# nature portfolio

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# Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

## Statistics

For all statistical analyses, confirm that the following items are present in the 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 |
| ☐ | ☒ | A statement on 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 statistical parameters 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 |

*Our web collection on statistics for biologists contains articles on many of the points above.*

## Software and code

Policy information about availability of computer code

Data collection | CV7000 software to collect Yokogawa CV7000 microscope imaging

Data analysis |
bedtools (2.27.1) https://academic.oup.com/bioinformatics/article/26/6/841/244688
samtools (1.15.1) https://academic.oup.com/bioinformatics/article/25/16/2078/204688
macs2 (2.1.1) https://genomebiology.biomedcentral.com/articles/10.1186/gb-2008-9-9-r137
DESeq2 (1.14.1) https://genomebiology.biomedcentral.com/articles/10.1186/s13059-014-0550-8
CUT&RUNTools (1.0) https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1802-4
bowtie2 (2.2.9) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3322381/
HIFI (1.0) https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1913-y
deeptools (3.0.2) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4086134/
Slamdunk (1.0) https://bmcbioinformatics.biomedcentral.com/articles/10.1186/s12859-019-2849-7
bamtools (2.5.1) https://academic.oup.com/bioinformatics/article/27/12/1691/255399
Trimmomatic (0.36) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4103590/
HiC-Pro (2.10.0) https://genomebiology.biomedcentral.com/articles/10.1186/s13059-015-0831-x
bedops (2.4.30) https://academic.oup.com/bioinformatics/article/28/14/1919/218826
Hiccup (1.6) ( https://www.cell.com/fulltext/S2405-4712(16)30219-8
ImageJ (version 1.45f)
Python (version 3.85)
pandas (version 1.1.3)
Seaborn (version 0.11.0)

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See our guidelines for submitting code & software for further information.

matplotlib (version 3.3.2).
scipy (version 1.7.3)

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data that support this study are available from the corresponding author upon reasonable request. Hi-C, CUT&RUN, ChIP-seq, ATAC-seq, RNA-seq and SLAM-seq data sets generated this study have been deposited in the GEO database, under accession code GSE247105. Source data are provided with this paper.

## Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

| | |
|---|---|
| Reporting on sex and gender | N/A |
| Reporting on race, ethnicity, or other socially relevant groupings | N/A |
| Population characteristics | N/A |
| Recruitment | N/A |
| Ethics oversight | N/A |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

# Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf

# Life sciences study design

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

| | |
|---|---|
| Sample size | Sample sizes was chosen based on published data using similar approaches. https://pubmed.ncbi.nlm.nih.gov/32817427/; https://pubmed.ncbi.nlm.nih.gov/28841410/ |
| Data exclusions | No data were excluded. |
| Replication | All experiments were reliably repeated at least two times, and all the attempts at replication were successful. |
| Randomization | Experimental groups of cells were assigned according to their genotype, time of drug treatment and development stages |
| Blinding | Imaging data collection was made blinded to group allocation. Blinding was not relevant to the genomic data because investigator's bias would not affect the data collection. Data analysis was not blinded because it is strictly quantitative. |

# Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

## Materials & experimental systems

| n/a | Involved in the study |
|---|---|
| ☐ | ☒ Antibodies |
| ☐ | ☒ Eukaryotic cell lines |
| ☒ | ☐ Palaeontology and archaeology |
| ☒ | ☐ Animals and other organisms |
| ☒ | ☐ Clinical data |
| ☒ | ☐ Dual use research of concern |
| ☒ | ☐ Plants |

