Genome-wide distribution of linker histone H1.0 is independent of MeCP2

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Previous studies suggested that MeCP2 competes with linker histone H1, but this hypothesis has never been tested in vivo. Here, we performed chromatin immunoprecipitation followed by sequencing (ChIP-seq) of Flag-tagged-H1.0 in mouse forebrain excitatory neurons. Unexpectedly, Flag-H1.0 and MeCP2 occupied similar genomic regions and the Flag-H1.0 binding was not changed upon MeCP2 depletion. Furthermore, mild overexpression of H1.0 did not alter MeCP2 binding, suggesting that the functional binding of MeCP2 and H1.0 are largely independent.

Rett syndrome is a postnatal neurodevelopmental disorder caused by mutations in the gene encoding methyl-CpG binding protein 2 (MeCP2)1–2. Despite extensive research, the precise molecular function of MeCP2 remains unclear. MeCP2 contains a methyl-CpG binding domain and a transcription repression domain, and was initially thought to work as a transcriptional repressor3–4. However, transcriptomic analysis of mouse brain RNA revealed that MeCP2 depletion leads to both decreases and increases in gene expression5, indicating that MeCP2 is not a typical repressor. Because MeCP2 is abundant in neurons and is distributed genome-wide, tracking DNA methylation6–7, an emerging possibility is that MeCP2 is an architectural chromatin component with specialized mechanistic properties in the brain.

How could MeCP2 regulate neuronal chromatin? One hypothesis posits that MeCP2 may function like the linker histone H1, which binds to linker DNA and compacts nucleosomes8. This hypothesis was supported by in vitro studies which showed that MeCP2 binds to linker DNA and displaces linker H19–10. It was further supported by a study showing that the H1 protein level in male MeCP2 knockout (Mecp2−/−) mice is increased twofold relative

Fig. 1 | Generation of mice expressing Flag-H1.0 in forebrain excitatory neurons. a, Immunofluorescence images showing Fl-H1.0 expression in Cam-tTA:Fl-H1.0 mice (8–9 weeks old). Scale bar, 3 mm. The result was reproduced in four mice per genotype. b, Fl-H1.0 was expressed in nuclei and was enriched in DAPI foci of layer 2 Camk2α+ cortical cells (b) and of hippocampal CA1 pyramidal cells (c) in Cam-tTA:Fl-H1.0 mice (8–9 weeks old). Scale bars, 10 μm. The results were confirmed in four mice. d, RNA expression levels of various genes in the cortex. N = 5 (wild-type; WT), 6 (Cam-tTA), 6 (Fl-H1.0), 5 (Cam-tTA:Fl-H1.0) mice (8–9 weeks old) from at least two litters. One-way ANOVA for Fl-H1.0: P = 0.026 (WT vs. Cam-tTA); P = 0.26 (WT vs. Fl-H1.0); P < 0.0001 (WT vs. Cam-tTA:Fl-H1.0); P > 0.9999 (WT vs. Fl-H1.0); P < 0.0001 (Cam-tTA vs. Cam-tTA:Fl-H1.0); P < 0.0001 (Fl-H1.0 vs. Cam-tTA:Fl-H1.0). One-way ANOVA for others: P = 0.052, F(3,18) = 3.12 (H1.c); P = 0.11, F(3,18) = 2.32 (H1.x); P = 0.090, F(3,18) = 2.53 (Mecp2). e, Western blot showing Fl-H1.0 expression in the cortex of Cam-tTA:Fl-H1.0 mice (8–9 weeks old). Blot using anti-H1 antibody showed both endogenous (endo) and Flag-tagged H1.0. As the blot for H1.x resulted in a background band, we analyzed the H1.x-corresponding band around 28 kDa. The experiment was repeated five times using independent mice with similar results. f, Relative protein expression levels of H1.0 and other chromatin components, which were first normalized to H3 levels and then by the average of WT mice. N = 5 mice per genotype from at least two litters. One-way ANOVA for total H1.0: P = 0.00224, F(3,16) = 7.466. Tukey’s multiple comparison for total H1.0: P = 0.88 (WT vs. Cam-tTA); P = 0.68 (WT vs. Fl-H1.0); P = 0.0024 (WT vs. Cam-tTA:Fl-H1.0); P = 0.98 (WT vs. Fl-H1.0); P = 0.011 (Cam-tTA vs. Cam-tTA:Fl-H1.0); P = 0.023 (Fl-H1.0 vs. Cam-tTA:Fl-H1.0). One-way ANOVA for others: P = 0.54, F(3,16) = 0.75 (endogenous H1.0); P = 0.67, F(3,16) = 0.53 (MeCP2); P = 0.3613, F(3,16) = 1.14 (H1.c); P = 0.96, F(3,16) = 0.090 (H1.x); P = 0.84, F(3,16) = 0.2851 (histone H3 Lys9 trimethylation (H3K9me3)); P = 0.59, F(3,16) = 0.66 (H3K27me3). g, Fl-H1.0 enrichment at major satellite (Sat), Gapdh TSS, and Gapdh promoter (Pro) was measured by ChIP-qPCR using the frontal cortex of Cam-tTA:Fl-H1.0 and control (Cam-tTA) mice. Fl-H1.0 enrichment was very low to undetectable (0–0.1%) in Cam-tTA. N = 3 mice per genotype, 8–9 weeks old. h, ChIP-qPCR using frontal cortex showed MeCP2 enrichment was comparable between WT and Cam-tTA:Fl-H1.0 mice in various MeCP2 targets (N = 5 mice per genotype from at least two litters, 14–21 weeks old). ChIP-qPCR from Mecp2−/− mice (N = 3 mice, 8–9 weeks old) resulted in very low enrichment (0.00–0.02%). Two-tailed t-test between WT and Cam-tTA:Fl-H1.0: P = 0.98, t0 = 0.22 (major satellite (sat)); P = 0.79, t0 = 0.28 (bdn); P = 0.90, t0 = 0.13 (Myc-4583); P = 0.56, t0 = 0.60 (Myc-3673); P = 0.65, t0 = 0.47 (Myo-1170). Two-tailed Mann-Whitney U test: P = 0.55 (Myc-8373). i, Scatter plot showing correlation for MeCP2 ChIP-seq reads between WT and Cam-tTA:Fl-H1.0 mice using 264,922 data points pooled from the three mice per genotype. ChIP-seq reads were normalized to input and averaged over 10-kb windows. Box-and-whisker plots show median, 25th and 75th percentiles, and minimum and maximum values (df). Graphs with individual data show average ± s.e.m. (g.h). *P < 0.05, **P < 0.01, ***P < 0.0001. Full-length blots are available in Supplementary Fig. 5.

