus musculus (mus) origin, which allows allele-specific analysis. To increase available allelic read depth, we pooled two highly reproducible biological replicates performed in the wild-type (WT) and XaWT XiΔXist cell lines (Supplementary Fig. 1a and Supplementary Dataset 2). In WT cells, ATAC-seq data demonstrated a clear bias in accessibility on the Xa, as shown by the depletion of mus reads relative to cas reads (Fig. 1a), consistent with a previously published profile14.

1Howard Hughes Medical Institute, Boston, MA, USA. 2Department of Molecular Biology, Massachusetts General Hospital, Boston, MA, USA. 3Department of Genetics, Harvard Medical School, Boston, MA, USA. 4Institute for RNA Medicine, Department of Pathology, Cancer Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA. 5These authors contributed equally: Teddy Jégu, Roy Blum.

*e-mail: lee@molbio.mgh.harvard.edu
In Xist-deleted cells, the number of ATAC-seq peaks was substantially increased on the Xi (Fig. 1b), indicating restoration of chromatin accessibility after ablating Xist. By identifying peaks with significant differences in allelic read counts in WT versus mutant cells, we categorized regions into four accessibility classes (Fig. 1c,d and Table 1). First, the ‘Xi-only’ regions (6.4%) were those accessible only on the Xi in both WT cells and mutant cells (Fig. 1c,d and Table 1). Second, the ‘monoallelic’ class was accessible exclusively on the Xa in both cell lines and accounted for the majority (84.3%) of all X-linked regions. Third, ‘bi-allelic’ regions (6.4%) were those accessible on both alleles and in both cell lines and positioned adjacent to promoters of escapee genes that are immune to XCI (21–26) (Fig. 1c,d and Table 1).

Finally, there was a fourth new class—‘Xi-restored’ (8.8%; 37 regions)—that became bi-allelically accessible when Xist was deleted (Fig. 1c,d and Supplementary Fig. 1b). In all 37 cases, the restored peaks corresponded to regions exclusively accessible on the Xa in WT cells and were not randomly scattered de novo accessibility events. Principal component analysis clearly distinguished among the three major accessibility classes using only two principle components, suggesting that a linear model can be trained to classify allelic read counts based on features that enabled derivation of the three major accessibility classes (Fig. 1e). Targeted allele-specific formaldehyde-assisted isolation of regulatory elements (FAIRE)-qPCR (Supplementary Fig. 1c) and ATAC-seq replicates performed on an independently derived XaWT XiΔSat cell line, as...
Table 1 | Genes displaying a classified accessibility peak at their proximal promoter site (±500 bp of the TSS) on the Xi in WT cells

| Xi only  | Bi-allelic |
|---------|-----------|
| Firre   | 181003007Rik |
| Xist    | Med14     |
| Ddx3x   | Kdm6a     |
| Utp14a  | Eif2s3x   |
| Jpx     | Ftx       |
| S530601H04Rik | Tmem29 |
| Kdm5c   | Mid1      |

These genes correspond to previously reported escapee genes.

well as on a second WT fibroblast cell type, yielded similar results and confirmed the existence of an Xi-restored class (Supplementary Fig. 2a–f). Together, these data suggest that Xist is responsible for chromatin-accessibility repression at specific Xi regions.

To assess Xist binding patterns on the Xi-restored regions, we employed allele-specific capture hybridization analysis of RNA targets with deep sequencing (CHART-seq) of WT cells (Fig. 2). This analysis revealed a gradient of Xist binding on the Xi. Accessible bi-allelic and Xi-only (Firre (functional intergenic repeating RNA element)) regions (Fig. 2a–c) showed the least Xist binding in WT cells—consistent with their chromatin’s being accessible and their corresponding genes being expressed. Monoallelic regions showed intermediate Xist coverage, and the positive coverage was consistent with their being silent and inaccessible on the Xi (Fig. 2a–c). Xist preferentially targets Xi-restored regions (Fig. 2a–c). Thus, regions sensitive to Xist depletion were in general those that exhibited the greatest Xist binding levels in WT cells. Taken together, CHART and ATAC data suggest that Xi chromatin is not homogeneously regulated and that Xi regions are differentially dependent on Xist for suppression of chromatin accessibility during XCI maintenance.

Because Xist depletion induces Xi chromatin re-accessibility, we carried out allele-specific RNA-seq to investigate gene reactivation. To identify genes sensitive to Xist depletion, we subclassified X-linked genes by employing a support vector machine (SVM) classifier model trained on allelic read counts of the three major accessibility classes. The vast majority of RefSeq genes (90.7%) were classified as monoallelic in expression, while the remaining 9.3% were classified as bi-allelic in expression (Supplementary Fig. 2g and Supplementary Dataset 4). None of the RefSeq genes were classified as Xi-restored (for example, reactivated) by the SVM model across two biological replicates. This demonstrated that Xi-restored accessibility peaks were not accompanied by gene reactivation. We observed a direct correlation between the distance from the transcription start site (TSS) to the closest ATAC peak and the gene expression level (Supplementary Fig. 2h). Genes with accessible regions within 1 kb of their TSS manifested the highest expression levels. These findings suggest that the Xi-restored regions are not directly linked to gene-expression alterations (Supplementary Fig. 2g) and thus likely to correspond to distal regulatory elements rather than to gene promoters (Supplementary Fig. 2h).

Epigenetic attributes of Xi re-accessible regions. Next, we examined peak distributions of the accessibility classes over genomic features and observed that Xi-restored peaks were distinct from all other classified ATAC peaks (Fig. 3a, b); a large percentage of regions that became accessible after Xist loss therefore appeared to correspond to distal regulatory elements, rather than promoter elements, whereas the monoallelic and bi-allelic peaks tend to localize more frequently in the promoter region.

We then searched for known transcription factor motifs, as well as de novo motifs inside ATAC peaks to identify potential defining features for each class. We observed that the monoallelic class was enriched for the binding motif of transcription factor SP1, whereas the bi-allelic class was enriched for the architectural factor CTCF (Fig. 3c, Supplementary Note 1a,b and Supplementary Dataset 5). These observations are consistent with earlier studies showing that SP1 binds preferentially to the Xa and cannot be detected over Xi regulatory elements (Fig. 3c, Supplementary Note 1c and Supplementary Dataset 5). Of particular interest, these motifs were recently found enriched in distal regulatory regions bound by BRG1-containing SWI/SNF nucleosome-remodeling complexes—an association of potential relevance, given that SWI/SNF complexes help regulate chromatin accessibility. We wondered whether Xist ablation could also influence chromatin compaction around Xi-restored regions. Thus, we performed allele-specific compaction studies using microccocal nuclease (MNase) digestion over time, followed by qPCR (MNase preferentially releases nucleosomes from decompacted chromatin). Greater MNase digestion was observed only on the Xi allele over the Xi-restored regions of the mutant, as evidenced by the significantly decreased signals across the MNase time course (Fig. 3d and Supplementary Fig. 3a). Thus, ablating Xist in the post-XCI state clearly impacts both chromatin accessibility and compaction in an Xi-specific manner.

Xist is known to recruit Polycomb repressive complex 2 (PRC2) to the Xi, marking the Xi with the repressive histone modification H3K27me3. We performed allele-specific chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) analysis of WT cells and observed a high degree of concordance between Xist CHART and H3K27me3 ChIP signals over the classified accessible regions (Figs. 2a, b and 3e, f). Indeed, metagene analysis revealed that the bi-allelic class was depleted of both Xist and H3K27me3; the monoallelic class showed moderate Xist and H3K27me3 coverage, consistent with a repressed and inaccessible state on the Xi; and the Xi-restored class showed highest levels of Xist and H3K27me3 coverage of all (Figs. 2a, b and 3e, f). Taken together, these data demonstrate that (1) Xist RNA and the PRC2 mark are highly concordant at the regional level, and (2) Xi-restored regions are generally marked by high levels of Xist and H3K27me3 in WT cells.

We then examined consequences of ablating Xist by performing allele-specific ChIP-qPCR in Xa WT XiXa cells. We observed a decrease in H3K27me3 levels on the Xi in monoallelic regions, as represented by Mecp2, Taf1, Rgpr and Prdx4 (Fig. 3g and Supplementary Fig. 3b), supporting previously published studies. However, after Xist depletion, the decrease in H3K27me3 levels was more pronounced in Xi-restored regions (Fig. 3g), consistent with the idea that these regions are especially sensitive to perturbation. ChIP-qPCR of H2AK119ub, a mark set by Polycomb repressive complex 1 (PRC1), showed similar results (Fig. 3h and Supplementary Fig. 3c). Together, these observations indicate that (1) Xi-restored regions normally have high Xist coverage and (2) Polycomb marks in this class are highly sensitive to the loss of Xist.

Xist interacts with BRG1–SWI/SNF complexes. Our analysis revealed that Xi-restored peaks showed strong enrichment for motifs known to be occupied by the SWI/SNF nucleosome remodeler
BRG1 (Fig. 3c)27, which promotes chromatin accessibility30,31. In addition, BRG1 has been identified as interactor of Xist13. Therefore, we asked whether BRG1 plays a role in defining accessibility of the Xi-restored regions. ChIP-qPCR for bulk histone H3 revealed significant Xi-specific reduction in H3 levels over Xi-restored regions in a comparison of Xist-deletion cells to their WT counterparts (Fig. 4a and Supplementary Fig. 3d). This finding supports the idea that chromatin re-accessibility at the nucleosomal level involves nucleosome remodeling, potentially by SWI/SNF complexes.

To test the idea that Xist RNA directly interacts with BRG1, we carried out an ultraviolet-crosslinked RNA immunoprecipitation (UV-RIP) assay with BRG1 antibodies in WT female dermal fibroblasts. qRT-PCR of BRG1 pulldown material showed significant interaction with Xist but not with the different negative controls tested (Fig. 4b). The interaction was significantly lower in the absence of UV crosslinking, suggesting a direct interaction.