## Methods

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

## Antibodies

| | |
|---|---|
| Antibodies used | anti-Matrin3  proteintech, 12202-2-AP 1:40 for CUT&RUN<br>anti-Matrin3  Sigma HPA036565 1:1000 for WB<br>anti-Matrin3  abcam151739 1:200 for IF<br>anti-DMD a gift from Kunkel Laboratory 1:500 for IF, 1:1000 for WB<br>anti-DCAF8 Bethyl Laboratories® Catalog # A301-556A 1:500 for IF, 1:5000 for WB<br>anti-myosin heavy chain  DSHB Hybridoma Product MF 20, deposited to the DSHB by Fischman, D.A 1:40 for IF, 1:400 for WB<br>anti-GAPDH  abcam: ab9485 1:10,000 for WB<br>anti-HA-Tag (C29F4) Rabbit mAb  cell signaling, HA-Tag (C29F4) Rabbit mAb #3724 1:1000 for WB<br>anti-CTCF Abcam, ab70303 5 -10µg antibody  per ChIP reaction<br>anti-CTCF Millipore 07-729 1:100 for CUT&RUN<br>anti-Rad21 Abcam, ab992 5 -10µg antibody per ChIP, 1:100 for CUT&RUN<br>MyoD Santa cruz, Anti-MyoD Antibody (5.8A), sc-32758 X 1:100 for CUT&RUN<br>anti-YY1 santa cruz,  sc-7341X 1:100 for CUT&RUN<br>Histone H3K4me3 antibody (pAb) active motif, 39159 1:50 for CUT&RUN<br>Anti-acetyl-Histone H3 (Lys27) Antibody, clone RM172 millipore, MABE647 1:50 for CUT&RUN |
| Validation | anti-Matrin3  proteintech, 12202-2-AP for CUT&RUN, PMID: 37000624.<br>anti-Matrin3  Sigma HPA036565, western blots, https://www.sigmaaldrich.com/US/en/coa/SIGMA/HPA036565/R33715<br>anti-Matrin3  abcam151739 for IF, https://www.abcam.com/products/primary-antibodies/matrin-3-antibody-epr10634b-ab151739.html, PMID: 30015619<br>anti-DCAF8 Bethyl Laboratories® Catalog # A301-556A 1 for IF and  WB, https://www.thermofisher.com/antibody/product/WDR42A-Antibody-Polyclonal/A301-556A<br>anti-myosin heavy chain  DSHB Hybridoma Product MF 20, https://dshb.biology.uiowa.edu/MF-20<br>anti-GAPDH  abcam: ab9485, https://www.abcam.com/products/primary-antibodies/gapdh-antibody-loading-control-ab9485.html<br>anti-HA-Tag (C29F4) Rabbit mAb  cell signaling, HA-Tag (C29F4) Rabbit mAb #3724 1:1000 for WB<br>anti-CTCF Abcam, ab70303 (https://www.abcam.com/ctcf-antibody-ab70303.html).<br>anti-CTCF Millipore 07-729  https://www.emdmillipore.com/US/en/product/Anti-CTCF-Antibody,MM_NF-07-729?ReferrerURL=https%3A%2F%2Fwww.google.com%2F<br>anti-Rad21 Abcam, ab992, (https://www.abcam.com/rad21-antibody-ab992.html)<br>MyoD Santa cruz, Anti-MyoD Antibody (5.8A), sc-32758 X, https://www.scbt.com/p/myod-antibody-5-8a<br>anti-YY1 santa cruz,  sc-7341X, https://www.scbt.com/p/yy1-antibody-h-10<br>Histone H3K4me3 antibody (pAb) active motif, 39159, https://www.activemotif.com/catalog/details/39159/histone-h3-trimethyl-lys4-antibody-pab<br>Anti-acetyl-Histone H3 (Lys27) Antibody, clone RM172 millipore, MABE647, https://www.emdmillipore.com/US/en/product/Anti-acetyl-Histone-H3-Lys27-Antibody-clone-RM172,MM_NF-MABE647 |

## Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | C2C12 cells were purchased from ATCC. |
| Authentication | Cell lines were not authenticated. |
| Mycoplasma contamination | All cell lines were tested negative for mycoplasma contamination by PCR. |
| Commonly misidentified lines<br>(See ICLAC register) | None |

# Plants

| | |
|---|---|
| Seed stocks | *Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.* |
| Novel plant genotypes | *Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.* |
| Authentication | *Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.* |

# 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<br>*May remain private before publication.* | *For "Initial submission" or "Revised version" documents, provide reviewer access links.  For your "Final submission" document, provide a link to the deposited data.* |
| Files in database submission | *Provide a list of all files available in the database submission.* |
| Genome browser session<br>(e.g. UCSC) | *Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review.  Write "no longer applicable" for "Final submission" documents.* |

## Methodology

| | |
|---|---|
| Replicates | In general, ChIP-seq experiments were performed in at least two replicates. The number of replicates is indicated in each file name. |
| Sequencing depth | *Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.* |
| Antibodies | anti-CTCF Abcam, ab70303<br>anti-Rad21 Abcam, ab992 |
| Peak calling parameters | *Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.* |
| Data quality | verified by  FastQC |
| Software | *Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.* |

# 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 | C2C12 cells were tripsinized and washed with growth medium. Cells were filtered and resuspended in 1X PBS with 1%FCS and 7-AAD. |
| Instrument | JF Aria II |
| Software | FlowJo |

Cell population abundance | select the top 0.2% GFP positive cells

Gating strategy | a figure exemplifying the gating strategy is provided in the Supplementary Information

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