

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Immunoblot image acquisition was operated with Li-COR Image Studio (v2.0.38). Orbitrap Exploris 480 mass spectrometer was operated through Thermo MS Tune with Xcalibur (v4.1-4.1.292.16/4.1.335.19). Bruker timsTOF Pro 2 mass spectrometer was operated by timsControl (v4.1.12.0) and Bruker Compass HyStar (v6.2.1.13).
Data analysis	The following softwares were used: R (4.2.1), Rstudio (2023.03.1+446), Li-COR Image Studio Lite (v5.2.5), ImageJ (NIH, v1.52k), Proteome Discoverer (2.4), DIA-NN (1.8), and GraphPad Prism (v10.1.1). The following R packages were used for computational analysis: limma (v3.52.4), tidyverse (v1.3.1), readxl (v1.4.0), magrittr (v2.0.3), ggrepel (v0.9.1), ggplot2 (v3.3.6), HGNChelper (v0.8.1), dplyr (v1.1.1), stringr (v1.5.0), multcomp (v1.4-23), truncnorm (v1.0-9), tidyr (v1.3.0), Rcpp (v1.0.10), seqinr (v4.2-30), RColorBrewer (v1.1-3), diann (v1.0.1). In-house scripts necessary to reproduce the statistical analysis for quantitative proteomics can be found on https://doi.org/10.5281/zenodo.10633173 .

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](#)

For proteomics analysis, the Swissport human database (January 2021) was used. All raw files from mass spectrometry experiments described in this study have been deposited to the PRIDE repository (<https://www.ebi.ac.uk/pride/>). Information on the accession code, associated figures, and sample description for each dataset can be found in Supplementary Data 1-4, and is briefly summarized below.

Dataset 1: PXD042673 (associated with Fig. 2a,b)

Dataset 2: PXD042691 (associated with Fig. 2e and Extended Data Fig. 7a; VHL-BirA)

Dataset 3: PXD042687 (associated with Fig. 2e and Extended Data Fig. 7a; CRBN-BirA)

Dataset 4: PXD047583 (associated with Extended Data Fig. 3d)

Dataset 5: PXD042693 (associated with Extended Data Fig. 3e)

Dataset 6: PXD042674 (associated with Extended Data Fig. 4a)

Dataset 7: PXD042697 (associated with Fig. 3)

Dataset 8: PXD042700 (associated with Extended Data Fig. 4b,c,d)

Dataset 9: PXD042698 (associated with Fig. 4a,b,c)

Dataset 10: PXD042702 (associated with Fig. 4d,e,f)

Dataset 11: PXD042694 (associated with Extended Data Fig. 7b,c)

Dataset 12: PXD042706 (associated with Fig. 5c,d,f)

Dataset 13: PXD021255 (associated with Fig. 3b,c,e,f,h,i,k,l)

Dataset 14: PXD042657 (associated with Fig. 4b,c,e,f)

Dataset 15: PXD047455 (associated with Extended Data Fig. 2c,d,e)

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](https://www.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 decision to perform biological triplicates for E-STUB and a mix of biological duplicates and triplicates for total proteomics was influenced by the need to acquire statistically robust quantification results and to reduce cost and resources.

Data exclusions

Data were filtered to have a 1% FDR and to include only proteins that had a minimum of 2 abundance counts per protein in at least 2 replicates.

Replication

Number of replicates are described in the figure legends, where applicable.

Randomization

The order at which cells received biotin during E-STUB experiments were randomized to minimize systematic differences in labeling duration.

Randomization	To control covariates, we performed cross-sample normalization procedures such as total protein abundance normalization and run sample standards in-between every experiment set to ensure data quality is consistently of a high quality.
Blinding	Investigators were not blinded during data acquisition and analysis in this study because blinding is logistically challenging for the experiment types described in this study. Instead, we randomized sample treatment, included positive and negative controls, repeated experiments, and used orthogonal approaches to validate the results—strengthening the conclusions drawn 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
☐	☒ 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