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to wild-type mice. Notably, HMG-A proteins, which have AT-hook domains homologous to MeCP2, were also shown to compete with H1. These observations suggest that MeCP2 depletion may lead to accumulation of H1 in genomic regions where MeCP2 normally binds, causing chromatin structural changes. This model has been widely discussed as a potential mechanism to describe MeCP2's function. However, it has never been tested in vivo due to lack of optimal antibodies for H1.

Linker H1 has several subtypes, among which H1.0, H1.c and H1.x are expressed in the brain. To obtain insight into which H1 might be involved in Rett syndrome, we examined H1 levels in neonatal nuclei from the cortex of Mecp2–/y, MeCP2-overexpressing (Mecp2Tg3/y) and wild-type mice. The result showed a mild trend toward increase in H1.0 in Mecp2–/y mice (Supplementary Figs. 1f,g and 5), while H1.c and H1.x levels remained unchanged. None of the H1 subtypes was affected in Mecp2Tg3/y mice (Supplementary Figs. 1h,i and 5). Given that H1.0 was shown to compete with MeCP2 for DNA binding in vitro, we chose to study H1.0 in vivo.

To study the effects on H1.0, we generated mice expressing Flag-tagged H1.0 (Fl-H1.0) in forebrain excitatory cells, given the contributions of this cell type to Rett syndrome pathogenesis. Fl-H1.0 mice, which carried a Fl-H1.0 transgene under a tetracycline response element, were crossed with the Camk2a-promoter-driven tetracycline transactivator (Cam-tTA) line to induce Fl-H1.0 expression in Camk2α-positive cells (Fig. 1a–f). Total H1.0 protein was increased 1.5-fold in the cortex of Cam-tTA:Fl-H1.0 mice (Fig. 1e,f), while the levels of MeCP2, other H1 subtypes, and H3 modifications associated with linker H1 remained unchanged (Fig. 1e,f and Supplementary Fig. 5). Consistent with previous reports, ChIP followed by quantitative PCR (qPCR) showed that
Fl-H1.0 enrichment was high at the heterochromatic major satellite repeats and low at the transcriptional start site (TSS) of Gapdh (Fig. 1g). To address whether Fl-H1.0 expression affects MeCP2 binding to DNA, we measured MeCP2 enrichment by ChIP-qPCR at sites identified as rich in MeCP2 binding 7,16 and found no change in Cam-tTA:Fl-H1.0 mice (Fig. 1h). To further examine the effect of H1.0 overexpression on the genomic distribution of MeCP2, we performed ChIP-seq using frontal cortex of 3-month-old wild-type and Cam-tTA:Fl-H1.0 mice. We used three mice per genotype for sequencing and obtained sufficiently high read depth to cover the broad distribution of MeCP2 (Supplementary Table 1). Consistent with the previous studies 7,17,18, we found that MeCP2 enrichment positively correlated with the levels of both CG and CH methylation (where H represents A, T or C) 19 while it was lower around CG islands (Supplementary Fig. 2). These results were observed similarly in both wild-type and Cam-tTA:Fl-H1.0 mice (Supplementary Fig. 2b–e). Further analysis revealed little change in MeCP2 distribution upon Fl-H1.0 expression: principal component analysis revealed no clear separation between wild-type and Cam-tTA:Fl-H1.0 mice (Supplementary Fig. 2f), and the Spearman correlation coefficient of MeCP2 enrichment between wild-type and Cam-tTA:Fl-H1.0 mice was 0.93 at the binning size of 10 kb (Fig. 1i). We also performed window-based comparison, whereby average MeCP2 enrichment from each mouse was computed for every 200-bp window throughout the genome and was tested for significant differences. The result showed that MeCP2 enrichment was comparable in 99.96% of the genome at an uncorrected P-value threshold of 0.001. Together, these results suggested that Fl-H1.0 was properly expressed with no measured effect on MeCP2.

