nature portfolio

Corresponding author(s): Yi-Ming Li

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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	Shimadzu Prominence HPLC systems (Shimadzu Corp., Japan); LC/MS 2020 (SHIMADZU); Fusion FX EDGE SPECTRA (Vilber); Confocal Zeiss LSM 880; flow cytometer (CytoFLEX, Beckman); UV lamp (SCIENTZ; Model: SCIENTZ03-11); MicroCal PEAQ-ITC (Malvern)
Data analysis	Microsoft Excel (2016);LabSolutions (SHIMADZU);Proteome Discoverer software (PD, version 1.4); GraphPad Prism

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 mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) with the dataset identifier PXD055058, PXD055061, PXD055055 and PXD045620. 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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	The work performed here did not involve statistical analyses that would be impacted by sample size.
Data exclusions	No data were excluded from the analysis.
Replication	Almost all experiments were repeated from 2-4 times. Several experiments were repeated independently (at different times and by different individualsand some dependently (in parallel, by the same individual). All attempts at replication were successful.
Randomization	Randomization was not relevant to this study because no grouped samples were involved.
Blinding	Blinding was not relevant to this study because no grouped samples were involved.

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	Antibodies used include: Anti-His6 antibody (Abcam; ab18184, 1:2000 dilution), Anti-β-Actin antibody (Cell Signaling Technology; #4970, 1:2000 dilution), anti-AF9 antibody (Cell Signaling Technology; #47577, 1:2000 dilution), Anti-H3K9la antibody (PTM BioLab, Inc.; PTM-1419RM, 1:1000 dilution).
Validation	Abcam validates all their antibodies tested in their own labs, by their suppliers, or by selected trusted collaborators. This statement can be found at their website at https://www.abcam.com/help/abpromise-guarantee

Cell Signaling Technology validates all their antibodies in-house, in multiple research applications. This statement can be found at their website at https://www.cellsignal.com/about-us/cst-antibody-performance-guarantee
PTM BioLab, Inc states that the Anti-L-Lactyl-Histone H3 (Lys9) Rabbit mAb(PTM-1419RM) were tested in numerous studies, and a list of references can be found at their website at http://www.ptm-biolab.com.cn/productDetail.html?id=5381

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	RAW264.7 was purchased from cellcook (CC9001) and HeLa was a generous gift from Yangzhong Liu (University of Science and Technology of China, Hefei)
Authentication	Cell lines were authenticated by STR testing.
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	n/a

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Cells were incubated with FBS free culture medium containing different probes. After 30 min, cells were washed three times to remove unabsorbed probes. Cells were resuspended in PBS and analyzed.
Instrument	flow cytometer (CytoFLEX, Beckman)
Software	CytExpert
Cell population abundance	n/a
Gating strategy	n/a

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