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Corresponding author(s): Jiekai Chen

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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 .

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	RSEM(v1.2.22), bowtie2(v2.2.5), STAR(2.5.2a), DESeq2 (1.22.1), EDASeq(2.16.0), deeptools(2.5.4), HISAT2 (v2.2.0), TrimGalore (v0.6.4), MACS2 (v2.1.2), samtools (v1.9).
Data analysis	Follow the software documentations, no special changes

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. 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

The authors declare that the data and codes supporting the results of this study are available within the article and its supplementary information files or from the corresponding author upon reasonable request.

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 study design

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

Sample size	Sample size was determined based on previously published studies and ensure reproducibility. All experiments in this study were performed at least 2 and typically 3 independently unless otherwise specified.
Data exclusions	No data exclusions.
Replication	All attempts at replication were successful.
Randomization	This study was based on molecular and cellular biology techniques, which did not involve live organisms and no randomization techniques were required.
Blinding	The results were obtained using objective quantitative methods, so the researchers were not blinded to sample allocation during experiments and outcome assessment.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology
[ ]	[x] Animals and other organisms
[x]	[ ] Human research participants
[x]	[ ] Clinical data

Methods

n/a	Involved in the study
[ ]	[x] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies

Anti-Flag Sigma F1804, Anti-YTHDC1 CST 87459S, Anti-METTL3 Proteintech 15073-I-AP, Anti-SETDB1 GeneTex GTX115305, Anti-GAPDH Bioworld AP2063, Anti-Halo Promega G9211, Anti-HA CST 3724S, Anti-NANOG Bethy A300-397A, Anti-Flag CST 14793S, Anti-H3K9me3 Abcam Ab8898, Anti-YTHDC1 CST 77422, Anti-m6A NEB E1610S, Anti-ELF5 Santa-Cruz sc-166653 , Anti-mCherry LS-C204207 LSBio. Anti-CDX2 BioGenex MU392A-UC, Anti-SETDB1 Proteintech, 11231-1-AP, Anti-Mouse IgG Abcam ab18413, Anti-Rabbit IgG Abcam ab37415.

Secondary antibodies

Donkey anti Goat IgG 568 invitrogen A-11057, Donkey anti Mouse IgG 488 invitrogen A32766, Goat anti Rabbit IgG HRP Aksomics KC-RB-035, Goat anti Mouse IgG HRP Aksomics KC-MM-035.

Validation

All antibodies used in this study are commercially available and have been validated by manufacturer. Antibody validations and validation criteria are available on the following websites:

Anti-Flag https://www.sigmaaldrich.com/catalog/product/sigma/f1804?lang=zh&region=CN

Anti-YTHDC1 https://www.cellsignal.cn/products/primary-antibodies/ythdc1-p561-antibody/87459?site-search-type=Products&N=4294956287&Ntt=87459s&fromPage=plp&_requestid=2667843

Anti-METTL3 http://www.ptgcn.com/products/METTL3-Antibody-15073-1-AP.htm

Anti-SETDB1 https://www.genetex.cn/Product/Detail/SETDB1-antibody-N1-N-term/GTX115305

Anti-GAPDH https://www.bioworlde.com/GAPDH-polyclonal-antibody(AP0063).html

Anti-Halo https://www.promega.com.cn/Products/Protein-Detection/Primary and Secondary Antibodies/Anti-HaloTag-Monoclonal-Antibody/?catNum=G9211

Anti-HA https://www.cellsignal.cn/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724?site-search-type=Products&N=4294956287&Ntt=3724s&fromPage=plp&_requestid=2668171

Anti-NANOG https://www.bethyl.com/product/A300-397A/Nanog+Antibody

Anti-Flag https://www.cellsignal.cn/products/primary-antibodies/dykddddk-tag-d6w5b-rabbit-mab-binds-to-same-epitope-as-sigma-s-anti-flag-m2-antibody/14793?site-search-type=Products&N=4294956287&Ntt=14793s&fromPage=plp&_requestid=2668236

Anti-H3K9me3 https://www.abcam.cn/histone-h3-tri-methyl-k9-antibody-chip-grade-ab8898.html

Anti-YTHDC1 https://www.cellsignal.cn/products/primary-antibodies/ythdc1-e4i9e-rabbit-mab/77422?site-search-type=Products&N=4294956287&Ntt=77422&fromPage=plp&_requestid=2668393