Next, we asked which region of Xist interacts with BRG1 in vivo. Xist is a 17- to 19-kb-long noncoding RNA harboring six

Fig. 2 | CHART-seq analysis demonstrates that Xi-restored regions normally have the greatest Xist RNA coverage and may be most dependent on Xist for silencing. a, Average Xist CHART-seq signals on Xi in WT cells in the proximity of classified ATAC-seq peak centers (±5 kilobases (kb)). Unscaled reference-based profile (top panel) and scaled regions profile (bottom panel). b, Boxplots for each of the accessibility classes showing the distribution of Xist signals per ATAC-seq peak in WT cells; monoallelic (n = 356), bi-allelic (n = 27) and Xi-restored (n = 37). Box boundaries represent 25th and 75th percentiles; center line represents the median; whiskers indicate ±1.5 × IQR, and points are actual values of outliers. P values were determined using a one-sided non-parametric Wilcoxon test. c, Representative profiles of four ATAC accessibility classes and Xist CHART-seq signals on Xi in WT cells.
Fig. 3 | The epigenetic landscape of Xi-restored regions. a, Percentage of ATAC-seq peaks from the three accessibility classes and two control groups (autosomal and X chromosomal ATAC-seq peaks) in indicated genomic regions. The upper panel illustrates a schematic of the analyzed genomic features. TSS, transcription start site. b, Barplots of ATAC-seq peaks showing the percentage of each class annotated to indicated genomic features, monoallelic (n = 356), bi-allelic (n = 27) and Xi-restored (n = 37). P values were determined using a one-sided two-proportion z-test. Mon. in each of the three accessibility classes. d, Time-course MNase sensitivity, as measured by qPCR at representative Xi-restored genes and controls. Higher values indicate a stronger tendency of chromatin to remain refractory to MNase digestion and are thus indicative of increased chromatin compaction. Error bars represent mean ± s.d. P value indicates significant difference relative to mutant cells (*P < 0.005; two-tailed Student’s t-test). e, Enrichment levels of Polycomb repressive marks H3K27me3 (g) and H2AK119Ub (h) were measured by ChIP-qPCR in mutant and WT cells. Error bars represent mean ± s.d. P value indicates significantly higher difference between the two cell lines relative to the difference observed on Xi chromosome in the monoallelic region (*P < 0.01; two-tailed Student’s t-test, n = 3 independent experiments). Min, minutes.
Fig. 4 | Xist directly interacts with BRG1. a, H3 ChIP-qPCR performed in WT and mutant cells. Error bars represent mean ± s.d. P value indicates significant differences compared with mutant cells (*P < 0.001; two-tailed Student’s t-test, n = 3 independent experiments). b, UV- RIP experiments performed in female dermal fibroblasts using BRG1 or IgG antibodies, followed by qRT-PCR for Xist and Gapdh. Error bars represent mean ± s.d. A, C and E relative to repeats B and D were also calculated (*P < 0.005; two-tailed Student’s t-test, n = 3 independent experiments). c, RIP experiments performed in female dermal fibroblasts using BRG1 or IgG antibodies, followed by qRT-PCR for five different RNAs and six different regions of the Xist transcript (repeat A (RepA) to repeat F (RepF)). Error bars represent mean ± s.d. P values indicate significant differences relative to IgG control (*P < 0.01, **P < 0.001; two-tailed Student’s t-test, n = 3 independent experiments). d, Coomassie stain of purified full-length FLAG-BAF60 (left) and purified full-length FLAG-BRG1 (right). e, In vitro RNA pulldown assay using purified FLAG-BAF60, FLAG-BRG1 or M2 beads and RNA isolated from WT female dermal fibroblasts. qRT-PCR was performed for Xist and Gapdh. Error bars represent mean ± s.d. P value indicates significant difference relative to FLAG-BAF60 and beads controls (*P < 0.0001; two-tailed Student’s t-test, n = 3 independent experiments). f, Map of Xist locus (top panel). Black and green boxes correspond to exons and repeats, respectively; red lines represent in vitro transcribed RNAs. Agarose gel showing transcribed RNAs (bottom panel). g, RNA pulldown assay using purified FLAG-BAF60 or FLAG-BRG1 and XaWT XαWT female dermal fibroblasts RNAs with addition of Xist exon 1 RNA. qRT-PCR as in c. Error bars represent mean ± s.d. P value indicates significant differences relative to FLAG-BAF60 control (*P < 0.0002; two-tailed Student’s t-test, n = 3 independent experiments). h, RNA pulldown assay using purified FLAG-BAF60 or FLAG-BRG1 and XaWT Xα WT female dermal fibroblasts RNAs with addition of Xist repeats transcripts. qRT-PCR as in c. Error bars represent mean ± s.d. P values indicate significant differences relative to FLAG-BAF60 control (*P < 0.05, **P < 0.005; two-tailed Student’s t-test, n = 3 independent experiments). Significant differences between repeats A, C and E relative to repeats B and D were also calculated (P < 0.005; two-tailed Student’s t-test). Uncropped gel images for d and f are shown in Supplementary Dataset 1.
repeat elements defined as A–F that are known to interact with specific protein partners to regulate XCI. To identify the interacting domain(s), we used a formaldehyde-crosslinked RIP (RIP)-qPCR assay that includes a brief sonication step aimed to lightly fragment RNAs. While no significant enrichment was observed for two negative controls (Gapdh and Smad4), positive controls (Neat1 and Malat1) pulled down BRG1 (Fig. 4c). By applying this technique to Xist, we observed higher enrichment of Xist repeats A, C, E and F relative to other Xist regions (Fig. 4c). These data suggest that BRG1 interacts with broad regions in the Xist transcript.

We tested this interaction in vitro by an RNA pulldown assay using purified recombinant FLAG-tagged BRG1 proteins (Fig. 4d). To examine whether BRG1 preferentially binds Xist in a complex pool of cellular RNAs, we purified total RNA from female dermal fibroblasts and quantified the interaction between BRG1 and Xist. Pulldown of Xist by FLAG-BRG1 was significantly enriched compared to pulldown using negative controls such as uncoated beads or FLAG-BAF60—a SWI/SNF accessory subunit that was not identified as an Xist binder in previous proteomic screenings. Significant enrichment of Xist exon 1 was observed regardless of where the PCR amplicons were placed in exon 1 (Fig. 4g). We then repeated the pulldown experiment by supplementing with Xist subfragments encompassing repeat A, B, C, D, E or F (Fig. 4h). Consistent with in vivo RIP (Fig. 4c), we observed strong BRG1 interactions with repeats A, C, E and F, but to a significantly lesser extent with Repeats B or D (Fig. 4h). Taken together, our in vivo and in vitro results strongly argue that Xist RNA interacts with BRG1 in a direct and specific manner, preferentially mediated by repeats A, C, E and F.

Xist binding inhibits BRG1 activities in vitro. These findings highlight a paradox, as BRG1 ATPase interacts with Xist (Fig. 4b–h) but promotes open chromatin. Recent studies have suggested that interaction between RNA and epigenetic complexes could be inhibitory to their catalytic activity. Thus, to investigate whether Xist influences BRG1 activity, we performed two in vitro assays using purified recombinant BRG1-containing SWI/SNF complex with in vitro–transcribed RNA species and an assembled nucleosome array. First, we tested BRG1 nucleosome-remodeling activity in the presence of increasing concentrations of Xist fragments and two control RNAs: Evf2 and tRNA (Fig. 5a and Supplementary Fig. 4a). All tested RNA molecules had the capacity to inhibit BRG1’s remodeling activity to some extent. However, the degree of inhibition varied considerably between RNA fragments. Indeed, RNA species that can interact directly with BRG1 exerted greatest functional inhibition of BRG1’s remodeling activity, independently of RNA length (Fig. 5a and Supplementary Fig. 4a,c). In multiple biological replicates, Xist repeats C, E, F and A imposed greatest functional interference, as quantified by the IC_{50} (the concentration of RNA at which 50% of BRG1 is inhibited). Repeats B and D—which bound BRG1 to a substantially lower extent (Fig. 4d)—showed less inhibition. The negative control tRNAs showed least inhibition, consistent with poor binding to BRG1.

We also examined BRG1’s ATPase activity, a function that is required for BRG1’s remodeling activity. Similar to nucleosome remodeling, RNA generally inhibited ATP turnover. However, the degree of inhibition varied according to the RNA species that can bind BRG1, with good binders exhibiting a lower IC_{50} and nonspecific binders (tRNA) exhibiting a high IC_{50} (Fig. 5b and Supplementary Fig. 4b,c). We conclude that specific regions of Xist RNA bind BRG1 and inhibit BRG1’s activities, resolving the paradox that a gene-activating complex (BRG1) interacts with a gene-repressing factor (Xist).

Xist re-accessibility is BRG1 dependent. Since Xist represses BRG1 activity, we predicted that ablating Xist would induce Xist chromatin re-accessibility in a BRG1-dependent manner. To assess this, we performed ATAC-seq on BRG1 depletion in WT cells using siRNAs (Supplementary Fig. 5a–c). As expected, depleting BRG1 resulted in a genome-wide decrease in chromatin accessibility (Fig. 5c and Supplementary Dataset 6). For the X chromosome, the decrease was accentuated at regions classified as X-restored relative to the two other classes (Fig. 5d and Supplementary Dataset 6). Allele-specific analysis showed that the change occurred on the Xa in WT cells, as these regions are inaccessible even in presence of BRG1 on the X (Supplementary Fig. 5d and Supplementary Dataset 6). Thus, on the active X chromosome (Xa), the regions classified as ‘X-restored’ require BRG1 for full chromatin accessibility in WT cells.

We then examined changes in accessibility in Xist-deletion cells by performing ATAC-seq after BRG1 depletion (Supplementary Fig. 5e,f and Supplementary Dataset 6). Again, Xi-restored regions showed an accentuated decrease (Supplementary Fig. 5g and Supplementary Dataset 6). By comparing both cells lines, we observed that the effect on Xi-restored regions was greater and more notable in mutant cells than in WT cells, which was not the case for the two other classes, suggesting that in Xist-deleted cells, the X allele is also impacted.

