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	Primary data was not collected using software.
Data analysis	Custom algorithms or softwares was not developed for this research. Data preprocessing was performed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), STAR (https://github.com/alexdobin/STAR), Picard (http://broadinstitute.github.io/picard/) , MACS2 (https://pypi.org/project/MACS2/), BEDTools (https://bedtools.readthedocs.io/en/latest/), deepTools (https://deeptools.readthedocs.io/en/develop/index.html), CCseqBasicS (https://github.com/Hughes-Genome-Group/CCseqBasicS). Data analysis was also performed in R (v. >3.5) using a variety of published packages: DESeq2 (v>1.32.0), DiffBind (v>3.12.0) , circlize (v>0.4.15). Tracks were visualized using pyGenomeTracks (v>3.6), WashU Epigenome Browser. For sequence-to-signal modelling was used BPNet with BPNet-lite implementation (v 0.8.1), motif discovery TF-MoDISco-lite (v 2.2.0) with MEME suite (v 5.5.5), mutagenesis tangermeme (v 0.2.1).

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

All generated sequence data is available at the Gene Expression Omnibus (GEO) repository under accession code GSE300907. Additionally to the data generated for this study, we used RNA-seq, DNase-seq and MED1 ChIP-seq data downloaded from GEO accession GSE113253, enhancer capture Hi-C data from GSE140782, and Hi-C data from GSE140782 and GSE52457.

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	NA
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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	No statistical methods were used to calculate necessary sample size. Independent differentiation experiments were performed 2 times using 2-3 clonal cell lines per given condition and/or control. Sequencing depth is the only sampling process in the associated sequencing data. Sequencing depth were guided based on standards in the field (e.g. the ENCODE Data standards).
Data exclusions	No data were excluded from the analysis.
Replication	Identification of dynamic events (differential gene expression, transcription factors occupancy or regulatory elements interactions) all rely on 2 independent differentiation rounds in 2-3 clonal cell lines. All attempts of replication were successful.
Randomization	For any individual experiments, cell lines were expanded until a sufficient number was obtained. Cells from the individual cell lines were then pooled into a single homogeneous cell suspension, which were then randomly assigned to treatment conditions.
Blinding	Data acquisition was not performed blindly due to the specific phenotypes and/or characteristics of the cell lines generated. Samples within a specific experiments underwent the same experimental treatment and metrics were derived from absolute quantitative methods without human subjectivity.

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

For Western blotting:
anti-Lamin A: Sigma-Aldrich, Cat# L1293, RRID:AB_532254,
anti-PPARγ: Cell Sygnaling, Cat#2443S, RRID:AB_823598,
anti-FLAG: Sigma-Aldrich, Cat# F1804, RRID:AB_262044

For ChIP-seq assays:
anti-MED1: Bethyl Laboratories, Cat #A300-793A, RRID:AB_577241,
anti-CTCF: Active Motif, Cat# 61311, RRID: AB_2614975,
anti-H3K4me1: abcam, Cat# ab8895, RRID:AB_306847,
anti-C/EBPβ: Santa Cruz, Cat# sc-7962, RRID:AB_626772,
anti-C/EBPα: Cell Sygnaling, Cat# 8178S, RRID:AB_11178517,
anti-PPARγ: Cell Sygnaling, Cat#2443S, RRID:AB_823598

Validation

anti-Lamin A (Cat# L1293): Validated by the vendor by western blot and immunofluorescence; https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/181/610/l1293dat.pdf
anti-FLAG (Cat# F1804): Validated by the vendor by western blot, immunoprecipitation and immunofluorescence; https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/119/160/f1804bul-mk.pdf
anti-PPARγ (Cat# 2443S): Validated by the vendor by western blot, immunoprecipitation, immunofluorescence and chromatin immunoprecipitation; https://www.cellsignal.com/products/2443/datasheet?images=0&protocol=0&size=A4
anti-MED1 (Cat# A300-793A): Validated by the vendor by western blot, immunoprecipitation and immunohistochemistry; https://t31165-s51694.tp1.mozu.com/cms/files/A300-793A-13.pdf
anti-CTCF (Cat# 61311): Validated by the vendor by chromatin immunoprecipitation, western blot, immunohistochemistry; https://www.activemotif.jp/documents/tds/61311.pdf
anti-H3K4me1 (Cat# ab8895): Validated by the vendor by chromatin immunoprecipitation, western blot, immunoprecipitation, immunohistochemistry and immunofluorescence; https://doc.abcam.com/datasheets/active/ab8895/en-us/histone-h3-mono-methyl-k4-antibody-chip-grade-ab8895.pdf
anti-C/EBPβ (Cat# sc-7962): Validated by the vendor by western blot, immunoprecipitation, immunofluorescence and immunohistochemistry; https://datasheets.scbt.com/sc-7962.pdf
anti-C/EBPα (Cat# 8178S): Validated by the vendor by western blot, immunoprecipitation, immunohistochemistry, immunofluorescence and flow cytometry; https://www.cellsignal.com/products/8178/datasheet?images=1&protocol=0&size=A4