Anti-m6A https://international.neb.com/products/e1610-epimark-n6-methyladenosine-enrichment-kit#Product%20Information
Anti-ELF5 https://www.scbt.com/p/elf-5-antibody-g-2
Anti-mCherry https://www.lsbio.com/antibodies/mcherry-antibody-if-immunofluorescence-ihc-wb-western-ls-c204207/212463
Anti-CDX2 https://www.labome.com/product/Biogenex/MU392A-UC.html
Anti-SETDB1 http://www.ptgcn.com/products/SETDB1-Antibody-11231-1-AP.htm
Anti-Mouse IgG https://www.abcam.cn/mouse-igg2a-kappa-monoclonal-mopc-173-isotype-control-chip-grade-ab18413.html
Anti-Rabbit IgG https://www.abcam.cn/rabbit-igg-polyclonal-isotype-control-ab37415.html
Donkey anti Goat IgG 568 https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057
Donkey anti Mouse IgG 488 https://www.thermofisher.com/cn/zh/antibody/product/Donkey-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32766
Goat anti Rabbit IgG HRP http://www.aksomics.com/products/secondary-antibody-1.html
Goat anti Mouse IgG HRP http://www.aksomics.com/products/secondary-antibody-1.html

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	129 mouse embryonic stem cells were derived from 3.5 d.p.c inner cell mass (ICM) from female 129 mice (Stock No.: 217, Beijing Vital River Laboratory Animal Technology Co., Ltd.) HEK293T (ATCC, #CRL-1126) NIH3T3 (ATCC, #CRL-1658)
Authentication	129 mouse embryonic stem cells were authentication by Chimera experiment.
Mycoplasma contamination	Mycoplasma detection tests were conducted routinely to ensure mycoplasma-free conditions throughout this study.
Commonly misidentified lines (See ICLAC register)	Cells used are not in th ICLAC database.

Animals and other organisms

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

Laboratory animals	Female and male CD-1 mice were used in chimeric blastocyst assay between 8 and 12 weeks of age.
Wild animals	No wild animals were used in this study.
Field-collected samples	All animal procedures were performed according to NIH guidelines. Animals were housed in individually ventilated cages containing sterile quarter-inch corncob bedding for environmental enrichment. Mice were maintained on 12 h light/dark cycle and provided with food and water in individually ventilated units in a temperature controlled room (22 - 23℃).
Ethics oversight	Animal care and experimental protocols were approved by the Guangzhou Institutes of Biomedicine and Health Ethical Committee under NO. N2020106.

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

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=GSE146467 Reviewer token: evuvmckervorfuh
Files in database submission	Ythdc1-cKO.DMSO.Dux-Het-H3K9me3.rep1,Ythdc1-cKO.DMSO.Dux-Het-H3K9me3.rep2,Ythdc1-cKO.4OHT.Dux-Het-H3K9me3.rep1,Ythdc1-cKO.4OHT.Dux-Het-H3K9me3.rep2,Ythdc1-cKO.DMSO.Dux-KO-H3K9me3.rep1,Ythdc1-cKO.DMSO.Dux-KO-H3K9me3.rep2,Ythdc1-cKO.4OHT.Dux-KO-H3K9me3.rep1,Ythdc1-cKO.4OHT.Dux-KO-H3K9me3.rep2, YTHDC1(Ythdc1-KO).rep1,YTHDC1(Ythdc1-KO).rep2,Mettl3.KO.H3K9me3.rep1,Mettl3.KO.H3K9me3.rep2,Mettl3.WT.H3K9me3.rep1,Mettl3.WT.H3K9me3.rep2,YTHDC1(Ythdc1-WT).rep1,YTHDC1(Ythdc1-WT).rep2,INPUT(WT), shMettl3.H3K9me3, ChIP.Input, shScramble.H3K9me3, WT.rep1-H3K9me3, WT.rep2-H3K9me3, Ythdc1-KO.rep1-H3K9me3,Ythdc1-KO.rep2-H3K9me3, YTHDC1-RIP, YTHDC1-RIP-INPUT, Ythdc1_cKO_ESC#1_+4OHT.rep1,Ythdc1_cKO_ESC#1_DMSO.rep2, Ythdc1_cKO_ESC#1_+4OHT.rep2, Ythdc1_cKO_ESC#1_DMSO.rep1, Ythdc1_cKO_ESC#1_DMSO.rep2, Ythdc1_cKO_ESC#2_+4OHT.rep1, Ythdc1_cKO_ESC#2_+4OHT.rep2, Ythdc1_cKO_ESC#2_DMSO.rep1, Ythdc1_cKO_ESC#2_DMSO.rep2, m6A-RP.Mettl3-KO1.INPUT,m6A-RP.Mettl3-KO1.IP,m6A-RP.Mettl3-KO2.INPUT,m6A-RP.Mettl3-KO2.IP,m6A-RP.Mettl3-WT.INPUT.rep2,m6A-RP.Mettl3-WT.IP.rep2,m6A-RP.Mettl3-KO.Input,m6A-RP.Mettl3-KO.IP,m6A-RP.Mettl3-WT.Input.rep1,m6A-RP.Mettl3-WT.IP.rep1 ChIRP.Mettl3-KO.IAP.Even,ChIRP.Mettl3-KO.IAP.Odd,ChIRP.Mettl3-KO.Input,ChIRP.Mettl3-KO.LacZ.Even,ChIRP.Mettl3-

