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	No software was used for data collection.
Data analysis	No custom software or algorithm was used in this study. All software used in this study for data analysis are either commercially available or open source. Proteome Discoverer 2.3 for AP-MS analysis (https://www.thermofisher.com/hk/en/home/industrial/mass-spectrometry/liquid-chromatography-mass-spectrometry-lc-ms/lc-ms-software/multi-omics-data-analysis/proteome-discoverer-software.html). Microsoft Excel (professional Plus2013) for basic statistical analysis. Image J (v.1.8.0) for quantification of western blot images. (https://imagej.en.softonic.com/). prism (v.10.3.1) for graphs (https://www.graphpad.com/scientific-software/prism/). SRA toolkit (v.2.9.2)(https://www.ncbi.nlm.nih.gov/sra/docs/toolkitsoft/). Fastqc (v.0.11.9)(http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Trim Galore (v.2.11)(https://github.com/FelixKrueger/TrimGalore). Bowtie2 (v.2.1.0)(https://www.uio.no/english/services/it/research/hpc/abel/help/software/bowtie2.html). Samtools (v.1.11)(https://github.com/samtools/samtools). R (v.3.1.0)(https://www.r-project.org/). MACS2 (v.2.1.1)(https://github.com/macs3-project/MACS). deepTools2 (v.2.0) (https://deeptools.readthedocs.io/en/develop/content/installation.html). IGV software (v.2.0)(http://software.broadinstitute.org/software/igv/download). Fiji software (v.2.9.0)(http://imagej.net/software/fiji/downloads). MetaboAnalyst (v.6.0)(https://www.metaboanalyst.ca/)

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

Data availability. All the data supporting the findings of this study are included in the manuscript and its supplementary files are available. Source data are provided as a Source Data file. The RNA-seq data for WT and Rpd3-2SA generated in this study have been deposited in the NCBI under accession number GSE260970. [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE260970]. The RNA-seq data for WT and Ada3-3KR are available in the GEO database under accession number GSE161887. [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE161887]. The RNA-Seq accession number for the Flag-HDAC1 and Flag-HDAC1-S434A treated with or without H89 is GSE284216.
[https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE284216].
The CUT &Tag accession number for the HDAC1 treated with or without H89 is GSE285017. [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE285017]. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium with the dataset identifier PXD058634. [https://www.ebi.ac.uk/pride/archive/projects/PXD058634].

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 were determined based on previous experience or similar published studies. To determine whether the outcome is statistically significant, at least three biological independent replicates were performed for each experiment. For other experiments, to determine the outcome is reproducible, at least 2-3 biological replicates were performed for each experiment.
Data exclusions	No data were excluded from this study.
Replication	We confirmed that all attempts to replicate experiments were successful. All experiments were performed for at least 2-3 biological replicates, which were specified in the figure legends. To determine statistical significance, at least three biological replicates were used.
Randomization	Samples were allocated into groups by random.
Blinding	Not applicable. The experiments were performed by comparing various treatments or WT and mutants. It was necessary for the researchers to be aware of the treatment applied and mutants used. Meanwhile, we also need to use appropriate controls in each experiment.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used

Anti-Histone H3 (9715S; Cell Signaling Technology; WB (1:5000))
Anti-Histone H3 (ab1791; abcam; 2.5 ul/ChIP)
Anti-Flag(AE024; Abclonal; WB (1:10000))
Rabbit anti-Histone H4 (ab10158; Abcam; WB (1:1000))
Mouse anti-FLAG M2 (F1804-1MG; Sigma-aldrich; IF (1:500))
PKA C-alpha (PRKACA) Mouse mAb (A21869; Abclonal; WB (1:2000))
PKA C-beta (PRKACB) Rabbit mAb (A22721; Abclonal; WB (1:2000))
Rabbit anti-H3K9ac (9649S; Cell Signaling Technology; WB (1:10000); 2.5 ul/ChIP)
Mouse anti-His (HRP-66005; proteintech; WB (1:5000))
Rabbit anti-H3K14ac (7627S; Cell Signaling Technology; WB (1:1000); 2.5 ul/ChIP)
Anti-GAPDH (10494-1-AP; proteintech; WB (1:10000))
Rabbit anti-Actin (20536-1-AP; proteintech; WB (1:5000))
Anti-Myc (60003-2-Ig; proteintech; WB (1:5000))
Goat polyclonal anti-mouse IgG (SA00001-1; proteintech; WB (1:5000))
Goat polyclonal anti-rabbit IgG (SA00001-2; proteintech; WB (1:5000))
Anti-CBP (Abs130593; Absin Bioscience Inc; WB (1:2000))
Alexa Fluor 594-conjugated Goat anti-Rabbit IgG (H+L) (AS039, Abclonal; IF (1:500))
Alexa Fluor 488-conjugated Goat anti-Mouse IgG (H+L) (AS037, Abclonal; IF (1:500))
Rabbit anti-HDAC1 (34589S; Cell Signaling Technology;WB(1:2000); CUT&Tag (8 ul))
Rabbit anti-Ada3K14ac (Custom made in Abclonal; WB (1:2000); IF (1:400))
Rabbit anti-Ada3K182ac (Custom made in Abclonal; WB (1:2000); IF (1:400))
Rabbit anti-Rpd3S50p (Custom made in Abclonal; WB (1:2000); IF (1:500))
Rabbit anti-Rpd3S354p (Custom made in Abclonal; WB (1:2000); IF (1:500))
The custom made antibodies against Ada3, Gcn5 (1:2000) and Spt3 (1:2000) were kind gift from Jerry L. Workman (Stowers Institute for Medical Research)