By performing allelic analyses, we observed that both Xa and Xi were impacted in the X-restored regions in Xist-deleted cells on BRG1 knockdown (Fig. 5f, Supplementary Fig. 5h and Supplementary Dataset 6), where the loss was substantially greater than the loss observed in monoallelic and bi-allelic regions. Allele-specific FAIRE-qPCR experiments corroborated the loss of chromatin accessibility on both alleles at Xist-restored regions in Xist-deletion cells after BRG1 knockdown (Supplementary Fig. 5i). Taken together, these results demonstrate that the restoration of chromatin accessibility on the X after Xist ablation depends on BRG1.

Previous reports showed that Xist could also interact with two other chromatin-remodeling enzymes, SNF2H and CHD4. However, ATAC-seq analysis of WT cells in which SNF2H or CHD4 was knocked down (Supplementary Fig. 6) showed no significant differences in chromatin accessibility for any of the three classes (Supplementary Fig. 6). Collectively, these data argue for the specificity of BRG1 and suggest BRG1 as the primary chromatin-remodeling enzyme that interacts with Xist and positively regulates chromatin accessibility on the X chromosome.

Xist repels BRG1 in vivo. A Xist-mediated repulsion model was previously established by analysis of chromosome architectural factors. To determine whether Xist could repel BRG1, we carried out Xist RNA-FISH and BRG1 immunostaining in WT female fibroblasts. Relative to the surrounding nucleoplasm, we observed a substantial depletion of BRG1 in the Xist territory, marked by the Xist RNA ‘cloud’ (Fig. 6a and Supplementary Fig. 7a). Next, we asked if ectopically expressed Xist RNA could force BRG1 exclusion on an autosome. In a male mouse embryonic fibroblast line carrying an inducible Xist transgene, overnight induction of Xist resulted in a substantial depletion of BRG1 signals in the ectopic Xist cloud (Fig. 6a). Thus, Xist actively repels BRG1 from chromatin in cis.

To obtain higher resolution, we used native chromatin immunoprecipitation (nChIP)-seq (Supplementary Fig. 7b) and confirmed that BRG1 binding was elevated at nucleosomes flanking ATAC-seq peaks (Fig. 6b and Supplementary Fig. 7c), consistent with BRG1’s mediating the chromatin accessibility. We then performed allelic BRG1 nChIP-seq analysis and pooled two highly reproducible biological replicates for both WT cells and Xist-deletion cells to increase available allelic read depth (Supplementary Fig. 7d and Supplementary Dataset 2). As expected, in WT cells, there was significant enrichment for BRG1 reads on the Xa (Supplementary Fig. 7e), consistent with the Xa’s harboring active
chromatin. On the other hand, the Xi is nearly devoid of BRG1 binding, supporting our microscopy observations (Fig. 6a).

After Xist deletion, there was an increase in BRG1 occupancy on the Xi overall (Supplementary Fig. 7f). Next, we classified BRG1 peaks on the X chromosome into three deposition classes by using our SVM model trained on allelic read counts of three major accessibility classes (Fig. 6c, Supplementary Fig. 7g and Supplementary Dataset 7). The vast majority of the BRG1 peaks were classified as monoallelic (78%), while 5% were bi-allelic and 17% were classified as Xi-restored (Fig. 6c and Supplementary Fig. 7g).
Fig. 6 | Xist evicts BRG1 from the Xi. a. Immuno-RNA-FISH for BRG1 and Xist. Top row, female WT (♀ WT) fibroblasts, immortalized with SV40 large T-antigen and therefore tetraploid (4n). Bottom row, male MEF lines harboring a WT Xist transgene (♂ X+P). Arrows indicate locations of Xist clouds. Percentages of cells displaying a decrease in BRG1 intensity inside of Xist cloud are shown. Scale bar, 5 μm. For each cell line, BRG1 intensities outside (green) and inside of the Xist cloud (red) were calculated using ImageJ software. Box boundaries represent 25th and 75th percentiles; center line represents the median; whiskers indicate IQR, and points are actual values of outliers. n = 105 cells. b. Top, ATAC-seq summit-centered heat map of BRG1 ChIP-seq signal performed in WT cells. ATAC-seq peaks are clustered into BRG1-independent (indep.), down-regulated in siBRG1 (Down), and up-regulated in siBRG1 (Up) groups based on ATAC-seq analysis performed in siCTL- and siBRG1-treated WT cells. Bottom, distribution profile presenting mean BRG1 ChIP-seq signal for each group. c. Left panel, BRG1 ChIP-seq summit-centered heat map presenting allele-specific BRG1 ChIP-seq signal in mutant and WT cells. BRG1 ChIP-seq peaks are clustered into three BRG1 deposition classes (relative percentage is designated). Right panel, profiles presenting mean allele-specific BRG1 ChIP-seq signal around BRG1 peak summit for each of BRG1 deposition classes. d. ATAC-seq summit-centered heat map of allele-specific BRG1 ChIP-seq signal in both cell lines per accessibility class. For each class, percentage of classified ATAC-seq peaks presenting the same corresponding BRG1 classified ChIP-seq peaks at the two flanking nucleosomes is designated (corresponding pie charts to the right). e, Profiles presenting mean allele-specific BRG1 ChIP-seq signal around ATAC-seq peak summit for each accessibility class. f, Representative examples of Xi-restored ATAC-seq peak regions displaying restoration of BRG1 binding.
We asked whether restored BRG1 peaks corresponded to ATAC-based Xi-restored regions. While there was overlap of only 49% between ATAC-based versus BRG1-based monoallelic regions and overlap of only 37% for bi-allelic regions, 73% of accessible restored regions also display BRG1 re-occupancy (Fig. 6d–f and Supplementary Fig. 7h). This strongly argues that re-accessibility is functionally related to the return of BRG1 to the Xi. Allele-specific nChIP-qPCR confirmed the general findings (Supplementary Fig. 7i). Taken together, these data support the ideas that (1) BRG1 is responsible for rendering chromatin accessible over ‘Xi-restored’ regions and (2) one of Xist’s roles is to repel BRG1 from these Xi regions.

BRG1 potentiates Xi reactivation following drug treatment.

Previous studies have demonstrated that although Xi silencing is robust, partial Xi reactivation can be achieved by ablation of Xist and treatment with various drug inhibitors in combination13,44–48. To determine whether BRG1 affects Xi reactivation, we analyzed transcriptomic data13 from WT fibroblasts treated with a DNA methylation inhibitor (5'-azacytidine (aza)) and a topoisomerase 2b inhibitor (etoposide (eto)), treated with a control shRNA (shCTRL) versus those treated with shBRG1. The majority (77.3%) of Xi-reactivated genes were less reactivated in shBRG1-treated cells than in shCTRL-treated cells (Fig. 7a), suggesting that BRG1 stimulates Xi reactivation after drug treatment.

We then investigated if there is differential drug sensitivity among the three accessibility classes. We analyzed the chromatin-accessibility level after aza + eto treatment in WT cells treated with siBRG1 compared to siCTRL-treated cells by performing ATAC-qPCR assays. Our molecular assays revealed that Xi-restored regions were significantly more susceptible to a gain in accessibility after drug treatment than were monoallelic regions (Fig. 7b). Drug-dependent re-accessibility of these Xi-restored regions was substantially diminished in siBRG1 cells (Fig. 7b). These data demonstrate that BRG1 potentiates Xi reactivation following drug treatment.

Xist inhibits accessibility around escapee genes.

We looked for patterns in the epigenomic landscape that might predispose Xi-restored regions to re-accessibility. Peak distributions along the Xi confirmed the close correlation between ATAC- and BRG1-restored peaks (Fig. 7c). The non-random pattern of re-accessibility suggests the possible existence of structural ‘hotspots’. Re-accessible regions were often found in close proximity to restored TADs (Fig. 7c), which were shown to re-appear at select Xi locations when Xist is deleted1,55 (Fig. 7c). Overall, 85% of Xi-restored regions occurred in restored TADs, which was substantially different from the ~30% observed for monoallelic regions (Fig. 7d). Interestingly, >80% of restored BRG1 peaks were also significantly associated with restored TADs (Fig. 7d). This positive association with restored TADs was also observed for the bi-allelic class, as defined by both ATAC and BRG1 peaks, indicating that restoration of BRG1 binding and accessibility tends to occur in proximity to active chromatin.

We noticed that restoration of BRG1 binding after Xist ablation tends to originate from pre-existing BRG1 peaks on the Xi (Fig. 7e and Supplementary Fig. 8). Notably, 85% of all BRG1 bi-allelic peaks displayed nearby BRG1 re-occupation (Supplementary Fig. 8). This observation further supported a non-random pattern of restoration and suggested that new BRG1 complexes are guided by pre-bound BRG1. Strikingly, the overall distance from Xi-restored BRG1 peaks to the nearest bi-allelic peaks was substantially shorter than that for peaks residing in the monoallelic class (Fig. 7f). The same was true for comparison of ATAC peaks (Fig. 7f). These pre-bound BRG1 peaks were generally located around genes that escape XCI (Figs. 1 and 6). Thus, in the unperturbed state, Xist safeguards inaccessible chromatin from nearby open regions that harbor escapees. Loss of Xist leads to susceptibility of nearby chromatin to BRG1 encroachment and re-accessibility.

We furthermore observed that SMCH1a, an architectural factor that was shown to re-appear in restored TADs after Xist deletion13,55, was restored along the Xi in a pattern resembling Xi-restored ATAC and BRG1 peaks (Fig. 7c). By analyzing SMCH1a ChIP-seq data performed in both cell lines13, we found that 73% of Xi-restored ATAC regions displayed a restored SMCH1a peak (Fig. 7g and Supplementary Fig. 9a,b). Thus, SMCH1a tends to re-appear in the Xi-restored locations when Xist is eliminated. On a genomewide scale, we found that a quarter of all SMCH1a peaks displayed ATAC peaks within ±1.5 kb (Supplementary Fig. 9c). The vast majority of SMCH1a peaks closely overlapped accessible peaks, and their loss following BRG1 depletion occurred to a greater degree than that seen at more distal SMCH1a sites (located ~400 base pairs (bp) away) (Supplementary Fig. 9c). These findings indicate that BRG1 modulates accessibility at SMCH1a binding sites on a genomewide basis.

