nature portfolio

Corresponding author(s): Bing Yang

Last updated by author(s): Jan 16, 2025

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	Thermo Scientific Xcalibur 4.5.445.18 was used to collect the LC-MS/MS data; SCIEX OS 3.1.6.44 was used to collect the LC-MS data. Amersham™ ImageQuant™ 800 was used to collect the immunoblots. ImageView 4.11 was used to detect fluorescent images. ChampGel™ 6000 was used to detect protein gel electrophoresis images. CytExpert 2.5.0.77 was used to collect flow cytometry data.
Data analysis	pLink 2.3.11 was used for identification of cross-linking peptides. pFind 3.2.0 was used for identification of modified peptides. MaxQuant 2.0.3.0 was used for label-free quantitative proteomics analysis. PyMOL 2.5.0 was used to visualize of crystal structures of proteins. Graphpad Prism 9.0 and R 4.2.1 was used for statistical analysis. ChemDraw 19.0 was used to draw structures of molecular. FlowJo 10.8.1 was used for flow cytometry data analysis. ImageJ 2.14.0 was used to fluorescent images analysis.

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 MS proteomics data have been deposited to the ProteomeXchange Consortium (PXD ID: PXD 051167)

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	Our manuscript is a mass spectrometry methodology study. For AP-MS experiments, samples were collected as one 10 cm dish cells for each group, at least three biological replicates were performed to catculate the statistical significance. For purified protein samples, E. coli samples at least 25mL,and HEK293T samples at least two 10 cm dishes.
Data exclusions	No data was excluded from our analysis.
Replication	The reproducibility of the developed mass spectrometry methodology was verified by repeated experiments. Both quantitative and comparative experiments were performed with at least twice biological replicates.
Randomization	For studies using cells, samples were randomly allocated into experimental or control groups.
Blinding	Blinding is not relevant to this study because animal or human subjects was not involved in this study.

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 and archaeology
[x]	[ ] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

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

Antibodies

Antibodies used

The following antibodies were used:
anti-6×His tag, Mouse, Proteintech, cat. no. HRP-66005, 1:10000
anti-FLAG tag, Mouse, HUABIO, cat. no. M1403-2, 1:10000
anti-Strep-tag II, Mouse, Wuhan Dian Biotechnology, cat. no. 2098, 1:10000
anti-Methacryllysine, Mouse, PTO BIO, cat. no. PTM-1501, 1:2000
anti-Crotonyllysine, Mouse, PTO BIO, cat. no. PTM-502, 1:2000
Goat anti-Mouse IgG (H+L) HRP conjugate,Jackson ImmunoResearch, Code. 115-035-003, 1:10000
anti-β-Tubulin, Mouse, Abmart, cat, no. M20005, 1:5000
anti-V5 tag, Mouse, Thermo, cat. no. MA5-15253, 1:2000
anti-Cyclophilin A, Rabbit,HUABIO,at. no. ET1703-33,1:5000

Validation

These antibodies have been validated by suppliers, by relevant citations. The manufacturer's websites are provided as follows.
anti-His tag: https://www.ptgcn.com/products/His-Tag-Antibody-66005-1-Ig.htm
anti-FLAG tag: http://www.huabio.cn/products/DYKDDDDK-Tag-FLAG-antibody-M1403-2
anti- Strep-tag II: https://dia-an.com/index/product.html?pro_id=4470&aid=48,49#view_zjy
anti-Methacryllysine: http://www.ptm-biolab.com.cn/productDetail.html?id=7845
anti-Crotonyllysine: http://www.ptm-biolab.com.cn/productDetail.html?id=4759
Goat anti-Rabbit IgG (H+L) Secondary Antibody: https://www.jacksonimmuno.com/catalog/products/115-035-003
anti-β-Tubulin: http://www.ab-mart.com.cn/page.aspx?node=59&id=983
anti-V5 tag: https://www.thermofisher.cn/cn/zh/antibody/product/V5-Tag-Antibody-clone-E10-V4RR-Monoclonal/MA5-15253
anti-Cyclophilin A: http://www.huabio.cn/products/Cyclophilin-A-antibody-ET1703-33

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T and HeLa cell was obtained from American Type Culture Collection (ATCC).
Authentication	Cells were authenticated by STR profiling by vendors. Cell lines were not additionally authenticated in our hands.
Mycoplasma contamination	All cells were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

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.

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	HEK293T cells were washed with PBS, for the analysis of unnatural amino acid insertion efficiency treated with or without MeaK for 36h; for ROS level analysis treated with or without H2DCFDA (DCFH-DA) probe for 20 min. Then the cells were washed with PBS and resuspended in 500 µL PBS for further analysis.
Instrument	All flow cytometry acquisitions were realized using Beckman CytoFlex.
Software	Collection: CytExpert ver 2.5.0.77 (Beckman Coulter) Analysis: FlowJo ver 10.8.1 (BD Biosciences)
Cell population abundance	According to the estimated size and particle size of the forward and lateral scattering cells, as well as the accompanying mCherry-positive or negative signals, the cell population is established for flow analysis. Positive cells (n=5000) were analyzed for FTIC-A for ROS level analysis. Positive cells (n=20000) were analyzed for unnatural amino acid insertion efficiency analysis.
Gating strategy	Cells were first gated based on their size (FSC-A) and granularity (SSC-A). Further gating is done in an FSC-H and FSC-A dot plot to eliminate doublets. Then target cell population for further analysis were gated by mCherry (ECD-A) and GFP (FTIC-A) fluorescent signal.

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