Validation

All antibodies have been validated for the application and species.
Anti-Histone H3 (9715S; Cell Signaling Technology) has been validated for Western blots in yeast (https://www.cellsignal.com/products/primary-antibodies/histone-h3-antibody/9715).
Anti-Histone H3 (ab1791; abcam) has been validated for ChIP in yeast (https://www.abcam.cn/products/primary-antibodies/histone-h3-antibody-nuclear-marker-and-chip-grade-ab1791.html).
Anti-Flag (AE024; Abclonal) has been validated for Western blots in yeast (https://abclonal.com.cn/catalog/AE024).
Anti-H4 (ab10158; Abcam) has been validated for Western blots (PMID33500413, 30759223) (https://www.abcam.com/histone-h4-antibody-chip-grade-ab10158.html).
Mouse anti-FLAG M2 (F1804-1MG; Sigma-aldrich) has been validated for IF in yeast (https://www.sigmaaldrich.cn/CN/zh/product/sigma/f1804).
PKA C-alpha (PRKACA) Mouse mAb (A21869; Abclonal) has been validated for Western blots in human and mouse (https://abclonal.com.cn/catalog/A21869)
PKA C-beta (PRKACB) Rabbit mAb (A22721; Abclonal) has been validated for Western blots in human and mouse (https://abclonal.com.cn/catalog/A22721)
Rabbit anti-H3K9ac (9649S; Cell Signaling Technology) has been validated for Western blots (PMID33027655) and for ChIP in yeast (https://www.cellsignal.cn/products/primary-antibodies/acetyl-histone-h3-lys9-c5b11-rabbit-mab/9649). The specificity of this antibody was validated within our lab group by Western blots of WT,H3K9R yeast cells (PMID34183849).
Mouse anti-6xHis (HRP-66005; proteintech) has been validated for Western blots of recombinant protein and 6xHis-tagged proteins (http://www.ptgcn.com/products/6-His,-His-Tag-Antibody-HRP-66005.htm).
Rabbit anti-H3K14ac (7627S; Cell Signaling Technology) has been validated for Western blots and ChIP (https://www.cellsignal.cn/products/primary-antibodies/acetyl-histone-h3-lys14-d4b9-rabbit-mab/7627). The specificity of this antibody was validated within our lab group by Western blots of WT, H3K14R yeast cells (PMID34183849)
Anti-GAPDH (10494-1-AP; proteintech) has been validated for Western blots in yeast (PMID30759223, 32663628) (https://www.ptgcn.com/products/GAPDH-Antibody-10494-1-AP.htm).
Rabbit anti-Actin (20536-1-AP; proteintech) has been validated for Western blots in cell (PMID30576924)(https://www.ptgcn.com/products/ACTB-Antibody-20536-1-AP.htm).
Anti-Myc (60003-2-Ig; proteintech) has been validated for Western blots in yeast (https://www.ptgcn.com/products/MYC-Antibody-60003-2-Ig.htm).
Goat polyclonal anti-mouse IgG (SA00001-1; proteintech) has been validated for Western blots in yeast (https://www.ptgcn.com/products/HRP-conjugated-Affinipure-Goat-Anti-Mouse-IgG-H-L-secondary-antibody.htm).
Goat polyclonal anti-rabbit IgG (SA00001-2; proteintech) has been validated for Western blots in yeast (https://www.ptgcn.com/products/HRP-conjugated-Affinipure-Goat-Anti-Rabbit-IgG-H-L-secondary-antibody.htm).
Anti-CBP (Abs130593; Absin Bioscience Inc) has been validated for Western blots of recombinant protein and TAP-tagged proteins (https://www.absin.cn/rabbit-cbp-polyclonal-antibody/abs130593.html).
Alexa Fluor 594-conjugated Goat anti-Rabbit IgG (H+L) (AS039, Abclonal) has been validated for IF (https://abclonal.com.cn/catalog/AS039).
Alexa Fluor 488-conjugated Goat anti-Mouse IgG (H+L) (AS037, Abclonal) has been validated for IF (https://abclonal.com.cn/catalog/AS037).
Rabbit anti-HDAC1 (34589S; Cell Signaling Technology) has been validated for Western blots and ChIP in human and mouse (https://www.cellsignal.cn/products/primary-antibodies/hdac1-d5c6u-xp-rabbit-mab/34589).
The specificity of the custom-made antibodies anti-Rpd3S50p and anti-Rpd3S354p was examined by dot blots with phosphorylated