Next we crossed Fl-H1.0 and Cam-tTA mice with Mecp2−/− mice to obtain Cam-tTA:Fl-H1.0:Mecp2−/− (fl-Mecp2−/−) and Cam-tTA:Fl-H1.0:Mecp2+− (fl-Mecp2+−) mice, which expressed Fl-H1.0 in the presence and absence of MeCP2, respectively. Overall health of Mecp2−/− and Mecp2+− mice was not affected by Fl-H1.0 expression (Supplementary Fig. 3a,b). The levels of Fl-H1.0 were similar in fl-Mecp2−/− and fl-Mecp2+− mice (Supplementary Figs. 3c–e and 5). Together with our results on the endogenous H1.0 (Supplementary Fig. 1f,g), these results indicate that it is likely that H1.0 level is not robustly affected by MeCP2. However, because binding patterns might change even in the face of unchanged protein levels, we reasoned that we needed to clarify the genome-wide distribution of Fl-H1.0 and its relations to MeCP2. We therefore performed Fl-H1.0 ChIP-seq using the frontal cortex of fl-Mecp2−/− and fl-Mecp2+− mice (8–9 weeks old). Sequencing was again performed with a high read count to achieve sufficient resolution and was performed using three mice as biological replicates to account for variability (Supplementary Table 1). Consistent with previous reports 13, Fl-H1.0 was distributed throughout the genome but depleted around TSS of transcribed genes (Fig. 2a). To look for changes in genome-wide distribution of Fl-H1.0, we computed Spearman correlation coefficients for the normalized ChIP-seq reads between replicates.

**Fig. 2 | ChIP-seq revealed no changes in genomic distribution of Fl-H1.0 upon MeCP2 loss.** a. Representative tracks of Fl-H1.0 ChIP-seq from individual replicates (R1-R3) and pooled datasets (P) are shown together with MeCP2 ChIP-seq from the frontal cortex (FC) of the Fl-H1.0 expressing mice. Fl-H1.0 distribution is widely similar between replicates, and Fl-H1.0 is depleted around two alternative start sites of Bdnf (orange box). b. Scatter plot showing correlation for Fl-H1.0 ChIP-seq reads between fl-Mecp2−/− and fl-Mecp2+− mice (264,901 data points from pooled data). c. Scatter plot showing correlation between MeCP2 and Fl-H1.0 ChIP-seq from fl-Mecp2−/− mice (264,903 data points from pooled data). d. Scatter plot showing correlation between MeCP2 and Fl-H1.0 ChIP-seq from fl-Mecp2+− mice (264,907 data points from pooled data). ChIP-seq reads were normalized to input and averaged in 10-kb windows (b–d).
We observed high correlations between replicates regardless of the genotypes (Supplementary Fig. 4a and Table 2), indicating that MeCP2 depletion had little impact on Fl-H1.0 distribution. Consistently, principal component analysis showed no separation between genotypes (Supplementary Fig. 4b).

To clarify the relationship between H1 and MeCP2, we computed correlations between Fl-H1.0 and MeCP2 ChIP-seq data from the Cam-tTA;Fl-H1.0 mice, which were genetically identical to fh-Mecp2+/y mice. If MeCP2 competes with H1, we would expect to see a negative correlation between MeCP2 and Fl-H1.0, which should
shift toward a positive correlation upon MeCP2 depletion. Contrary to our expectation, however, the correlations between MeCP2 and Fl-H1.0 were positive in both \(fh\)-MeCP2\(^{+/y}\) and \(fh\)-MeCP2\(^{-/-}\) mice (Supplementary Fig. 4a), and the correlation coefficient did not change when MeCP2 was depleted. This result suggested that Fl-H1.0 and MeCP2 occupied similar genomic regions, while the distribution of Fl-H1.0 was unaffected by the presence of MeCP2. To further validate our finding, we pooled the three replicates for each genotype together. The correlation for Fl-H1.0 between \(fh\)-MeCP2\(^{+/y}\) and \(fh\)-MeCP2\(^{-/-}\) was again very high (Fig. 2b), and the correlation between Fl-H1.0 and MeCP2 ChIP-seq data was also consistently high in both \(fh\)-MeCP2\(^{+/y}\) and \(fh\)-MeCP2\(^{-/-}\) mice (Fig. 2c,d). We also performed correlation analysis using larger (1 Mb and 10 Mb) and smaller (1 kb and 0.2 kb) window sizes and obtained consistent results (Supplementary Fig. 4c and Table 3).