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

BM-hMSC-TERT4 cells (RRID: CVCL_Z017) were obtained directly from Dr. Moustapha Kassem, Molecular Endocrinology Department, Odense University Hospital who derived the cell line (PMID: 15596132) from its parental cell line, the BM-hMSC-TERT cell (RRID: CVCL_Z015), which was also derived by Dr. Moustapha Kassem from bone marrow aspirates from a 33-year-old male donor (PMID: 12042863).
hMSC-TERT4-iCas9-4 cell line and respective clonal cell lines with individual enhancer deletion were derived from the BM-hMSC-TERT cell line.

Authentication

The cells were authenticated by karyotyping and functional differentiation assays.

Mycoplasma contamination

All cell lines were routinely tested negative for Mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

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

[x] 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=GSE300907

Files in database submission

D081_S9_R1_001.fastq.gz
D081_S9_R2_001.fastq.gz
D084_S12_R1_001.fastq.gz
D084_S12_R2_001.fastq.gz
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Genome browser session
(e.g. UCSC)

No longer applicable.

Methodology

Replicates

ChIP-seq experiments have been replicated on at least two independent differentiation experiments of BM-hMSC-TERT4. ChIP-seq experiments of hMSC-TERT4-iCas9-4 control or enhancer deletion cell lines have ben replicated on at least two independent differentiation experiments (n=2 independent biological replicates, n=2 clonal cell line per condition).

Sequencing depth

Sequencing depth was >10 million reads per sample.

Antibodies

As mentioned above:
anti-MED1: Bethyl Laboratories, Cat #A300-793A, RRID:AB_577241,
anti-CTCF: Active Motif, Cat# 61311, RRID: AB_2614975,
anti-H3K4me1: abcam, Cat# ab8895, RRID:AB_306847,
anti-C/EBPβ: Santa Cruz, Cat# sc-7962, RRID:AB_626772,
anti-C/EBPα: Cell Sygnaling, Cat# 8178S, RRID:AB_11178517,
anti-PPARγ: Cell Sygnaling, Cat#2443S, RRID:AB_823598

Peak calling parameters

ChIP–seq reads were aligned to the human hg19 genome using STAR with the following parameters: --genomeLoad LoadAndRemove --readFilesCommand zcat --runThreadN 16 --outSJfilterIntronMaxVsReadN 0 --alignIntronMax 1 --alignSJDBoverhangMin 200.

BAM files from these alignments were used as input for MACS2 peak calling (callpeak -t $BAMFILE -c input.bam -f BAM -n ${BAMFILE/.bam} --outdir macs2). Peaks were called separately for each replicate using the matched input DNA as control. Reproducible peaks were defined as those overlapping between replicates, identified with bedtools intersect -wo -f 0.3 -F 0.3 -r.

Data quality

PCR duplicates were identified and removed using Picard MarkDuplicates, retaining only one read per set of identical genomic coordinates. Peaks were called with MACS2 for each replicate, and high-confidence peaks were defined as those overlapping between replicates using bedtools intersect

Software

Sequencing quality was assessed with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were aligned using STAR, and BAM files were sorted and deduplicated with Picard (https://broadinstitute.github.io/picard/). Read density quantification and visualization were performed using deepTools (https://deeptools.readthedocs.io/en/develop/content/list_of_tools.html) and pyGenomeTracks (https://pygenometracks.readthedocs.io/en/latest/).