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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	SH800Z Cell Sorter Software version 2.1.3
Data analysis	TopHat2 v2.1.0 was used to align total RNAseq reads. Freebayes version 1.1.0-4 was used to identify single nucleotide variants. Samtools 1.2 was used to extract reads containing variants. Prism v7 was used for certain statistical analyses. Single-cell clustering was performed with Seurat and differential expression was performed with Monocle 2. Custom scripts were written in R version 3.3.3 to count the number of reads that contain allele-specific single nucleotide polymorphisms in the single-cell RNA sequencing data. These are available upon request.

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 sequencing data reported in this study have been deposited in the NCBI Gene Expression Omnibus under accession GSE113673

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	No statistical methods were used to pre-determine sample size but our samples sizes are similar or larger to those reported in previous publications.
Data exclusions	Cells or nuclei with greater than 500 unique genes detected per cell were included for further consideration. Cells or nuclei with greater than 15,000 unique molecular identifiers detected were omitted to minimize inclusion of data that represented the common barcoding of two or more cells.
Replication	We observed the same findings within each rodent and human individual studied.
Randomization	mice of the correct genotype, age, and sex were randomly selected for inclusion in the study. To generate randomly transcriptotyped groups of cells, the sample function in R version 3.2.2
Blinding	Cell encapsulation was blinded. Analysis was conducted using automated scripts, but knowledge of sample IDs to write the code.

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

Antibodies

Antibodies used	MECP2 C-terminal antibody (made in-house); goat anti-rabbit 647 secondary antibody (Life Technologies) cat#A21244
Validation	Chen, et al. Science 2003, Cohen et al. Neuron 2011. Signal is absent in MeCP2 KO tissue.

Animals and other organisms

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

Laboratory animals	Mecp2tm1.1Bird/J mutant mice and their wild-type controls were obtained from Jackson labs (Stock No. 003890). Male mice were 8 weeks old, female mice were 12-20 weeks old.
Wild animals	This study did not use wild animals
Field-collected samples	This study did not use field-collected samples

Human research participants

Policy information about studies involving human research participants

Population characteristics	Female Rett syndrome brain donors were 8-24 years old. All donors had the R255X mutation in MECP2.
Recruitment	Rett syndrome donor brain tissue was obtained from the NIH NeuroBioBank/Harvard Brain Bank in coordination with Rettsyndrome.org

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.*	GEO accession GSE113673
Files in database submission	Raw Samples: CamkIIa_cortex_MeCP2_ChIP_rep1, CamkIIa_cortex_input_rep1, CamkIIa_cortex_MeCP2_ChIP_rep2, CamkIIa_cortex_input_rep2 Processed: GSE113903_CamkIIa_mecp2_chip_counts.txt.gz
Genome browser session (e.g. UCSC)	N/A

Methodology

Replicates	2 biological replicates of MeCP2 ChIP and input from CamkIIa-positive nuclei from mouse cortex.
Sequencing depth	MeCP2 ChIP rep 1: 59108980 total reads; 32580048 mapped, de-duplicated reads Input rep 1: 62068745 total reads; 44909766 mapped, de-duplicated reads MeCP2 ChIP rep 2: 55833090 total reads; 27752219 mapped, de-duplicated reads Input rep 2: 59495391 total reads; 40515255 mapped, de-duplicated reads Read length: 85bp single-end
Antibodies	MeCP2 antibody from Chen WG et al. Science 2003
Peak calling parameters	Read mapping: Used Bowtie2 (version 2.2.4) to map to mm10 genome using default parameters Peak calling: N/A; Mapped ChIP and input reads were counted in mm10 gene body locations using Bedtools map (version 2.23.0)
Data quality	N/A; did not call peaks; see MeCP2 gene body densities in processed counts tables.
Software	Trimmomatic (version 0.33) was used to trim reads Bowtie2 (version 2.2.4) was used to map reads to mm10 genome Samtools (version 0.1.19) was used to remove duplicate reads UCSC-tools was used to extend reads to 250bp Bedtools (version 2.23.0) was used to count mapped ChIP and input reads in mm10 gene body locations

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	To stain nuclei for sorting, nuclei were incubated with a C-terminal MeCP2 antibody at 1:500 for 1 hour at 4°C, washed once with Wash buffer (PBS with 1% BSA and 0.16% IGEPAL), incubated with a goat anti-rabbit 647 secondary antibody (Life Technologies) at 1:500 for 30 min at 4°C, then washed once with Wash buffer. All washes were performed by centrifuging at 500g 5 min at 4°C. Nuclei were then resuspended in PBS with 1% BSA.
Instrument	Sony SH800Z Cell Sorter
Software	SH800 Software
Cell population abundance	The MECP2_high population was 13.55% and MECP2_low population was 35.31%. We confirmed purity through Sanger sequencing and total RNA sequencing of these cell populations.
Gating strategy	DAPI-positive singlets were gated on fluorescence intensity of MeCP2 staining to define high and low populations. A no primary antibody control was used to confirm specificity of staining.

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