To examine more local effects on Fl-H1.0, we examined Fl-H1.0 binding in the regions where MeCP2 is enriched, which included heterochromatic repeat elements and MeCP2 peaks identified from ChIP-seq. We detected no change in Fl-H1.0 in either region (Fig. 3a,b). The results from the repeat elements were confirmed by ChIP-qPCR in the frontal cortex and hippocampus (Fig. 3c,d). To examine Fl-H1.0 in euchromatic regions and to correlate the results with gene transcription, we mapped Fl-H1.0 reads around TSS of genes, which were grouped according to RNA expression levels\(^5\). Consistent with previous reports\(^6,7\), the magnitude of depletion around TSS correlated with the gene expression (Fig. 3e). However, this correlation was observed similarly in \(fh\)-MeCP2\(^{+/y}\) and \(fh\)-MeCP2\(^{-/-}\) mice (Fig. 3e and Supplementary Fig. 4d). We also examined Fl-H1.0 distribution around the TSS of those genes differentially expressed in the cortical layer 5 excitatory cells of MeCP2\(^{-/-}\) mice\(^8\) and found no change (Fig. 3f and Supplementary Fig. 4e,f). Taken together, ChIP-seq data revealed no change in Fl-H1.0 distribution upon MeCP2 depletion. This was further validated by the window analysis, in which we compared average enrichment for Fl-H1.0 in 200-bp windows using biological replicates. The result showed Fl-H1.0 enrichment was comparable in 99.10% of the regions at an uncorrected \(P\)-value threshold of 0.001.

In conclusion, we found that H1.0 and MeCP2 tend to bind to similar genomic regions but, notably, we detected little change in H1.0 distribution upon MeCP2 depletion, suggesting that MeCP2 works independently of competition with linker H1.0. High-depth sequencing using multiple biological replicates was crucial to reach our expectation, however, the correlations between MeCP2 and MeCP2 distribution upon mild overexpression of H1.0 (Fig. 1) using both ChIP-seq and ChIP-qPCR clearly showed that there is no direct MeCP2-H1.0 competition in cortical excitatory neurons. Our findings argue for the need for more studies to pinpoint the molecular mechanism by which MeCP2 alters chromatin architecture.

### Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41593-018-0155-8.

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Methods

Animals. Mice were housed in an AAALAS-certified Level 3 facility on a 14 h light cycle. The mice were weaned 21 d after birth and housed with 3–5 littermates per cage. F1.H1.0 mice were generated in our laboratory (see below) and maintained on an FVB background. Cam-TATA mice (TATA) and Cam-TATA mouse (TATA) were purchased from Jackson Laboratory and maintained on a 129S6 background. Mecp2-Tg3 and Mecp2-null mice were maintained on FVB and 129S6 backgrounds, respectively. Male Cam-TATA heterozygous mice were mated with female F1.H1.0 heterozygous mice, and male offspring with a 129/ FVB hybrid background was used for analysis. To study F1.H1.0 in Mecp2−/− mice and their Mecp2+/- littermates, we first mated Cam-TATA heterozygous male mice with female Mecp2−/− mice to obtain females heterozygous for both Cam-TATA and Mecp2. These females (129S6 background) were crossed with male F1.H1.0 mice (FVB background) to obtain male Cam-TATA:F1.H1.0:Mecp2+/− and Cam-TATA:F1 H1.0:Mecp2−/− mice (129/FVB hybrid background). These mice were scored based on their littermate phenotype while the embryos were bled to the genotypes. Other experiments were performed without blinding to genotypes. No randomization was performed but all the experiments were conducted using appropriate littermate controls. All procedures for maintaining and using these mice were approved by the Institutional Animal Care and Use Committee for Baylor College of Medicine and Affiliates.