Finally, because chromatin of similar properties is thought to self-associate69–71, we analyzed allele-specific Hi-C performed in WT and Xist-deletion cells13 and found de novo hotspots of interaction between Xi-restored regions (Supplementary Fig. 10). This indicates the formation of ‘superloops’ between distant Xi-restored regions. These superloops were specifically observed on the Xi of the Xist-depleted cells, not that in WT cells (Supplementary Fig. 10) or on the Xa of either cell line (data not shown). Taken together, these data provide strong support for (1) large-scale 3D reorganization of the Xi when Xist RNA is ablated, and (2) the notion that re-accessible chromatin has a tendency to self-associate.

Discussion

Here we have shown that BRG1–SWI/SNF complexes play an important role in defining chromatin accessibility for specific regions of the X chromosome. Xist directly binds BRG1 and functionally antagonizes recruitment of associated SWI/SNF complexes to the Xi. The SWI/SNF core subunits BAF155, BAF170 and BAF47 are critical for enabling the remodeling activity of the complex72,73, and all four core subunits interact with Xist RNA74. The functional antagonism between Xist and BRG1 is based on two properties. In vitro, physical interaction between Xist and BRG1 represses BRG1’s remodeling and ATPase activities. In vivo, Xist evicts BRG1-containing SWI/SNF complexes from the Xi (Fig. 8a). Although these properties are distinct, interactions inside cells are likely to be very dynamic. In one possible model, Xist first binds BRG1, blocks its ATPase/remodeling activity and then evicts BRG1 complexes to the Xi. The SWI/SNF core subunits BAF155, BAF170 and BAF47 are critical for enabling the remodeling activity of the complex72,73, and all four core subunits interact with Xist RNA74. The functional antagonism between Xist and BRG1 is based on two properties. In vitro, physical interaction between Xist and BRG1 represses BRG1’s remodeling and ATPase activities. In vivo, Xist evicts BRG1-containing SWI/SNF complexes from the Xi (Fig. 8a). Although these properties are distinct, interactions inside cells are likely to be very dynamic. In one possible model, Xist first binds BRG1, blocks its ATPase/remodeling activity and then evicts BRG1 from the Xi chromatin in a step-wise fashion, but future research will be required to fully elucidate the precise order of events. In the absence of Xist, BRG1 returns to particular regions of the Xi (the ‘Xi-restored regions’), restoring chromatin accessibility and binding of cohesins (Fig. 8a). In line with previous studies72,73,74, restoration of BRG1’s binding on the Xi is, however, insufficient for Xi reactivation during XCI maintenance, suggesting that other chromatin-modification pathways must be perturbed in parallel. Indeed, our data show that BRG1 potentiates Xi reactivation when DNA methylation and topoisomerase 2b are inhibited (Fig. 7a,b). Our findings thereby provide further insight into the mechanism of Xist action: conceptually, this long noncoding RNA repels positive chromatin factors in addition to recruiting inhibitory factors. Thus, even as Xist recruits Polycomb repressive complexes and other epigenetic complexes1,73,97,100, the RNA actively repels a growing list of complexes, including SWI/SNF (Figs. 4 and 6) and cohesins13.

Another finding is that after Xist ablation, BRG1 binding tends to be restored around pre-existing BRG1-binding regions, corresponding to escapees (Fig. 7e and Supplementary Fig. 8). This tendency may have a basis in the Xi chromosome territory organization. The Xi is partitioned into INCs and collapsed ANCs, where Xist is enriched—’the WT ANC is very limited and is restricted to domains harboring escapees’73–75 (Fig. 8b). We speculate that after
Fig. 7 | BRG1 re-occupancy extends from pre-existing BRG1 sites, and associate with restored cohesins and TADs. a, Pie chart depicting percentage of genes that are more reactivated (FC > 2) (red) or less reactivated after stable BRG1 knockdown (FC < 2) (green). P values were determined using a two-tailed Student’s t-test, n = 3 independent experiments. Neg Ctl, negative control, representing an inaccessible region. Chromosomal locations of three accessibility and three BRG1-deposition classes, as well as restored TADs and cohesins regions across the Xi. b, Chromatin-accessibility profiles, measured by ATAC-qPCR in treated WT cells. Higher values indicate more accessible chromatin. Error bars represent mean ± s.d. Genes that are more reactivated upon shBRG1 (top) and BRG1 (bottom) peaks in restored TADs, monoallelic (n = 356), bi-allelic (n = 27) and Xi-restored (n = 37). P values were determined using a one-sided two-proportion z-test, P < 0.001.* P < 0.01. 

Mon. = monoallelic, Bi-a. = bi-allelic, Xi rest. = Xi-restored. c, Representative examples of Xi-restored BRG1 binding patterns nearby pre-existing bi-allelic BRG1-bound sites (highlighted). Red arrows indicate origin and direction of expansion of BRG1 re-occupancy on the Xi in Xist-deleted cells. f, Boxplots showing distribution of distances in megabases (Mb) from monoallelic (blue) or Xi-restored (red) peaks to nearest bi-allelic peaks. Analyses were carried out for ATAC-seq (top) and BRG1-seq (bottom) classified peaks, monoallelic (n = 356) and Xi-restored (n = 37). P values were determined using a one-sided non-parametric Wilcoxon test. Box boundaries represent 25th and 75th percentiles; center line represents the median; whiskers indicate ± 1.5x IQR, and points are actual values of outliers.

Left panel, ATAC-seq peak summit-centered heat map of SMC1a ChIP-seq signal (using composite reads) in both cell lines for each accessibility class. For each class, percentages of classified ATAC-seq peaks presenting significant SMC1a peaks within ±1.5 kb of the flanking region is designated (right). Right panel, profiles representing mean SMC1a ChIP-seq signal around ATAC-seq peak sums for each accessibility class.
Xist removal, the ANC is extended to neighboring regions, concomitant with the restoration of BRG1 occupancy and accessibility (Fig. 8). Thus, we propose that the proximity of Xi-restored regions to the ANC facilitates their restoration.

Our study also has implications for Xi 3D organization. Given that chromatin-remodeling complexes can regulate the binding of cohesins\textsuperscript{56,57}, and owing to the ability of BRG1 to govern accessibility at SMC1a binding sites (Fig. 7 and Supplementary Fig. 9), we suggest that BRG1 is required for cohesin restoration. We do not currently know the precise order of return, but our data indicate that co-restoration of BRG1, accessibility, cohesins and TADs results in formation of de novo Xi superloops. Because the action of BRG1-containing SWI/SNF complexes is ATP dependent, we predict that restoration of accessibility, TADs and cohesins, as well as formation

---

**Fig. 8** | **Model for Xist-BRG1 dynamics on the Xi.** **a**, On the unperturbed Xi (WT), Xist actively blocks remodeling and ATPase activities of BRG1 and repels BRG1 from the Xi (inset). BRG1 is normally found only in bi-allelic escapee regions (and Xi-only regions) (highlighted green box). When Xist is deleted (Xa\textsuperscript{WT} Xi\textsuperscript{ΔXist}), BRG1 returns to Xi-restored regions (highlighted brown box), restoring chromatin accessibility (blue arrows) and binding of cohesins (SMC1a). BRG1 re-occupancy occurs nearby pre-existing BRG1 binding sites in bi-allelic regions. NFR, nucleosome-free region. **b**, In the Xi chromosome territory of WT cells, Xist RNA is enriched in the collapsed active nuclear compartment (ANC), and escapee genes are localized in normal ANCs (left panel). After Xist deletion, these ANCs expand to include Xi-restored regions (right panel). We propose that proximity to escapee ANCs predisposes Xi regions to restoration, potentially due to enrichment of activating chromatin factors. Purple squares denote region described in Fig. 8a. INC, inactive nuclear compartment.
of superloops, would require ATP hydrolysis. Formation of superloops is in line with the idea of self-association of chromatin with the same properties\textsuperscript{49–51}. However, escapee regions on the upregulated Xi do not form similar superloops (at least not detectably in our female dermal fibroblast Hi-C experiments). One possibility is that escapee regions in WT cells are encompassed in crammed ANC regions that limited their interaction and movement in the Xi chromosome territory. The extension of these ANC regions induced by Xist depletion allows them to be efficiently re-organized. We suggest that Xist actively safeguards against formation of accessible 3D structures, including de novo superloops and conventional TADs.

Finally, our study brings to light the differential dependence of the X chromosome on BRG1 and further underscores the emerging concept of regional epigenetic differences along the 166-Mb chromosome\textsuperscript{11–13}. BRG1 is generally associated with enhancement of chromatin accessibility and in fact binds throughout the Xa at actively transcribed regions (Figs 5 and 6). However, only 10–20% of the X chromosome is affected by perturbations to the Xist–BRG1 interaction, whether by deletion of Xist or by knockdown of BRG1 (Figs 1 and 5). This implies that other SWI/SNF or other remodeling complexes may cooperate and/or regulate the accessibility of other regions of the X chromosome. The fact that the Xi is not monolithic from an epigenetic perspective has pharmacological implications for X-linked diseases. As Xi-activating approaches gain feasibility for restoring the expression of missing proteins in Rett, CDKL5 syndromes and other X-linked disorders\textsuperscript{6,15–16}, further understanding of how to selectively turn on genes or regions on the Xi would be of tremendous benefit. Our current study reveals that BRG1 potentiates Xi reactivation after drug treatment (Fig 7a,b) and thus suggests future approaches by which targeting the BRG1–Xist interaction, possibly in combination with DNA-demethylation agents, could enhance the selectivity of Xi reactivation.

Online content Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41594-018-0176-8.

