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	The following software were used for proteomics data collection: Bruker HyStar (version 6.3) and Bruker timsControl (version 6.0.6); Chromeleon (version 7.2.10). The Odyssey Fc Imaging System (LI-COR Corp.) was used to acquire the Western blot images. 7500 Real-Time PCR System (Applied Biosystems) was used to acquire the RT-qPCR data.
Data analysis	The following software were used for proteomics raw data processing and downstream statistical analysis: DIANN (v1.8.2beta9, 1.9.1, 1.8.2beta27), MaxLFQ (as implemented in DIA-NN), R (version 4.4.3). Licor Image Studio 6.0 (LI-COR Biotech) was used for the analysis of Western blot images. RT- PCR and DC50 data were analyzed and figures were generated using GraphPad Prism version 10 (GraphPad Software).

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 raw data generated in this study have been deposited in the MassIVE repository (https://massive.ucsd.edu/ProteoSAFe/static/massive.jsp) under and can be accessed via https://proteomecentral.proteomexchange.org/ui with the identifier PXD059111 (https://proteomecentral.proteomexchange.org/cgi/GetDataset?ID=PXD059111).

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	No sample size calculation was done for proteomics experiments. We acquired three or four replicate samples/condition (independent cell treatments/sample preparation) for experiments. This number is sufficient for robust statistical analyses.
Data exclusions	No data was excluded
Replication	For proteomics-based experiments: Three or four independent treatments (individual wells in cell culture plates) for each time point were analyzed. Replication experiments (e.g. testing of the same compound(s) across different cell lines) were successful. Independent treatment experiment in -/+ MLN4924 reproduced the effects observed in the primary proteomics screens. Relevant compounds (i.e., the ones yielding novel CRBN neosubstrates) were retested by ubiquitinomics, yielding to a independent validation of the screening hits. Reproducibility between replicates was confirmed by Pearson correlation coefficients, by coefficients of variation and/or by principal component analysis (PCA). All Western blot data for DC50 calculations and other quantifications were obtained from duplicates or triplicates. RT-qPCR results were from three independent technical replicates
Randomization	All proteomics MS measurements were randomized according to a computationally optimized plate layout. The DMSO controls were distributed homogenously over the 96-well plate along with the replicates of the individual compound treatments. Ubiquitinomics MS measurements were block randomized (e.g., DMSO_rep1, compound 1_rep1, compound 2_rep1, compound 3_rep1, DMSO_rep2,...).
Blinding	For proteomics no blinding was intentionally applied. However, each sample was assigned a number (without any association to the treatment) and the compounds have not been characterized previously by proteomics. Moreover, all data collected by MS is inherently not directly biased by the operator.

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

GAPDH (14C10), Cell Signaling Technology, Cat# 3683S; CRBN (D8H3S), Cell Signaling Technology, Cat# 71810S; G3BP2, Bethyl Laboratories, Cat# A302-040A; USP10 (D7A5) , Cell Signaling Technology, Cat# 8501S; KDM4A, ABclonal, Cat# A9267; KDM4B, Bethyl Laboratories, Cat# A301-478A; KDM4C, ABclonal, Cat# A8485; KDM4D, ProteinTech, Cat# 22591-1-AP; Flag M2 (F1804), SIGMA, Cat# F1804; α-Tubulin (6A204), Santa Cruz Biotechnology, Cat# sc-69969; HSP90α/β (F-8), Santa Cruz Biotechnology, Cat# sc13119; Vinculin (E1E9V), Cell Signaling Technology, Cat# 13901S

Validation

GAPDH, rabbit, western blot, GAPDH antibody detects endogenous levels of GAPDH protein in extracts from NIH/3T3, HeLa, and C6 cells. CRBN, rabbit, western blot, CRBN antibody detects endogenous levels of CRBN protein in extracts from various cell lines. G3BP2, rabbit, western blot, G3BP2 antibody detects endogenous levels of G3BP2 protein in extracts from various cell lines. USP10, rabbit, western blot, western blot analysis of extracts from 293T cells transfected with hUSP10-Myc/DDK. KDM4A, rabbit, western blot, KDM4A antibody detects endogenous levels of KDM4A protein in extracts from HeLa, NIH/3T3, and SH-SY5Y cells. KDM4B, rabbit, western blot, KDM4B antibody detects endogenous levels of KDM4B protein in extracts from various cell lines. KDM4C, rabbit, western blot, western blot analysis of mouse brain. KDM4D, rabbit, western blot, western blot analysis of PC-3 cells. Flag M2, mouse, western blot, validated by our previous data, DOI:10.1126/scitranslmed.abq2096. α-Tubulin, mouse, western blot, α-Tubulin antibody detects endogenous levels of α-Tubulin protein in extracts from various cell lines. HSP90α/β, mouse, HSP90α/β antibody detects endogenous levels of HSP90α/β protein in extracts from various cell lines. Vinculin, rabbit, western blot, vinculin antibody detects endogenous levels of vinculin protein in extracts from various cell lines.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

HEK293: Cytion (300192)
HuH7: Cytion (300156)
NB-4: Cytion (300299)
SUSA: DSMZ (ACC-747)
RH4 and RH30: Dr. Gerald Grosveld at St Jude
SK-N-BE(2)-C (BE(2)-C): ATCC (CRL-2268)
Kasumi-1: ATCC (CRL-2724)
Caki-1: ATCC (HTB-46)
SIMA: DSMZ (ACC 164)
EW-8: DSMZ (ACC 493)
SKNO-1: DSMZ (ACC 690)

Authentication

For HEK293, HuH7, NB-4 and DSMZ no authentication was performed as all cell lines were newly ordered from the indicated cell line provider. No further authentication was performed. RH4, RH30, SK-N-BE(2)-C, Kasumi-1, Caki-1, SIMA, EW-8, and SKNO-1 cell lines were validated by short tandem repeat (STR) profiling using PowerPlex 16 HS System.

Mycoplasma contamination

All cell lines were regularly tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

None

Plants

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