and unphosphorylated Rpd3 peptides and Western blots with the yeast cell lysates of WT, Rpd3-S50A and Rpd3-S354A mutants (Fig. 3c; Supplementary Fig. 3h).
The custom made antibodies against Ada3, Gcn5 and Spt3 have been validated for Western blots in yeast (PMID9674426, 12419247).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293T cells (STCC10301P) and HepG2 cells (STCC10114P) were obtained from the Servicebio Company. All cell lines were cultured for no more than 2 months and their morphology was confirmed periodically to avoid cross-contamination.
Authentication	HepG2 and HEK293T cells were authenticated through short tandem repeat (STR) analysis.
Mycoplasma contamination	All cell lines were regularly tested for mycoplasma contamination. The results showed that all cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

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

Laboratory animals	Our research complies with all relevant ethical regulations. All procedures were approved by the Animal Care and Use Committee of Hubei University (NO. 20240116). Wild-type C57BL/6J mice were obtained from Henan Skobes Biotechnology Co., Ltd, and housed in a specific pathogen-free (SPF) facility on a 12-hour light/dark cycle with ad libitum access to food and water. Animals were fed with a normal chow diet. Animals used for in vitro experiments were all healthy prior to experiments. For PKA inhibition analysis, 9-week-old wild-type mice were fed ad libitum or fasted for 12 h with or without H89 treated (6 mice per group, including 3 male and 3 female mice). For H89 injection, animals were injected with 5 mg/kg H-89 (HY-15979, MCE) at 9 pm, followed by feed or fast treated overnight. 5 mice were randomly chosen and sacrificed to harvest the liver tissues were for immunoblot, RT-qPCR and metabolite analysis.
Wild animals	The study did not involve wild animals
Field-collected samples	The study did not involve samples collected from the field
Ethics oversight	Animal procedures complied with all relevant ethical regulations and were approved by the Animal Care and Use Committee of Hubei University (NO. 20240116).

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.*	The GEO accession number for Cut&Tag of HDAC1 treated with or without PKA inhibitor H89 is GSE285017. (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE285017).
Files in database submission	FASTQ files, Bigwig files and BED files for WT and HDAC_S434A Cut&Tag are included.
Genome browser session (e.g. UCSC)	No applicable-visualized data using IGV.

Methodology

Replicates	For WT and HDAC1_S434A Cut&Tag, one biological replicate.
Sequencing depth	WT,HDAC1_S434A Cut&Tag samples: clean reads 22M IgG Cut&Tag control sample: clean reads 24M
Antibodies	Rabbit anti-HDAC1 (34589S; Cell Signaling Technology) Rabbit anti-IgG (A19711; Abclonal)
Peak calling parameters	Data analysis and peak calling parameters are provided in the methods. macs2 callpeak -t treated.bam -c control.bam -g hs -n -B -p. 0.01
Data quality	All raw data used for analysis with Per base sequence quality greater than 30 (Q30 cutoff). All assigned peaks were identified based on an FDR < 0.001.

Software

SRA toolkit (v.2.9.2)
Fastqc (v.0.11.9)
Trim Galore (v.0.3.1)
Bowtie2 (v.2.1.0)
Samtools (1.7-2)
MACS2 (v.2.1.1)
Bedtools (v.2.19.0)
R (v.3.1)
IGV software (v.2.0)
deepTools2(v2.0)
hisat2 (v.2.1.0)
DESeq2 （1.46.0）
ggplot2 (v.3.3.3)
ChIPseeker (v.1.42.0)