Received: 11 October 2018; Accepted: 3 December 2018; Published online: 21 January 2019

References

1. Smeets, D. et al. Three-dimensional super-resolution microscopy of the inactive X chromosome territory reveals a collapse of its active nuclear compartment harboring distinct Xist RNA foci. Epigenetics Chromatin 7, 8 (2014).
2. Cremet, T. et al. The 4D nucleome: evidence for a dynamic nuclear landscape based on co-aligned active and inactive nuclear compartments. FEBS Lett. 589, 2931–2943 (2015).
3. Dixon, J. R. et al. Topological domains in mammalian genomes identified by chromatin interaction analysis with the chromosome conformation capture–hyperbolic exponential mapping technique. Nature 485, 376–380 (2012).
4. Nora, E. P. et al. Spatial partitioning of the regulatory landscape of the X-inactivation centre. Nature 485, 381–385 (2012).
5. Rao, S. S. et al. A 3D map of the human genome at kilobase resolution reveals principles of chromosome looping. Cell 159, 1665–1680 (2014).
6. Rao, S. S. P. et al. Cohesin loss eliminates all loop domains. Cell 171, 305–320 (2017).
7. Lee, J. T. Gracefully ageing at 50, X-chromosome inactivation becomes a paradigm for RNA and chromatin control. Nat. Rev. Mol. Cell. Biol. 12, 815–826 (2011).
8. Distech, C. M. Dosage compensation of the sex chromosomes and autosomes. Semin. Cell Dev. Biol. 56, 9–18 (2016).
9. Mosfoot, A. & Wutz, A. Progress in understanding the molecular mechanism of Xist RNA function through genetics. Philos. Trans. R Soc. Lond. B Biol. Sci. 372, 20160368 (2017).
10. Robert Finestra, T. S. & Gribnau, J. X chromosome inactivation: silencing, topology and reactivation. Curr. Opin. Cell Biol. 46, 54–61 (2017).
11. Clement, C. M., McNeil, J. A., Willard, H. F. & Lawrence, J. B. XIST RNA paints the inactive X chromosome at interphase: evidence for a novel RNA involved in nuclear/chromosome structure. J. Cell Biol. 132, 259–275 (1996).
12. Simon, M. D. et al. High-resolution Xist binding maps reveal two-step spreading during X-chromosome inactivation. Nature 504, 465–469 (2013).
13. Minajig, A. et al. Chromosomes. A comprehensive Xist interactome reveals cohesin repulsion and an RNA-directed chromosome conformation. Science 349, aab2276 (2015).
14. Giorgetti, L. et al. Structural organization of the inactive X chromosome in the mouse. Nature 535, 575–579 (2016).
15. Darrow, E. M. et al. Deletion of DUXA on the human inactive X chromosome alters higher-order genome architecture. Proc. Natl. Acad. Sci. USA 113, E4504–E4512 (2016).
16. Costanzi, C. & Pehrson, J. R. Histone macroH2A1 is concentrated in the inactive X chromosome of female mammals. Nature 393, 599–601 (1998).
17. Heard, E. et al. Methylation of histone H3 at Lys-9 is an early mark on the X chromosome during X inactivation. Cell 107, 727–738 (2001).
18. Plath, K. et al. Role of histone H3 lysine 27 methylation in X inactivation. Science 300, 131–135 (2003).
19. Zhao, J., Sun, R. K., Erwin, J. A., Song, J. J. & Lee, J. T. Polycomb proteins targeted by a short repeat RNA to the mouse X chromosome. Science 322, 750–756 (2008).
20. Zhang, L. F., Huynh, K. D. & Lee, J. T. Perinucleolar targeting of the inactive X during S phase: evidence for a role in the maintenance of silencing. Cell 129, 693–706 (2007).
21. Splinter, E. et al. The inactive X chromosome adopts a unique three-dimensional conformation that is dependent on Xist RNA. Genes Dev. 25, 1371–1383 (2011).
22. Fabre, J. M. et al. Site-specific silencing of regulatory elements as a mechanism of X inactivation. Cell 151, 951–963 (2012).
23. Wu, H. et al. Cellular resolution maps of X chromosome inactivation: implications for neural development, function, and disease. Neuron 81, 103–119 (2014).
24. Finn, E. H., Smith, C. L., Rodriguez, J., Sidow, A. & Baker, J. C. Maternal bias and escape from X chromosome imprinting in the midgestation mouse placenta. Dev. Biol. 390, 80–92 (2014).
25. Berleth, J. B. et al. Escape from X inactivation varies in mouse tissues. PLoS Genet. 11, e1005079 (2015).
26. Marks, H. et al. Dynamics of gene silencing during X inactivation using allele-specific RNA-seq. Genome Biol. 16, 149 (2015).
27. Nakayama, R. T. et al. SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. Nat. Genet. 49, 1613–1623 (2017).
28. Blank, M. et al. A tumor suppressor function of Smurf2 associated with controlling chromatin landscape and genome stability through RNF20. Nat. Med. 18, 227–234 (2012).
29. Nozawa, R. S. et al. Human inactive X chromosome is compacted through a PRC2-independent SMCHD1–HBX1 pathway. Nat. Struct. Mol. Biol. 20, 566–573 (2013).
30. Bao, X. et al. A novel ATAC-seq approach reveals lineage-specific reinforcement of the open chromatin landscape via cooperation between BAF and p63. Genome Biol. 16, 284 (2015).
31. Miller, E. L. et al. TOP2 synergizes with BAF chromatin remodeling for both resolution and formation of facultative heterochromatin. Nat. Struct. Mol. Biol. 24, 344–352 (2017).
32. Liu, Z. & Carter, A. C. & Chang, H. Y. Mechanistic insights in X chromosome inactivation. Philos. Trans. R Soc. Lond. B Biol. Sci. 372, 20160356 (2017).
33. D. G. H., Kelley, D. R., Tenen, D., Bernstein, B. & Rinn, J. L. Widespread RNA binding by chromatin-associated proteins. Genome Biol. 17, 28 (2016).
34. Kawaguchi, T. et al. SWI/SNF chromatin-remodeling complexes function in noncoding RNA-dependent assembly of nuclear bodies. Proc. Natl. Acad. Sci. USA 112, 4304–4309 (2015).
35. Tang, Y. et al. Linking long non-coding RNAs and SWI/SNF complexes to chromatin remodeling in cancer. Mol. Cancer 16, 42 (2017).
36. Lino Cardenas, C. L. et al. An HDAC9-MALAT1-BRG1 complex mediates smooth muscle dysfunction in thoracic aortic aneurysm. Nat. Commun. 9, 1609 (2018).
37. McHugh, C. A. et al. The Xist IncRNA interacts directly with SHARP to silence transcription through HDAC3. Nature 521, 232–236 (2015).
38. Chu, C. et al. Systematic discovery of Xist RNA binding proteins. Cell 161, 404–416 (2015).
39. Cifuentes-Rojas, C., Hernandez, A., Sarma, K. & Lee, J. T. Regulatory interactions between RNA and Polycomb repressive complex 2. Mol. Cell 55, 171–185 (2014). ePub May 29, 2014.
40. Kaneko, S., Son, J., Bonasio, R., Shen, S. S. & Reinberg, D. Nascent RNA interaction keeps PRC2 activity poised and in check. Genes Dev. 28, 1983–1988 (2014).
41. Nalpas, J. et al. Evt2 IncRNA/BRG1/DLX1 interactions reveal RNA-dependent inhibition of chromatin remodeling. Development 142, 2641–2652 (2015).
42. Jeon, Y. & Lee, J. T. YY1 tethers Xist RNA to the inactive X nucleation center. *Cell* **146**, 119–133 (2011).
43. de Dieuleveult, M. et al. Genome-wide nucleosome specificity and function of chromatin remodelers in ES cells. *Nature* **530**, 113–116 (2016).
44. Lessing, D. et al. A high-throughput small molecule screen identifies synergism between DNA methylation and Aurora kinase pathways for X reactivation. *Proc. Natl Acad. Sci. USA* **113**, 14366–14371 (2016).
45. Carrette, L. L. G. et al. A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders. *Proc. Natl Acad. Sci. USA* **115**, E668–E675 (2018).
46. Bhatnagar, S. et al. Genetic and pharmacological reactivation of the mammalian inactive X chromosome. *Proc. Natl Acad. Sci. USA* **111**, 12591–12596 (2014).
47. Minkovský, A. et al. A high-throughput screen of inactive X chromosome reactivation identifies the enhancement of DNA demethylation by 5-aza-2′-dC upon inhibition of ribonucleotide reductase. *Epigenetics Chromatin* **8**, 42 (2015).
48. Sripathy, S. et al. Screen for reactivation of MeCP2 on the inactive X chromosome identifies the BMP/TGF-beta superfamily as a regulator of XIST expression. *Proc. Natl Acad. Sci. USA* **114**, 1619–1624 (2017).
49. Lieberman-Isser, E. et al. Comprehensive mapping of long-range interactions reveals folding principles of the human genome. *Science* **326**, 289–293 (2009).
50. Bickmore, W. A. & van Steensel, B. Genome architecture: domain organization of interphase chromosomes. *Cell* **152**, 1270–1284 (2013).
51. Bonev, B. et al. Multiscale 3D genome rewiring during mouse neural development. *Cell* **171**, 557–572 (2017).
52. Phelps, M. L., Sif, S., Narlikar, G. J. & Kingston, R. E. Reconstitution of a core chromatin remodeling complex from SWI/SNF subunits. *Mol. Cell* **3**, 247–253 (1999).
53. Wutz, A. & Jaenisch, R. A shift from reversible to irreversible X inactivation is triggered during ES cell differentiation. *Mol. Cell* **5**, 695–705 (2000).
54. Csankovszki, G., Panning, B., Bates, B., Pehrson, J. R. & Jaenisch, R. Conditional deletion of Xist disrupts histone macroH2A localization but not maintenance of X inactivation. *Nat. Genet.* **22**, 323–324 (1999).
55. Jegu, T., Aeby, E. & Lee, J. T. The X chromosome in space. *Nat. Rev. Genet.* **18**, 377–389 (2017).
56. Hakimi, M. A. et al. A chromatin remodeling complex that loads cohesin onto human chromosomes. *Nature* **418**, 994–998 (2002).
57. Weichens, N. et al. The chromatin remodelling enzymes SNF2H and SNF2L position nucleosomes adjacent to CTCF and other transcription factors. *PloS Genet.* **12**, e1005940 (2016).
58. Chadwick, B. P. & Willard, H. F. Multiple spatially distinct types of facultative heterochromatin on the human inactive X chromosome. *Proc. Natl Acad. Sci. USA* **101**, 17450–17455 (2004).

