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	RNAseq, CUT&Tag, ATAC-seq, and Hi-C libraries were sequenced on the Illumina NovaSeq 6000 platform in 150PE mode. Confocal images were taken by Zeiss Ls 880 confocal microscope with Zeiss Zen software (2012 s4).
Data analysis	RNA-seq: The quality of reads was assessed using the FastQC tool. The raw reads were trimmed using Trim Galore (v0.6.10) (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and then mapped to the transcriptome of mm10 by STAR (v2.7.1a) with default parameters. All mapped bam files were converted to bigwig using bedtools (v2.31.0) for visualization in IGV. Differentially expressed genes (adjusted p value < 0.05 and abs (log2 fold change) > 1) were identified using DEseq2. The data were scaled and visualized in R using the heatmap2 package. Gene ontology enrichment analysis was performed using ClusterProfiler (v4.7.1.003). Genes were clustered by K-means. FPKM is calculated by homer analyzeRepeats.pl(http://homer.ucsd.edu/homer/ngs/analyzeRNA.html). CUT&Tag: The quality of CUT&Tag reads was assessed using the FastQC tool. Raw reads were trimmed with Trim Galore (v0.6.10) (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) and subsequently mapped to the mouse transcriptome (mm10) using Bowtie2. PCR duplicates and mitochondrial reads were excluded using Samtools (v1.9), and the resulting SAM files were converted to BAM files. CUT&Tag peaks were called using MACS2 (v2.2.7.1, -f BAMPE) with a significance threshold of p < 0.05. Weak ATF4 peaks were filtered out using a cutoff of summit value > 100(CST antibody) or > 400(SCBT antibody) in C2C12 cells. CUT&Tag peaks in HeLa cells were called using combined replicated data by MACS2 (v2.2.7.1, -f BAMPE) with a significance threshold of p < 0.05 and IgG control. Weak peaksin HeLa cells were filtered out using a cutoff of summit value > 100. Genome-wide differential CUT&Tag peaks were identified using the DiffBind package (v3.6.1) based on edgeR analysis (FDR < 0.05, |log2FC| > 1). Peak annotation was performed using ChIPseeker (v1.30.2). To normalize signal intensity, Deeptools (v3.5.1) bamCoverage was applied to sorted BAM files. ComputeMatrix was used to calculate signals over designated

genomic regions. Bigwigcompare was employed to compare BigWig files and obtain log2 ratios, which were visualized with plotProfile and plotHeatmap. Motif discovery and analysis in the regions of interest were conducted using Homer's findMotifsGenome.pl (http://homer.ucsd.edu/homer/ngs/peakMotifs.html). Putative ATF4 and CEBPγ transcription factor binding sites (TFBS) were scanned using TFBSTools (v3.19). Position weight matrices (PWM) for ATF4 and CEBPγ motifs were obtained from the JASPAR database (https://jaspar.elixir.no/).

ATAC-seq:
For all ATAC-Seq datasets, the data processing and statistical analysis were performed following the same procedures as for the CUT&Tag data.

Hi-C:
Raw reads were firstly trimmed using TrimGalore (v0.6.10) with default settings. The data were then processed using a standard Hi-C processing pipeline recommended by the 4D Nucleome Data Portal (https://data.4dnucleome.org/resources/data-analysis/hic-processing-pipeline). Briefly, the trimmed reads were first mapped to the mouse genome (mm10) using BWA MEM (v0.7.17-r1188) with the -SP5M option. The mapped reads were then parsed using pairtools (v0.3.0). After data aggregation and normalization, contact matrices in the cool file format were generated using the cooler package (v0.8.11). Hi-C heatmaps at selected regions were generated using the cooltools package (v0.5.0).
For A/B compartment analysis, Eigenvalue decomposition was performed on cooler matrices binned at 10kb resolution using the call-compartments utility from the cooltools package (v0.5.0). The A/B compartment was defined using the first eigenvector values.
For TAD boundary analysis, TAD calling and insulation analysis was performed on cooler matrices binned at 10kb resolution using the diamond-insulation utility from the cooltools. TAD boundaries with boundary strength above 0.5 were considered high-confidence boundaries and used in subsequent analysis. Chi-square test was used to compare the proportion of ATF4-binding promoters located on a changed or stable TAD boundaries.
For loop analysis, mustache was used for calling the loops and different loops using $p < 0.1$ based on cooler matrices binned at 10kb resolution. Chi-square test was used to compare the proportion of ATF4-binding promoters located on a changed or stable loop anchors. The heatmaps comparing the loop intensity in different regions of multiple matrices were plotted with R package GENOVA(https://github.com/dewitlab/GENOVA).

