nature portfolio

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Last updated by author(s): Sep 11, 2024

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	Mass spectrometry: Q-Exactive HF Hybrid Quadrupole-Orbirap Mass spectrometer system (Thermo Fisher Scientific).
Data analysis	Statistical analysis was performed using Prism 8.0.2 software (GraphPad). For image analysis, Image J Fiji was used.

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 data associated with this study are available in the main text or the Supplementary Materials. All requests for reagents should be made to the corresponding authors upon reasonable request. They will be made available through appropriate administrative channels (MTA).

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	No participants in this study.
Reporting on race, ethnicity, or other socially relevant groupings	Not applicable
Population characteristics	Not applicable
Recruitment	Not applicable
Ethics oversight	Not applicable

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	Specific sample sizes are described in figures or figure legends for all experiments. The sample size used for animal experiment is based on previous experience from the Li labs. No statistical test was used to pre-determine sample size.
Data exclusions	No samples or animals were excluded from the analyses.
Replication	All experiments were performed in at least 3 biological replicates, which is indicated in the Figure Legends. All repeats support the same conclusion.
Randomization	Cells or mice tissue were randomly assigned to groups (chemical compound/other treatments).
Blinding	For animal related experiments, the investigators were divided into two groups: one group is responsible for collecting samples and the other group is responsible for experiment 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
☐	☒ 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	Pan anti-Kla (PTM-bio, Cat# PTM-1401, immunoblot, 1:2000), anti-acetylated tubulin (Sigma, Cat# T7451, immunoblot, 1:50000, immunostaining, 1:5000), anti-HA tag (Abmart, Cat# M20003, immunoblot, 1:2000),

anti-HA tag (Sigma, Cat# H6908, immunoblot, 1:3000),
anti-Flag tag (Abmart, Cat# M20008, immunoblot, 1:2000),
anti-Flag tag (Sigma, Cat# F7425, immunoblot, 1:2000),
anti-HDAC6 (Proteintech, Cat# 12834-1-AP, immunoblot, 1:2000),
anti-GFP (Thermo Fisher Scientific, Cat# A-11122, immunoblot, 1:2000),
anti-α-tubulin (Santa Cruz, Cat# sc-32293, immunoblot, 1:1000),
anti-α-tubulin (Proteintech, Cat# 11224-1-AP, immunoblot, 1:1000),
anti-β3-Tubulin (Cell Signaling technology, Cat# 5568, immunostaining, 1:1000),
anti-β3-Tubulin (Merck/Millipore, Cat# MAB1637, immunostaining, 1:1000),
anti-Sirt2 (PTM-bio, Cat# PTM-6318, immunoblot, 1:1000),
anti-HDAC3 (PTM-bio, Cat# PTM-5183, immunoblot, 1:1000),
Purified anti-Neurofilament Marker (SMI-312, BioLegend, Cat# 837904, immunostaining, 1:500),
anti-lactylated α-tubulin antibody (generated by HUA BIO, immunoblot, 1:5000, immunostaining, 1:500).

Validation

Pan anti-Kla (PTM-bio, Cat# PTM-1401):
Species: human, mouse; Application: immunoblot, immunoprecipitation; Validated in other publications (e.g., Nature. 2019;574 (7779):575-580.).

anti-acetylated tubulin (Sigma, Cat# T7451):
Species: bovine, frog, invertebrates, human, hamster, mouse, protista, pig, monkey, chicken, rat, plant; Application: immunoblot, immunostaining; Validated in other publications (e.g., PLoS One. 2011;6(2):e17138.).

anti-HA tag (Abmart, Cat# M20003):
Used for recombinant proteins tagged with HA; Application: immunoblot; Validated in other publications (e.g., Gene. 1988;67 (1):31-40.).

anti-HA tag (Sigma, Cat# H6908):
Used for recombinant proteins tagged with HA; Application: immunoblot; Validated in other publications (e.g., Oncogene. 2016;35 (39):5106-5118.).

anti-Flag tag (Abmart, Cat# M20008):
Used for exogenously expressed DYKDDDDK proteins; Application: immunoblot; Validated in other publications (e.g., Nat Med. 2017 Mar;23(3):337-346.).

anti-Flag tag (Sigma, Cat# F7425):
Used for exogenously expressed DYKDDDDK proteins; Application: immunoblot; Validated in other publications (e.g., Frontiers in microbiology, 2019;10, 1857-1857).

anti-HDAC6 (Proteintech, Cat# 12834-1-AP):
Species: human; Application: immunoblot; Validated in other publications (e.g., Mol Cell. 2017;65(5):941-955.e8.).

anti-GFP (Thermo Fisher Scientific, Cat# A-11122):
Used for exogenously expressed GFP proteins; Application: immunoblot; Validated in other publications (e.g., Sci Rep. 2023;13 (1):1956.).

anti-α-tubulin (Santa Cruz, Cat# sc-32293):
Species: mouse, rat and human; Application: immunoblot; Validated in other publications (e.g., Genetics. 2023;224(4):iyad105.).

anti-α-tubulin (Proteintech, Cat# 11224-1-AP):
Species: human; Application: immunoblot; Validated in other publications (e.g., Nat Biotechnol. 2023;41(6):858-869.).

anti-β3-Tubulin (Cell Signaling technology, Cat# 5568):
Species: human, mouse, rat; Application: immunostaining; Validated in other publications (e.g., Andrology. 2023;11(6):1188-1202.).

anti-β3-Tubulin (Merck/Millipore, Cat# MAB1637):
Species: avian, bovine, human, mouse, monkey, rat, sheep; Application: immunostaining; Validated in other publications (e.g., Dis Model Mech. 2015;8(9):1047-1057.).

anti-Sirt2 (PTM-bio, Cat# PTM-6318):
Species: human, mouse, rat; Application: immunoblot; Validated in this paper.

anti-HDAC3 (PTM-bio, Cat# PTM-5183):
Species: human, mouse, rat; Application: immunoblot; Validated in this paper.

Purified anti-Neurofilament Marker (SMI-312, BioLegend, Cat# 837904):
Species: human, mouse, rat; Application: immunostaining; Validated in other publications (e.g., Cereb Cortex. 2017;27 (2):1253-1269.).

anti-lactylated α-tubulin antibody (generated by HUA BIO).
Species: human, mouse in this paper; Application: immunoblot, immunostaining; Validated in this paper.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T was obtained from NSTI-BMCR.
Authentication	HEK293T cells were authenticated based on our vast experience working with these cells lines (such as cell morphology, culture conditions, etc.). Furthermore, we believe that the modification we described in the paper is widely existed in various cell lines, not specific to certain cell types.
Mycoplasma contamination	Cells were routinely tested for mycoplasma contamination, and only negative cells were used in experiments.
Commonly misidentified lines (See ICLAC register)	None of the cell lines used are listed in the database of commonly misidentifed cell lines maintained by ICLAC.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	P0 mice (Mus musculus, C57BL/6) were purchased from Shanghai Jihui Animal Care Co., Ltd. (China), and were used to culture primary neurons. MEC-17 KO mice (Mus musculus, C57BL/6, 8-10 weeks old) from Bao lab and HDAC6 KO mice (Mus musculus, C57BL/6, 8-10 weeks old) which were purchased from the GemPharmatech and dKO mice were used to detect PTMs of α-tubulin.
Wild animals	The study did not involve samples collected from wild animals.
Reporting on sex	Both male and female mice were used in studies.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal protocols were approved by the ethical guidelines of the Institutional Animal Care (IACUC) and Use Committee of ShanghaiTech University.

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

Plants

Seed stocks	None
Novel plant genotypes	None
Authentication	None