Acknowledgements
T. Jegu is a European Molecular Biology Organization (EMBO) postdoctoral fellow (EMBO ALTF 1313–2015) and a young researcher Bettencourt Schueller Foundation awardee. R.E.K. is supported by NIH grant, R37-GM048405. This work was supported by funding to J.T.L. from the Rett Syndrome Research Trust, the LouLou Foundation, the National Institutes of Health (R01-DA36895) and the Howard Hughes Medical Institute.

Author contributions
T.J., R.B. and J.T.L. designed the experiments and analyzed data. J.C.C. performed in vitro ATPase and remodeling assays. L.Y. performed the RNA-seq assays. D.C., A.S. and S.K.M. made all the plasmids. T.J. generated all data. R.B. performed all bioinformatics analyses, excepted the Hi-C analysis performed by C.Y.W. T.J. and M.E.G analyzed the immunofISH. R.E.K. supervised J.C.C and S.K.M. T.J., R.B. and J.T.L. wrote the manuscript.

Competing interests
J.T.L. is a co-founder of and serves on the Scientific Advisory Boards of Translate Bio and Fulcrum Therapeutics.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41594-018-0176-8.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to J.T.L.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2019
Articles

Methods

Cell lines and siRNA transfection. Fibroblast lines Xα+/Xα−Xi+/Xi− Q× P and Q × P have been described elsewhere⁶⁻⁹, and were negative for mycoplasma contamination. For depleting BRG1, SNF2H or CHD4, 30,000 cells were transfected with Dharmacon siRNAs (L-001810-01-0005, L-041484-01-0005, L-052142-00-0005, respectively) at a final concentration of 20 nM using a Lipofectamine RNAiMAX (Invitrogen) reverse transfection protocol. Control cells were transfected with control siRNAs (D-001810-01-0005) under identical conditions. Knockdown of target genes was confirmed by qRT-PCR and western blotting. All experiments were performed 48 h post transfection.

Assay for transposable-accessible chromatin with high-throughput sequencing. 50,000 cells were washed briefly in cold PBS and permeabilized with cold lysis buffer (10 mM Tris-HCl, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.1% GPEAL CA-630) containing proteinase inhibitor cocktail (Roche). Nuclei were resuspended in 1× TDT buffer (Illumina FC-121-1030) and 2.5 μl of Transposase (Illumina FC-121-1030) were added. Transposition reaction was performed at 37°C for 30 min, and DNA was purified using a Qiagen MinElute Kt. DNA libraries were amplified for a total of eight cycles. Libraries were assessed for quality control on the BioAnalyzer 2100 (Agilent) to ensure nucleosomal phishing and complexity. Sequencing was performed on the high-throughput sequencing HiSeq2500 (Illumina), using 50 bp paired-end reads.

For ATAC-qPCR, fibroblast cells were treated daily with dimethyl sulfoxide or with 0.3 μM acridine yellow + 0.3 mM etoposide for 3 days. Chromatin accessibility values were normalized to those obtained on the Gapdh and Actn1 (positive controls) loci. Xi-containing regions serially correspond to 1–4 corresponding loci: chrX:75,835,000, chrX:73,476,670, chrX:94,048,210 and chrX:96,077,970, respectively. All sequences of primers used are designated in Supplementary Dataset 8.

ATAC-seq analysis. ATAC-seq libraries were subjected to high-throughput sequencing using an Illumina HiSeq2500 apparatus according to the manufacturer’s instructions. On average, approximately 40 million paired-end 50 bp reads were generated per every ATAC-seq sample (Supplementary Dataset 2). Adapter sequences were trimmed with Trim Galore! v.0.4.1 (www.bioinformatics.babraham.ac.uk/projects/trim_galore/) (stringency 12 and allowed error rate 0.2). Identical genomic sequences (PCR duplicates) were removed by custom program before alignment. To account for the M. mus (M. castaneus) (cas) hybrid character of mouse dermal fibroblast lines that were employed in a ChIP-seq studies, reads were first aligned to custom mus/129 and cas genomes, and then mapped back to the reference mm10 genome. All alignments were performed by using novoalign (v.3.00.02) in paired-end mode (Novacraft Technology, Selangor, Malaysia). Post-processing of alignments was performed with custom scripts using SAMTools⁶⁵, and BEDTools (v.2.25.0). These included accounting, alignment file-type conversion, extracting and read sorting (SAMtools), and obtaining wig coverage files (SAMtools depth). Detailed description of all ATAC-seq analysis is provided in the Supplementary Note 2.

M. mus/natural mouse. Nm23Rd5, GAPDH, gapdh, Nucleolin, PIG-1, PIG-A, PIG-B, PIG-D, PIG-E, PIG-Fr, PIG-H, PIG-J, PIG-K, PIG-M, PIG-N or PIG-W (from mouse). The experiments were performed at least three times in biological triplicates. For ChIP-qPCR, fibroblast cells were treated daily with dimethyl sulfoxide or with 0.3 μM acridine yellow + 0.3 mM etoposide for 3 days. Chromatin accessibility values were normalized to those obtained on the Gapdh and Actn1 (positive controls) loci. Xi-containing regions serially correspond to 1–4 corresponding loci: chrX:75,835,000, chrX:73,476,670, chrX:94,048,210 and chrX:96,077,970, respectively. All sequences of primers used are designated in Supplementary Dataset 8.

RNA immunoprecipitation assay. 10 × 10⁶ female WT female dermal fibroblasts per immunoprecipitate were crosslinked by exposure to UV light at 200 mJ energy (Stratagene 2400) in 10 ml ice-cold PBS and collected by scraping in PBS. Cell pellets were incubated on ice in lysis solution (1% NP40, 0.5% S, 0.5% deoxycholate, additional 150 mM NaCl) for 40 min on ice, and DNA was purified using a Qiagen MinElute Kt. DNA libraries were amplified for a total of eight cycles. Libraries were assessed for quality control on the BioAnalyzer 2100 (Agilent) to ensure nucleosomal phishing and complexity. Sequencing was performed on the high-throughput sequencing HiSeq2500 (Illumina), using 50 bp paired-end reads.

GAPDH values were normalized to those obtained on the

UV-RNA immunoprecipitation assay. 10 × 10⁶ female WT female dermal fibroblasts per immunoprecipitate were crosslinked by exposure to UV light at 200 mJ energy (Stratagene 2400) in 10 ml ice-cold PBS and collected by scraping in PBS. Cell pellets were incubated on ice in lysis solution (1% NP40, 0.5% S, 0.5% deoxycholate, additional 150 mM NaCl) for 40 min on ice, and DNA was purified using a Qiagen MinElute Kt. DNA libraries were amplified for a total of eight cycles. Libraries were assessed for quality control on the BioAnalyzer 2100 (Agilent) to ensure nucleosomal phishing and complexity. Sequencing was performed on the high-throughput sequencing HiSeq2500 (Illumina), using 50 bp paired-end reads.

Dataset 8. For sequencing, 10 ng of immunoprecipitate or input DNA was used for ChIP-seq library construction using an NEBNext Ultra DNA Library Prep Kit for Illumina. Libraries were assessed for quality control on the BioAnalyzer 2100 (Agilent). Sequencing was performed on the HiSeq2500 (Illumina), using 50 bp paired-end reads.

ChIP-seq analysis. ChiP-seq libraries were subjected to high-throughput sequencing using an Illumina HiSeq2500 apparatus according to the manufacturer’s instructions. On average, approximately 45 million paired-end 50 bp reads were generated per every ChiP-seq sample (Supplementary Dataset 2). Adapter sequences were trimmed with Trim Galore! v.0.4.1 (www.bioinformatics.babraham.ac.uk/projects/trim_galore/) (stringency 12 and allowed error rate 0.2). Identical genomic sequences (PCR duplicates) were removed by custom program before alignment. To account for the M. mus (M. castaneus) (cas) hybrid character of mouse dermal fibroblast lines that were employed in a ChIP-seq studies, reads were first aligned to custom mus/129 and cas genomes, and then mapped back to the reference mm10 genome. All alignments were performed by using novoalign (v.3.00.02) in paired-end mode (Novacraft Technology, Selangor, Malaysia). Post-processing of alignments was performed with custom scripts using SAMTools⁶⁵, and BEDTools (v.2.25.0). These included accounting, alignment file-type conversion, extracting and read sorting (SAMtools), and obtaining wig coverage files (SAMtools depth). Detailed description of all nChIP-seq analysis is provided in the Supplementary Note 2.