Generation of Flag-H1.0 transgenic mice. Mouse gene encoding H1.0 was directly cloned from wild-type C57/BL6 mouse genomic DNA by PCR following primer sets to incorporate 3XFlag tag to the N-terminus of H1.0: Forward 5′- ggacggaaggccatcagcatcactgaggaagccaggtagagagtgaag-3′, Reverse 5′- gcggcggttttcctttgctggccct-3′. The PCR product was digested by restriction enzymes (EcoRI/HindIII) and was inserted into the p-3 Tight vector (derived from Addgene vector 23966). The vector was purified with a Nucleobond Xtra Midi Kit (Clontech) and digested with BclI and the fragment was used for pronuclear injection according to standard procedures. The mouse strain was maintained on an FVB background and stable germline transmission was confirmed for at least four generations. These Flag H1.0 transgenic mice have been donated to and will be available from the Baylor College of Medicine and Affiliates.

Gene expression analysis with RT-qPCR. Cortex from 8- to 9-week-old mice was dissected and immediately frozen in liquid nitrogen. The frozen tissue was placed in 1 ml Purezol (Bio-Rad) on ice and immediately homogenized with a Dounce homogenizer. Cortex from 8- to 9-week-old mice were used for western blot. Chromatin immunoprecipitation. Mice were approved by the Institutional Animal Care and Use Committee for Baylor College of Medicine and Affiliates.

Histone expression and western blot. Cortex from 8- to 9-week-old mice was dissected and immediately frozen in liquid nitrogen. The frozen tissue was placed in 1 ml PBS on ice and gently homogenized using a Dounce homogenizer. The samples were centrifuged at 3,300 g for 5 min. The supernatant was discarded and 500 μl of ice-cold 0.4 M sulfuric acid was added to the pellet. After overnight rotation at 4 °C, the sample was centrifuged at 16,000g for 10 min to collect the supernatant, and 125 μl of trichloroacetic acid (TCA) was added. After 1 h incubation, the precipitate containing histones was collected by centrifugation at 16,000g, 4 °C, for 30 min. The precipitate was dissolved in 2X SDS buffer (100 μl of Tris (pH 6.8), 4% SDS, 20% glycerol), protein concentration was adjusted using Pierce BCA protein assay kit (Thermo Fisher) and the sample was used for western blots in NuPAGE Bis-Tris gels. In the experiments in which we extracted histones from the sorted nuclei, acid extraction was performed by overnight incubation with 0.4 N sulfuric acid, followed by addition of one-tenth volume of 4 N sodium hydroxide, and the sample was processed for western blot without precipitation by TCA.

Isolation of neuronal nuclei by cell sorting. Nuclei were isolated from frozen cortices as previously described with a few modifications. Bilateral cortices were homogenized in 5 ml of cold HEPES-buffered saline, 5 mM CaCl2, 3 mM magnesium diacetate, 10 mM Tris (pH 8.0), 0.1 mM EDTA, 0.1% Triton-X, 0.1 mM PMSF, 1X complete protease inhibitors (CPI, Sigma, #A8392), anti-H1.1 (1:500, rabbit polyclonal, Proteintech, #19649-1-AP), anti-H1.1x (1:500, rabbit polyclonal, Abcam, #ab31972), anti-H3 (1:500, rabbit polyclonal, Abcam, #ab31972), anti-H3K27me3 (1:5,000, rabbit polyclonal, Millipore, #07-449), and anti-MeCP2 (1:5,000, rabbit polyclonal, in-house)8. Secondary antibodies conjugated to HRP were mouse anti-rabbit (Jackson ImmunoResearch Laboratories, 201-032-171) and donkey anti-mouse antibodies (Jackson ImmunoResearch Laboratories, 715-035-150). Immunoblot images were acquired with an ImageQuant LAS 4000 (GE Healthcare) and quantified using Image-Pro Analyzer 7 (Media Cybernetic).
Brief Communication Nature Neuroscience

Sequenced to a depth of ~90 million 100-bp paired-end reads. Cutadapt version
MeCP2 ChIP samples, 3
ChIP-seq analysis.

Data processing.
the DNA containing the target sequence was precipitated by the ChIP reaction.
either anti-Flag or anti-MeCP2 antibody. 100% enrichment over input means all

For minor satellite repeat, LINE L1 and SINE B1 are from Martens et al.26. Statistical

parameters ratio, --binsize 50, --smooth 150 and normalizeTo1x). Normalized

ChIP-seq enrichment around MeCP2 peak regions (5 kb upstream and downstream of the peaks) were generated using ComputeMatrix and plotProfile modules of deepTools28.

Peak calling. Peaks from the MeCP2 ChIP-seq were called using MACS226 with parameters --nomodel, --broad and --broad-cutoff 0.1. Aggregation plots of normalized FL-H1.0 ChIP-seq enrichment around MeCP2 peak regions (5 kb upstream and downstream of the peaks) were generated using ComputeMatrix and plotProfile modules of deepTools28.

Repeat quantification. To analyze FL-H1.0 enrichment in repetitive elements, repeat annotations and respective sequences of mm10 were downloaded from the UCSC table browser. Major satellite, minor satellite, L1 LINE and B1 SINE elements were quantified from fl-Mecp2+/− and fl-Mecp2−/− samples using Salmon27.