Microscopic Images:
The image were analyzed and processed with Zeiss Zen software (2012 s4)

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 complete raw data and processed data sets for RNA-seq (GSE281443, GSE303801, and GSE303803), CUT&Tag (GSE281440, GSE303802, and GSE303804), ATAC-seq (GSE281438 and GSE303800), and Hi-C (GSE281441) have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). The mass spectrometry (MS) proteomics data have been deposited in the ProteomeXchange database via the iProX partner repository under the dataset identifier PXD058631. 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	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study*

design questions and have nothing to add here, write "See above."

Recruitment: *Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*

Ethics oversight: *Identify the organization(s) that approved the study protocol.*

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 size estimates have been performed on previous experience to obtain statistical significance and reproducibility. Independent biological replicates of RNA-Seq, CUT&Tag, ATAC-seq , and co-IP/MS experiments were performed in line with the ENCODE consortium experimental standards.
Data exclusions	No data were excluded from analysis.
Replication	The RNA-seq, CUT&Tag, ATAC-seq and co-IP/MS experiments were performed on independent biological duplicates. Experiment repeat numbers are reported in the figure legends.
Randomization	Cells were randomly allocated into experimental groups.
Blinding	Blinding was not applicable in this study. This is a study with data collection and analyses relying on objective measures.

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-rabbit ATF4 (Cell Signaling Technology #11815; 1:1000 dilution for Western Blot; 1:200 dilution for immunofluorescence; 1:50 dilution for CUT&Tag; 1:50 dilution for immunoprecipitation)
anti-mouse ATF4 (Santa Cruz Biotechnology #sc-390063,1:50 dilution for CUT&Tag)
anti-rabbit β-Actin (Cell Signaling Technology #8457, 1:1000 dilution for Western Blot)
anti-mouse CEBPβ (Thermo Fisher #MA1-827, 1:1000 dilution for Western Blot; 1:50 dilution for CUT&Tag)
anti-rabbit CEBPγ (Thermo Fisher #PA5-80468, 1:1000 dilution for Western Blot; 1:50 dilution for CUT&Tag)
anti-mouse CHOP (Cell Signaling Technology #2895T, 1:1000 dilution for Western Blot; 1:50 dilution for CUT&Tag).
anti-rabbit H3K27Ac (Abcam #ab4729, 1:50 dilution for CUT&Tag)
anti-rabbit IgG (Beyotime #A7016, 1:200 dilution for immunofluorescence)
anti-rabbit IgG -Alexa Fluor 594 secondary antibody (Thermo Fisher, #A-11012, 1:1000 dilution for immunofluorescence)
anti-rabbit IgG-HRP secondary antibody (YEASEN #33101ES60, 1:10000 dilution for Western Blot)
anti-mouse IgG-HRP secondary antibody (YEASEN #33201ES60,1:10000 dilution for Western Blot)

Validation: Verification of all antibodies used in the study for species and application can be found at the manufacturer's website.

anti-rabbit ATF4 (https://www.cellsignal.com/products/primary-antibodies/atf-4-d4b8-rabb)
anti-mouse ATF4 (https://www.scbt.com/p/atf-4-antibody-b-3)
anti-rabbit β-Actin (https://www.cellsignal.com/products/primary-antibodies/b-actin-d6a8-rabbit-mab/8457)
anti-mouse CEBPβ (https://www.thermofisher.cn/cn/zh/antibody/product/C-EBP-beta-Antibody-clone-1H7-Monoclonal/MA1-827)
anti-rabbit CEBPγ (https://www.thermofisher.cn/cn/zh/antibody/product/C-EBP-gamma-Antibody-Polyclonal/PA5-80468)
anti-mouse CHOP (https://www.cellsignal.com/products/primary-antibodies/chop-l63f7-mouse-mab/2895)
anti-rabbit H3K27Ac (https://www.abcam.cn/products/primary-antibodies/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html)
anti-rabbit IgG (https://www.beyotime.com/product/A7016.htm)
anti-rabbit IgG -Alexa Fluor 594 secondary antibody (https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11012)
anti-rabbit IgG-HRP secondary antibody (https://www.yeasen.com/products/detail/319)
anti-mouse IgG-HRP secondary antibody (https://www.yeasen.com/products/detail/407)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	C2C12 cell line (# SCSP-505) and HeLa cell line (#SCSP-504) were purchased from the Cell Bank of the Chinese Academy of Sciences.
Authentication	Cell line used in this study were authenticated by performing STR profiling.
Mycoplasma contamination	Cell line tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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.*