UV-RNA immunoprecipitation assay. For UV-RNA immunoprecipitation assay, 10 × 10⁶ female WT female dermal fibroblasts per immunoprecipitate were crosslinked by exposure to UV light in 200 mJ energy (Stratagene 2400) in 10 ml ice-cold PBS and collected by scraping in PBS. Cell pellets were incubated on ice in lysis solution (1% NP40, 0.5% S, 0.5% deoxycholate, additional 150 mM NaCl) for 40 min on ice, and DNA was purified using a Qiagen MinElute Kt. DNA libraries were amplified for a total of eight cycles. Libraries were assessed for quality control on the BioAnalyzer 2100 (Agilent) to ensure nucleosomal phishing and complexity. Sequencing was performed on the high-throughput sequencing HiSeq2500 (Illumina), using 50 bp paired-end reads.

nChIP assay. Approximately 50 × 10⁶ cells were washed in PBS, scraped in lysis buffer (10 mM Tris-HCl, pH 7.5, 30 μM NaCl, 0.1% NP-40, 3 μM MgCl₂, and proteinase inhibitor cocktail, Roche) and incubated for 10 min on ice. After centrifugation, nuclei pellets were resuspended in benzenease buffer (25 mM Tris, pH 7.5, 300 mM NaCl, 0.5% NP-40, 2.5 mM MgCl₂, and proteinase inhibitor cocktail, Roche). Chromatin was sonicated by adding 25 μl of benzenease buffer for 30 min on ice and was spun at 16,000g at 4°C. Benzenease digestion was stopped by diluting twice the supernatant in 50 mM Tris pH 7.5, 100 mM NaCl, 0.5% NP-40, 15 mM EDTA containing proteinase inhibitor cocktail. After pre-clearing step using 100 μl Dynabeads protein G magnetic beads (Invitrogen), the chromatin was incubated with 4 μg of either anti-BRG1 (ab110641) or anti-IgG (Cell Signaling, 2729S) antibodies pre-bound to 50 μl Dynabeads protein-G magnetic beads (Invitrogen) rotating for 4 h at 4°C. An aliquot of untreated chromatin was processed in parallel and used as the total input DNA control. Beads were washed three times in 50 mM Tris, pH 7.5, 150 mM NaCl, 0.5% NP-40, 5 mM EDTA, followed by three times in 50 mM Tris, pH 7.5, 300 mM NaCl, 0.5% NP-40, 2.5 mM MgCl₂, and proteinase inhibitor cocktail (Roche). The purified DNA was eluted by incubation in elution buffer (1% SDS, 0.1% NaHCO₃), for 15 min. DNA was subjected to RNase and proteinase K digestion and then purified. Pulldown efficiencies were calculated by qPCR using input as reference. Error bars were calculated as standard deviation of three biological replicates. All sequences of primers used are designated in Supplementary
In vitro RNA pulldown assay. 40 pmol of BRG1-FLAG or BAF60-FLAG were immobilized with anti-FLAG M2 Magnetic Beads (Sigma) in PBS containing 0.05% bovine serum albumin (BSA), 0.1% NP40, 1 mM ATP, 15 mM β-mercaptoethanol and proteinase inhibitor cocktail (Roche) for 2 h. For in vitro RNA pulldown performed with RNA from WT cells, 2 μg of total RNA from WT fibroblast cells were incubated with protein–bead complexes at room temperature for 2 h in PBS containing 2 mM MgCl2, 0.2 mM ZnCl2, 15 mM β-mercaptoethanol, 100 μM RNase inhibitor, 0.05% BSA, 0.2% NP40, 1 mM ATP and proteinase inhibitor cocktail (Roche). For in vitro RNA pulldown performed with RNA from Xist-deleted cells with addition of Xist repeat RNA, 2 μg of total RNA from Xist-deleted fibroblast cells were incubated with 0.5 pmol of each in vitro–transcribed Xist repeat RNA (using MEGAscript, Invitrogen) and with protein–bead complexes at room temperature for 2 h in PBS containing 2 mM MgCl2, 0.2 mM ZnCl2, 15 mM β-mercaptoethanol, 100 μM RNase inhibitor, 0.05% BSA, 0.2% NP40, 1 mM ATP and proteinase inhibitor cocktail (Roche). RNAs were treated with TURBO DNase, TRizol purified and reanimated by heating and slow cooling. RNA–protein–bead complexes were washed four times with the same incubation buffer supplemented with additional 150 mM NaCl (total 300 mM NaCl). RNAs were eluted and extracted with TRizol and then were reverse-transcribed with random primers (Promega) and Superscript III reverse transcriptase (Invitrogen). Control reactions without reverse transcriptase (−RT) were also prepared. RNA pulldown was then analyzed by qPCR. An aliquot of untreated RNA was processed in parallel and used as the total input RNA control. All sequences of primers used are designated in Supplementary Dataset 8.

Remodeling assays. Nucleosome arrays were assembled using Hela native histones on a Cy5-labeled template containing 12 55 positioning sequences flanking a central dinucleosome (GSE4) site containing the recognition sequence for the H3a endonuclease32. Baculovirus expression of the SWI/SNF complex was done essentially as described60. Reactions were carried out in 20-μl volume containing 8 mM HEPES (pH 7.9), 10 mM Tris (pH 7.7), 60 mM KCl, 120 mM β-ASA, 4 mM MgCl2, and 8% glycerol, similar to prior reports61. Dilutions of the different RNAs were incubated with 5 μM SWI/SNF for 30 min at 30°C before the addition of 1 nM nucleosomes and 10 units of H3a. Reactions were incubated for 1 h at 30°C before the addition of 10 μl of stop buffer (1.5 μM/ml). Proteinase K, 70 mM EDTA, 10 mM Tris (pH 7.7), 1% SDS, 0.1% Orange-G. Reactions were incubated at 55°C for 1 h and then separated on a 1.2% agarose gel in 1X TAE. DNA was visualized on a Typhoon scanner and quantified using ImageQuant software. Apparent inhibition of remodeling was determined by the equation: ((fraction uncut with RNA and SWI/SNF) – (fraction uncut with SWI/SNF))/((fraction uncut without SWI/SNF)) and plotted using GraphPad Prism and fit to an equation for a sigmoidal curve.

ATPase assays. Mononucleosomes were assembled using recombinant histones on the Widom 601 positioning sequence with a 20-bp overhang62. Baculovirus expression of the SWI/SNF complex was done essentially as described63. Reactions were carried out in 20-μl volume containing 8 mM HEPES (pH 7.9), 10 mM Tris (pH 7.7), 60 mM KCl, 120 mM β-ASA, 4 mM MgCl2, and 8% glycerol, similar to prior reports61. Dilutions of the different RNAs were incubated with 5 μM SWI/SNF for 30 min at 30°C before the addition of 1 nM nucleosomes. Reactions were incubated for 1 h at 30°C before the addition of additional MgCl2 to 10 mM. Following the manufacturer’s instructions for the ADP-Glo Max Assay (Promega), 25 μl of ADP-Glo Reagent was added to each reaction and incubated for 40 min at 25°C. Then, 50 μl of ADP-Glo Max Reagent was added and the reactions were allowed to incubate for a further hour at 25°C. Luminescence was measured using a SpectraMax (Molecular Devices) plate reader. Apparent inhibition of ATPase activity was determined by the equation: 1 – ((luminescence with RNA and SWI/SNF) – (luminescence with SWI/SNF))/(luminescence without BRG1) – (luminescence with BRG1)) and plotted using GraphPad Prism and fit to an equation for a sigmoidal curve.

Immunostaining and RNA-FISH assays. Cells were grown on coverslips (pre-incubated with 0.2% gelatin for 30 min), rinsed in PBS and pre-extracted in 0.5% CSDK-Triton supplemented with 10 mM ribonucleoside-5’-diphosphate complex (RVP) (New England Biolabs) on ice for 2 min. Cells were then washed once in CSK supplemented with 10 mM VRC and incubated on ice for 2 min, followed by fixation by 4% paraformaldehyde in PBS at room temperature for 10 min. Cells were then washed twice with PBS and blocked in 1% BSA in PBS containing RNase inhibitor (Roche) at room temperature for 20 min. Incubation was carried out at room temperature for 1 h with primary antibodies raised against either BRG1 (ab110641) or H3K27me3 (Millipore, 07–449) in PBS containing 1% BSA and RNase inhibitor (Roche). Cells were washed three times in PBS containing 0.02% Tween-20 (PBS-T). After incubating with secondary antibody at room temperature for 30 min, cells were washed three times in PBS-T. Cells were fixed again in 4% paraformaldehyde at room temperature for 10 min, washed twice in PBS and dehydrated in ethanol series. RNA-FISH was performed using Cy3-labeled Xist probes for 6 h at 37°C in a humid chamber. Xist was detected using nick translation of pS9. Cells were washed three times in 2x SSC. The slides were then mounted with a drop of Vectashield with 4,6-diamidino-2-phenylindole. Cells were observed under Nikon Eclipse 90i microscope equipped with 60x/1.4 NA objective lens, Orca ER charge-coupled device camera (Hamamatsu) and Volocity software (Perkin Elmer). ImageJ software was used for analysis. For the Xist-induction experiments, Xist was induced overnight. Quantification of BRG1 intensity was represented as boxplots where horizontal line in each box interior represents median of BRG1 intensity values and the black cross indicates mean.

Statistics and reproducibility. All qPCR experiments (FAIRE-qPCR, ATAC-qPCR, MNase-qPCR, ChiP-qPCR, nChiP-qPCR, UV-RIP-qPCR, RIP-qPCR, in vitro RNA pulldown–qPCR and RT-qPCR assays) were repeated at least three times as independent biological replicates and results are presented as mean ± s.d. For all qPCR results, the P values were calculated using two-tailed Student’s t-test using GraphPad. All experiments presented as western blot images have been repeated twice with similar results. All sequencing experiments have been performed in two independent biological replicates, except for negative controls as ATAC-seq in siCHD4 and siSNF2H and nChiP-seq of lqG, where only one replicate has been sequenced. For all boxplot analysis from sequencing data, the P values were calculated using a one-sided non-parametric Wilcoxon test and the box boundaries represent 25th and 75th percentiles; center line represents the median; whiskers indicate ±1.5×IQR, and points are actual values of outliers. For all barplots analysis from sequencing data, the P values were calculated using a one-sided two-proportion z-test.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. ATAC-seq, nChiP-seq and RNA-seq data have been deposited in GEO under accession code GSE109395. All other data are available from the corresponding author upon reasonable request.