Gene expression data. Microarray data from the pyramidal cells in motor cortex in MeCP2+/− and MeCP2−/− mice were acquired from GEO (GSE8720)29. The genes were categorized based either on their expression levels (top 20%, bottom 20%, and the rest), or on the expression level difference between MeCP2+/− and MeCP2−/− mice (top 100 genes upregulated in MeCP2−/−, top 100 genes downregulated in MeCP2−/− mice, and the genes unaltered). The aggregation plots of FL-H1.0 ChIP-seq along the TSSs were generated with respect to these categories. Significance of the difference between fl-Mecp2+/− and fl-Mecp2−/− was computed using respective replicates.

Immunofluorescence. Brain sections were obtained as previously described24. The brains were fixed by transcardial perfusion of PBS-buffered 4% paraformaldehyde (PFA). Brains were removed, kept in 4% PFA overnight and cryoprotected in 25% sucrose solution, and frozen in Optimal Cutting Temperature medium (O.C.T.). Sagittal sections were obtained using a Leica CM3050S cryostat at 25 μm thickness. The slices were incubated in a PBS-buffered blocking solution containing 2% normal goat serum and 0.3% Triton-X for 1 h, followed by incubation in a primary antibody solution containing anti-MeCP2 antibody (1:1000, rabbit monoclonal, D4F3, Cell Signaling, #3456), anti-CamK2α antibody (1:1000, rabbit monoclonal, Abcam, #ab52476) and anti-Flag antibody (1:1000, mouse monoclonal, M2, Sigma, #F1804) overnight at 4°C. After three washes with PBS, the slices were incubated with secondary antibodies conjugated with either Alexa 488, 555 or 633 (1:100, Invitrogen) overnight at 4°C. After one wash with PBS, PBS containing DAPI (2.5 μg/ml) was applied for 5 min, followed by two more washes with PBS. To obtain high-magnification images of FL-H1.0, we used FITC-labeled anti-Flag antibody (1:1000, mouse monoclonal, Sigma, #F1409) with antigen retrieval. The antigen retrieval was performed by incubating slices in a buffer containing 10 mM sodium citrate (pH 6) and 0.05% Tween 20 and heating them to 95°C for 5 min. Stained brain sections were imaged using a Leica LCS SP8 confocal microscope with either a 60× glycerol lens (N.A. 1.3) or a 20× air lens (N.A. 0.7), and the images were processed using ImageJ and Image-Pro Analyzer 7.0 (Media Cybernetics).

All image sets were acquired at a same laser and gain settings when processing multiple genotypes, and they were processed using the same intensity thresholds.

Statistics summary. Statistical tests for each assay were chosen based on their appropriateness for the assay. All statistical calculations were carried out using Graphpad Prism software with α = 0.05. No statistical method was used to predetermine sample size, but our sample sizes are similar to or exceed previous publications24. For experiments comparing two groups, data distribution was examined by F-test. Two-tailed t-test was performed when the result was not significant, whereas non-parametric test was performed when the result from F-test was significant. For experiments comparing multiple groups, data distribution was assumed to be normal, but this was not formally tested. Variance was estimated as s.e.m. Multiple comparisons were corrected by Tukey’s post hoc test. Statistical methods and values including all P values are described in figure legends.

Data availability. All datasets in the manuscript will be available upon reasonable request. Datasets from MeCP2 and FL-H1.0 ChIP-seq were deposited to Gene Expression Omnibus database (GSE107533).

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Reporting Summary

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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

Data collection was performed using commercial built-in softwares provided with equipment: ImageQuant LAS 4000, Cell Sorter SH800, Bio-Rad CFX96 Real-Time System, Illumina HiSeq 2500, and Leica LCS SP8 confocal microscope.

Data analysis

Image J, Image-Pro Analyzer 7.0, GraphPad Prism 7, FastQC v0.10.1, Bowtie2 V2.1.0, SAMtools V0.1.19, BEDtools v2.17.0, deepTools, MACS2, Integrative Genome Browser (IGV), bwtool 1.0, R and Python.

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

All data sets in the manuscript will be available upon request. Data sets from MeCP2 and Fl-H1.0 ChIP-Seq were deposited to Gene Expression Omnibus database (GSE107533).

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

We did not predetermine sample size using statistical method but our sample numbers meet or exceed those used in previous related publications. These publications were referenced in corresponding paragraphs of the main text describing each experiment.

Data exclusions

We did not exclude any data points.

Replication

Experiments including Western blot, qRT-PCR and ChIP-qPCR were performed using sufficient number of biological replicates to ensure reproducibility. The number of samples are indicated in figure legends. Immunostaining using mouse brains was repeated at least three times for each condition and the results were successfully reproduced. ChIP-Seq was performed with three biological replicates per genotype. We analyzed both pooled data sets and independent replicates. All conclusions derived from the pooled data sets were confirmed by analyzing biological replicates.