KO.LacZ.Odd,ChIRP.Mettl3-KO.Malat1.Even,ChIRP.Mettl3-KO.Malat1.Odd,ChIRP.Mettl3-WT.IAP.Even,ChIRP.Mettl3-WT.IAP.Odd,ChIRP.Mettl3-WT.Input,ChIRP.Mettl3-WT.LacZ.Even,ChIRP.Mettl3-WT.LacZ.Odd,ChIRP.Mettl3-WT.Malat1.Even,ChIRP.Mettl3-WT.Malat1.Odd,ChIRP.Mettl3-KO.LINE1.1,ChIRP.Mettl3-KO.LINE1.2,ChIRP.Mettl3-WT.LINE1.1,ChIRP.Mettl3-WT.LINE1.2,
ChIRP.Ythdc1-cKO.4OHTCHIRP_Input,ChIRP.Ythdc1-cKO.4OHTIAP_Even,ChIRP.Ythdc1-cKO.4OHTIAP_Odd,ChIRP.Ythdc1-cKO.4OHTLINE1_Even,ChIRP.Ythdc1-cKO.4OHTLINE1_Odd,ChIRP.Ythdc1-cKO.4OHTLacZ_Even,ChIRP.Ythdc1-cKO.4OHTLacZ_Odd,ChIRP.Ythdc1-cKO.4OHTMalat1_Even,ChIRP.Ythdc1-cKO.4OHTMalat1_Odd,ChIRP_IAP_Even,ChIRP_IAP_Odd,ChIRP_Input,ChIRP_LINE1.Even,ChIRP_LINE1_Odd,ChIRP_LacZ_Even,ChIRP_LacZ_Odd,ChIRP_Malat1.Odd,ChIRP_Malat1_Even

Genome browser session
(e.g. UCSC)

No public available genome browser session

Methodology

Replicates

2 replicates for Ythdc1 knock out, 1 replicates for Mettle3 knock down, 2 replicates for Mettl3 knock out, 2 replicates for Dux +/-, 2 replicates for Dux-/-

Sequencing depth

Paire-ends, 20-40M raw sequencing reads for each sample

Antibodies

anti-H3K9me3 antibody (Abcam, #ab8898), anti-YTHDC1 antibody (CST, 77422), anti-SETDB1 antiody (Proteintech 11231-1-AP)

Peak calling parameters

Peaks were called by MACS2 with default parameters with just specify the genome "-g mm"

Data quality

H3K9me3 ChIP-seq quality was performed by comparing to previous published dataset with pearson correlation > 0.8. And we detect significant 18788 peaks. We detect 18525 peaks for peaks for YTHDC1 ChIP-seq data. Other QC filter are detailed in the method part of the manuscript

Software

ChIP-seq reads were aligned to the mouse mm10 genome using bowtie247 with the options '-p 20–very-sensitive–end-to-end–no-unal–no-mixed -X 2000'. The multimapped reads were kept for TE analysis, but only the best alignment is reported for those reads, if more than one equivalent best alignment was found, then one random alignment was reported. Whereas the reads mapping to mitochondrial DNA or unassigned sequences were discarded. The Bam files of biological replicates were merged using samtools (version 1.9), and peaks were called using MACS (version 2.1.2). BigWig files were generated using deeptools53 with the RPKM normalization method.

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

MERVL::tdTomato reporter mESCs were digested with 0.25% trypsin and resuspended by PBS with 1% FBS.

Instrument

LSRFortessa flow cytometer (BD Biosciences)

Software

FlowJo V10 software

Cell population abundance

One hundred thousand to distinguish the tdTomato positive and negative cells.

Gating strategy

Using the DMSO treated cells as negative control.

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