References. 59. Pinter, S. F. et al. Spreading of X chromosome inactivation via a hierarchy of defined polycomb stations. Genome Res. 22, 1864–1876 (2012). 60. Langmead, B. & Salzberg, S. L. Fast gapped-read alignment with Bowtie 2. Nat. Methods 9, 357–359 (2012). 61. Li, H. et al. The sequence alignment/Map format and SAMtools. Bioinformatics 25, 2078–2079 (2009). 62. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 26, 841–842 (2010). 63. Luger, K., Rechsteiner, T. J. & Richmond, T. J. Preparation of nucleosome core particle from recombinant histones. Methods Enzymol. 304, 3–19 (1999). 64. Narlikar, G. J., Phelan, M. L. & Kingston, R. E. Generation and interconversion of multiple distinct nucleosomal states as a mechanism for catalyzing chromatin fluidity. Mol. Cell 8, 1219–1230 (2001).
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- **n/a**
  - Confirmed

☐ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐ The statistical test(s) used AND whether they are one- or two-sided
  - *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐ A description of all covariates tested
☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever suitable.
☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐ Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated
☐ Clearly defined error bars
  - State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

**Data collection**

| Raw Illumina output was converted to fastq format using Illumina Bcl2fastq v2.18 |

**Data analysis**

- Analyses were performed with: Trim Galore! V0.4.1 http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/, SAMtools http://samtools.sourceforge.net/, Bedtools v2.25.0, Bowtie2 v2.2.7, IGV, MACS2, edgR, Homer, CEAS v1.0.2, MEME-ChIP http://meme-suite.org/doc/meme-chip.html, STAMP http://www.benoslab.pitt.edu/stamp/, Molecular Evolutionary Genetics Analysis (MEGA), SeqLogo Bioconductor R package, CLOVER, seqMINER, Deeptools, Novoalign v3.00.02, HITC Bioconductor R package, scatterplot3d R package, “e1071” svm R package, Illustrator software, Microsoft Excel, and GraphPad Prism v7.0a. Any scripts or code written by the authors are available upon request from the corresponding author.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.
**Data**

Policy information about availability of data. All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The sequencing data sets generated during the current study have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE109395 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109395).

---

**Field-specific reporting**

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

---

**Life sciences study design**

All studies must disclose on these points even when the disclosure is negative.

- **Sample size**: N/A. No human or animal subjects necessitating sample size calculations were used in this study.
- **Data exclusions**: No data were excluded from analysis or reporting.
- **Replication**: All sequencing experiments were performed in at least n=2 biologically independent experiments. For all biochemistry and qPCR experiments, n=3 independent biological samples were used, enabling statistical calculations. No replicates were excluded from analyses presented, and all attempts at replication were successful.
- **Randomization**: No such relevant experiments.
- **Blinding**: No requirement for the blinding test.

---

**Reporting for specific materials, systems and methods**

**Materials & experimental systems**

| n/a | Involved in the study |
|-----|-----------------------|
| x   | Unique biological materials |
| x   | Antibodies |
| x   | Eukaryotic cell lines |
| x   | Palaeontology |
| x   | Animals and other organisms |
| x   | Human research participants |

**Methods**

| n/a | Involved in the study |
|-----|-----------------------|
| x   | ChIP-seq |
| x   | Flow cytometry |
| x   | MRI-based neuroimaging |

**Antibodies**

- anti-BRG1 (ab110641) Lot#: GR150844-37 Western Blot: 1/10,000; nChIP-seq: 4 μg
- anti-IgG (Cell signaling, 2729S) nChIP-seq:4 μg
- anti-H3 (ab1791) Lot#: GR237144-2ChIP: 2 μg
- anti-H3K27me3 (Millipore 07-449) Lot#: 2382150 ChIP: 2 μg
- anti-H2AK119ub (CellSignaling 8240S) ChIP: 2 μg
- anti-SNF2H (ab3749) Lot#: GR113175-1 Western Blot: 1/500
- anti-CHD4 (ab72418) Lot#: GR271300-10 Western Blot: 1/4,000
- anti-ZNF143 (NOVUS BIOLOGICALS INC, H00007702-M01) Lot#: G1181-2B4 Western Blot: 1/500
- anti-Rabbit (Promega, W4011) Western Blot: 1/10,000
- anti-Mouse (Promega, W4021) Western Blot: 1/10,000
Validation

All antibodies used in this study have been thoroughly validated by our laboratory to be specific (using immunoblot in wild-type and knock-down cell lines).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

WT and XaWT Xi\delta\text{Xist} fibroblast lines (Zhang et al., 2007 Cell)
♀ X+P and σX+P fibroblast lines (Jeon and Lee, 2011 Cell)

Authentication

Cell lines were genotyped by PCR.

Mycoplasma contamination

All cell lines used in the study tested negative for mycoplasma.

Commonly misidentified lines

See ICLAC register

No commonly misidentified cell lines were used.

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links

May remain private before publication.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109395

Files in database submission

GSM2942180 ATAC-seq; WT cells; Rep1
GSM2942181 ATAC-seq; WT cells; Rep2
GSM2942182 ATAC-seq; XaWT Xi[delta]Xist cells; Rep1
GSM2942183 ATAC-seq; XaWT Xi[delta]Xist cells; Rep2
GSM2942184 ATAC-seq; XaWT Xi[delta]Xist #2 cells; Rep1
GSM2942185 ATAC-seq; XaWT Xi[delta]Xist #2 cells; Rep2
GSM2942186 ATAC-seq; WT cells; siCTL; Rep1
GSM2942187 ATAC-seq; WT cells; siCTL; Rep2
GSM2942188 ATAC-seq; WT cells; siBRG1; Rep1
GSM2942189 ATAC-seq; WT cells; siBRG1; Rep2
GSM2942190 ATAC-seq; WT cells; siSNF2H; Rep1
GSM2942191 ATAC-seq; WT cells; siCHD4; Rep1
GSM2942192 ATAC-seq; XaWT Xi[delta]Xist cells; siCTL; Rep1
GSM2942193 ATAC-seq; XaWT Xi[delta]Xist cells; siBRG1; Rep1
GSM2942194 ATAC-seq; XaWT Xi[delta]Xist cells; siBRG1; Rep2
GSM2942195 ChIP-seq; WT cells; BRG1 Rep1
GSM2942196 ChIP-seq; WT cells; BRG1 Rep2
GSM2942197 ChIP-seq; XaWT Xi[delta]Xist cells; BRG1 Rep1
GSM2942198 ChIP-seq; XaWT Xi[delta]Xist cells; BRG1 Rep2
GSM2942199 ChIP-seq; WT cells; Input Rep1
GSM2942200 ChIP-seq; WT cells; IgG Rep1
GSM2942201 ChIP-seq; XaWT Xi[delta]Xist cells; Input Rep1
GSM2942202 ChIP-seq; XaWT Xi[delta]Xist cells; IgG Rep1
GSM3393999 RNA-seq; WT cells; Rep1
GSM3393999 RNA-seq; WT cells; Rep2
GSM3393998 RNA-seq; XaWT Xi[delta]Xist cells; Rep1
GSM3393998 RNA-seq; XaWT Xi[delta]Xist cells; Rep2
GSM3393996 RNA-seq; XaWT Xi[delta]Xist #2 cells; Rep1
GSM3393997 RNA-seq; XaWT Xi[delta]Xist #2 cells; Rep2
GSM3394753 ATAC-seq; WT MEF cells; Rep1
GSM3394754 ATAC-seq; WT MEF cells; Rep2

Genome browser session

N/A

Methodology

Replicates

Two biological replicates, detailed information in Methods section

Sequencing depth

On average, approximately 40 million paired-end 50 bp reads were generated per every ATAC-seq sample. On average, approximately 45 million paired-end 50bp reads were generated per every ChIP-seq sample. Further details are provided in Methods section and supplementary Table 1

Antibodies

anti-BRG1 (ab110641) or anti-IgG (Cell signaling, 2729S)
Peak calling parameters

All details of the bioinformatic tools and software were listed in Methods section, and we used the default settings for data analysis unless otherwise designated. No new computer code was used. Generally, we called MACS2 peaks by employing –BAMPE (paired-end mode), and default cut-off p-value (or p=0.005, or q=0.05, as designated).

Data quality

Pearson correlation was computed between two biological replicates. Further details provided within Methods section

Software

Adaptor sequences were trimmed with Trim Galore! V0.4.1 (stringency 12 and allowed error rate 0.2). Identical genomic sequences (PCR duplicates) were removed by custom program prior to alignment. To account for the M. mus (mus) / M. castaneus (cas) hybrid character of mouse dermal fibroblast lines that were employed in a ATAC-seq and ChIP-seq studies, reads were first aligned to custom mus/129 and cas genomes, and then mapped back to the reference mm10 genome. All alignments were performed by utilizing novoalign (v3.00.02) in paired-end mode (Novocraft Technology, Selangor, Malaysia). Post-processing of alignments was performed with custom scripts using SAMtools v0.1.18, and BEDtools (v2.25.0). These included accounting, alignment file-type conversion, extracting and reads sorting (SAMtools), and obtaining wig coverage files (SAMtools depth). In each of these cases, pooled replicates were highly correlated to each other (r² > 0.91), indicating strong data reproducibility (for details about calculation of correlation coefficients between two replicates see the following section). For visualizing ChIP binding signal, we generated fragments per million (fpm)-normalized bigWig files from the raw ChIP read counts for all reads (comp), mus-specific (mus), and cas-specific reads separately. Uniquely aligned wig files were scaled to total number of fragments per million in each library (determined by SAMtools flagstat combining reads “with itself and mate mapped” and “singletons”). ChIP-seq tag density bigWig files were viewed in Integrative Genomics Viewer (IGV). To quantify reproducibility between two biological replicates of BRG1 ChIP-seq generated in WT cells (or XaWT X[delta]Xist cells), we first called MACS2 peaks (by employing –BAMPE (paired-end mode), qvalue=0.005, and –bdg) in individual replicates, by contrasting each sample (uniquely aligned comp reads) against its corresponding control sample (prepared in the same cell line). Fold-enrichment signal (FE; IP sample’s signal over its corresponding Input sample’s signal) was calculated per each sample by executing MACS2 bdgcmp using –method FE. Peak sets called in individual replicates were then pooled, and overlapping peaks were merged to form a union peak set. By applying Homer suit’s makeTagDirectory and annotatePeaks algorithms we obtained a read counts matrix of two biological replicates over union peak set, and normalized these values per each library’s reads size using edgeR. Log2 fold-enrichment peak signal was plotted, and Pearson correlation coefficient of two biological replicates was calculated. In both cell lines, WT and XaWT X[delta]Xist, BRG1 ChIP-seq correlation coefficients of biological replicates were high (r²>0.97, 0.91, respectively), indicating strong data reproducibility.