Randomization

The experiment was not randomized. Each experiment was performed with appropriate control mice with the same genetic background.

Blinding

Blinding was done only for the analysis to obtain phenotypic scores of Rett related features in mice (Supplementary Fig. 3). No blinding was done for other experiments and analysis as they were performed using unbiased methods. This was stated in Online Methods, Animals.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a Involved in the study

☒ ☐ Unique biological materials
☒ ☐ Antibodies
☒ ☒ Eukaryotic cell lines
☒ ☒ Palaeontology
☒ ☐ Animals and other organisms
☒ ☒ Human research participants

Methods

n/a Involved in the study

☒ ☐ ChIP-seq
☑ ☒ Flow cytometry
☒ ☒ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

All materials in the manuscript will be available upon request. Flag-H1.0 transgenic mice have been donated to and will be available from the Jackson Laboratory.
Antibodies

For western blot, we used anti-Flag (mouse monoclonal, Sigma, ab6556), HRP-conjugated anti-Flag (mouse monoclonal, Sigma, #A8592), anti-H1.0 (mouse monoclonal, Abcam, #ab11079), anti-H1.c (rabbit polyclonal, Proteintech, #19649-1-AP), anti-H1.x (rabbit polyclonal, Abcam, #ab31972), anti-H3 (rabbit monoclonal, Cell Signaling, #4499P), anti-H3K9me3 (rabbit polyclonal, Abcam, #ab8898), anti-H3K27me3 (rabbit polyclonal, Millipore, #07-449), and anti-MeCP2 antibody generated in house. For ChIP, we used anti-MeCP2 (chicken and rabbit polyclonal, Millipore, #17-10491) and anti-Flag antibody (mouse monoclonal, Sigma, #A8592). For FACS, we used Alexa 555-conjugated anti-NeuN antibody (mouse monoclonal, Millipore, #MAB377A5). For immunofluorescence, we used anti-MeCP2 (rabbit monoclonal, Cell Signaling, Cat #3456), anti-Camk2a (rabbit monoclonal, Abcam, #ab52476), unconjugated anti-Flag (mouse monoclonal, M2, Sigma, #F4049), and FITC-labeled anti Flag antibody (mouse monoclonal, Sigma, #F4049). Secondary antibodies conjugated with HRP were used for the western blot and they were mouse anti-rabbit (Jackson ImmunoResearch Laboratories, 211-032-171) and donkey anti-mouse antibodies (Jackson ImmunoResearch Laboratories, 715-035-150). Secondary antibodies (Goat IgG) conjugated with either Alexa 488, 555 or 633 were used for immunostaining and they were from Invitrogen.

Validation

Most of the antibodies above are commonly used commercial antibodies that have been validated for each experiment using mouse tissue. Anti-MeCP2 antibody for western blot generated in house was validated by probing brain samples from Mecp2-null mice (Supplementary Fig. 1).

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mecp2-null (Guy et al., 2001) and Mecp2-Tg3 mouse lines were maintained in 129S6 and FVB background, respectively. Mecp2-null and Mecp2-Tg3 mice were mated with wild-type mice of either FVB or 129S6 background, and male offsprings with 129/FVB hybrid background (8-9 wk) were used to quantify H1 levels in the neuronal nuclei from the forebrain. Fl-H1.0 mice were generated in our lab and maintained in FVB background. Cam-tTA mice [129S6.Cg-Tg(Camk2α-tTA)1Mmay/JLwsJ] were from Jackson Laboratory and maintained in 129S6 background. Male Cam-tTA heterozygous mice were mated with female Fl-H1.0 heterozygous mice, and the male offspring with 129/FVB hybrid background was used for biochemical and histological analysis (8-9 wk old). MeCP2-ChIP Seq was performed using frontal cortex of 3-4 months old Cam-tTA:Fl-H1.0 mice and their wild-type littersmates. To study Fl-H1.0 in Mecp2-/y mice and their Mecp2+/y littersmates, we first mated Cam-tTA heterozygous male mice with female Mecp2-/+ mice to obtain females heterozygous for both Cam-tTA and Mecp2. These females [129S6 background] were crossed with male Fl-H1.0 mice (FVB background) to obtain male Cam-tTA:Fl-H1.0:Mecp2+/y and Cam-tTA:Fl-H1.0:Mecp2-/y mice (129/FVB hybrid background). Fl-H1.0 ChIP-Seq was performed using frontal cortex of these mice (8-9 wk old).

Wild animals

This study did not involve wild animals.

Field-collected samples

This study did not involve samples collected from the field.

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=GSE107533

Files in database submission

Processed files: H1.WT1.ratio.bw, H1.WT2.ratio.bw, H1.WT3.ratio.bw, H1.KO1.ratio.bw, H1.KO2.ratio.bw, H1.KO3.ratio.bw, Mecp2_FH1.bw, Mecp2_FH2.bw, Mecp2_FH3.bw, Mecp2_WT1.bw, Mecp2_WT2.bw, Mecp2_WT3.bw

Peak file: GSE107533_Mecp2.FH.MACS_peaks.bed.gz

Raw fastq files:

Run1_HZ1411_ATCACGTT_L006_R1_001.fastq.gz
Run1_HZ1411_ATCACGTT_L006_R2_001.fastq.gz
Run1_HZ1411_ATCACGTT_L007_R1_001.fastq.gz
Run1_HZ1411_ATCACGTT_L007_R2_001.fastq.gz
Run1_HZ1411_ATCACGTT_L008_R1_001.fastq.gz
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Methodology

Replicates

For both MeCP2 Chip-Seq and Fl-H1.0 Chip-Seq, we used three mice per genotype as biological replicates. We also sequenced input for each corresponding ChIP sample.

Sequencing depth

Total number of reads and mappability for each sample is summarized in Supplementary Table 1. Average number of total reads was 249 million, and the average mappability was 95.9% (overall). Length of the reads was 100bp (pair-end reads).

Antibodies

1. Anti-MeCP2 antibody from Millipore (#17-10491, chicken & rabbit polyclonal)
2. Anti-Flag antibody from Sigma (#F1804, mouse monoclonal).

Peak calling parameters

MeCP2 peaks were called using MACS2 with parameters --nomodel, --broad, --broad-cutoff 0.1, band width of 300 and model fold = [5, 50].

Data quality

Read quality was ensured using fastqc: average read phred score was greater than 30. The average mappability of all the samples was 95.9%.

We further ensured data quality by correlating MeCP2/H1 density with a well established genomic signatures that are known to be characteristics of MeCP2/H1 binding. Consistent with previous literatures, our data showed that MeCP2 enrichment positively correlated with DNA methylation and that Fl-H1.0 enrichment was depleted around TSS. In addition, specificity of ChIP reaction was ensured by Chip-qPCR using Mecp2-null brains for MeCP2 ChIP and control (Fl-H1.0 non-expressing) brains for Fl-H1.0 ChIP.

Software

FastQC v0.10.1, Bowtie2 V2.1.0, SAMtools V0.1.19, BEDtools v2.17.0, deepTools, MACS2, Integrative Genome Browser (IGV), bwtool 1.0, R and Python.
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Nuclei were isolated from frozen cortices as previously described with a few modifications (Swiech, Nat Biotech, 2015). Bilateral cortices were homogenized in 5 ml of ice-cold homogenization buffer (320 mM sucrose, 5 mM CaCl2, 3 mM Mg(Ac)2, 10 mM Tris (pH8.0), 0.1 mM EDTA, 0.1% Triton-X, 0.1 mM PMSF, 1x complete protease inhibitors (CPI, Sigma, #04693132001)). After incubation for 5 min, the lysate was mixed with 50% OptiPrep medium (50% OptiPrep, 320 mM sucrose, 5 mM CaCl2, 3 mM Mg(Ac) 2, 10 mM Tris (pH8.0), 0.1 mM PMSF, and 1x CPI) and was gently loaded on top of 10 ml 29% iso-osmolar OptiPrep solution. Samples were centrifuged at 10,000 × g, 4 °C, for 30 min. The nuclei pellet was resuspended in ice-cold nuclei suspension buffer (65 mM β-glycerophosphate (pH 7.5), 2 mM MgCl2, 25 mM KC1, 340 mM sucrose, 5% glycerol, 0.1 mM PMSF, and 1x CPI), and incubated with Alexa 555-conjugated anti-NeuN antibody (1:500, mouse monoclonal, Millipore, #MAB377AS) and 2% normal goat serum for 1 hour at 4 °C. The sample was filtered through a cell strainer, diluted with the nuclei suspension buffer, and were sorted using Cell Sorter SH800 (Sony).

Instrument

Sony SH800 cell sorter

Software

Built-in SH800 software was used for analysis

Cell population abundance

NeuN-positive nuclei was mounted on coverslips and observed by a fluorescent microscope. The positive fraction was >95 %.

Gating strategy

First, FSC-A x BSC-A plot was used to separate larger particles from smaller debris. Next, FSC-A x FSC-H plot was used to remove duplicates. Finally, NeuN-positive population was identified based on the Alexa 555 fluorescent intensity. Fluorescence event histogram showed two clear peaks. NeuN-positive fraction was isolated by sorting 70-75% of the nuclei from the higher peak to increase specificity. We used the same gating threshold for the nuclei incubated with Alexa 555-labeled mouse IgG as a negative control and confirmed that the positive fraction was almost negligible.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.