- Anti-GAPDH (D16H11) (1:2000, Cell Signaling Technology, 5174S)
- Anti- α -Tubulin (DM1A) (1:2000, Cell Signaling Technology, 3873S)
- Anti-vinculin (V284) (1:2000, Sigma-Aldrich, SAB4200080)
- Anti-FLAG (M2) (1:2000, Sigma-Aldrich, F1804)
- Anti-HA (12CA5) (1:1000, Sigma-Aldrich, 11583816001)
- Anti-HA (6E2) (1:1000, Cell Signaling Technology, 2367S)
- Anti-V5 [SV5-P-K], Mouse IgG1, Kappa (1:1000, Absolute Antibody, Ab00136-1.1)
- Anti-V5 [SV5-P-K], Rabbit IgG (1:1000, Absolute Antibody, Ab00136-23.0)
- Anti-eRF1 (1:1000, Invitrogen, PA5-28777)
- Anti-GSPT1 (1:1000, Abcam, ab234433)
- Anti-CRBN [4D6] (1:1000, Abcam, ab244223)
- Anti-HDAC1 (10E2) (1:1000, Cell Signaling Technology, 5356T)
- Anti-HDAC2 (3F3) (1:1000, Cell Signaling Technology, 5113T)
- Anti-HDAC3 (7G6C5) (1:1000, Cell Signaling Technology, 3949T)
- Anti-HDAC6 (D2E5) (1:1000, Cell Signaling Technology, 7558T)
- Anti-CoREST (D6I2U) (1:1000, Cell Signaling Technology, 14567S)
- Anti-MCL1 (D2W9E) (1:1000, Cell Signaling Technology, 94296S)
- Anti-NOXA (D8L7U) (1:1000, Cell Signaling Technology, 14766S)
- Anti-His-Tag (27E8) (1:1000, Cell Signaling Technology, 2366T)
- IRDye® 680RD Goat anti-Rabbit IgG Secondary Antibody (1:5000, LI-COR, 926-68071)
- IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody (1:20000, LI-COR, 926-32211)
- IRDye® 800CW Goat anti-Mouse IgG Secondary Antibody (1:20000, LI-COR, 926-32210)
- Streptavidin, Alexa Fluor™ 680 conjugate (1:5000, Invitrogen, S21378)

Validation

The following antibodies were validated by the manufacturer and have been cited hundreds to thousands of times to detect common loading control proteins.

- Anti-GAPDH (D16H11) (1:2000, Cell Signaling Technology, 5174S)
- Anti- α -Tubulin (DM1A) (1:2000, Cell Signaling Technology, 3873S)
- Anti-vinculin (V284) (1:2000, Sigma-Aldrich, SAB4200080)

The following antibody was validated by the manufacturer for the specified reasons.

- Anti-eRF1 (1:1000, Invitrogen, PA5-28777) was validated based on siRNA mediated knockdown.

The following antibodies were validated in this study, as they detect exogenously expressed proteins bearing the specific epitope tags.

- Anti-FLAG (M2) (1:2000, Sigma-Aldrich, F1804)
- Anti-HA (12CA5) (1:1000, Sigma-Aldrich, 11583816001)
- Anti-HA (6E2) (1:1000, Cell Signaling Technology, 2367S)
- Anti-V5 [SV5-P-K], Mouse IgG1, Kappa (1:1000, Absolute Antibody, Ab00136-1.1)
- Anti-V5 [SV5-P-K], Rabbit IgG (1:1000, Absolute Antibody, Ab00136-23.0)
- Anti-His-Tag (27E8) (1:1000, Cell Signaling Technology, 2366T)

The following antibodies were validated in this study, as the detected species respond as expected to drug treatment (e.g. protein degrader, cycloheximide, and biotin). The expected response was based on prior description of the protein degraders or protein half-

lives described in the literature.

- Anti-GSPT1 (1:1000, Abcam, ab234433)
- Anti-HDAC1 (10E2) (1:1000, Cell Signaling Technology, 5356T)
- Anti-HDAC2 (3F3) (1:1000, Cell Signaling Technology, 5113T)
- Anti-HDAC3 (7G6C5) (1:1000, Cell Signaling Technology, 3949T)
- Anti-HDAC6 (D2E5) (1:1000, Cell Signaling Technology, 7558T)
- Anti-CoREST (D6I2U) (1:1000, Cell Signaling Technology, 14567S)
- Anti-MCL1 (D2W9E) (1:1000, Cell Signaling Technology, 94296S)
- Anti-NOXA (D8L7U) (1:1000, Cell Signaling Technology, 14766S)
- Streptavidin, Alexa Fluor™ 680 conjugate (1:5000, Invitrogen, S21378)

The following antibody was validated in this study, as the detected species disappears upon genetic knockout.

- Anti-CRBN [4D6] (1:1000, Abcam, ab244223)

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	293T (CRL-3216), A375 (CRL-1619), and U2OS (HTB-96) were obtained from ATCC.
Authentication	293T (CRL-3216), A375 (CRL-1619), and U2OS (HTB-96) were authenticated by STR profiling (Labcorp, Burlington, NC).
Mycoplasma contamination	All cell lines were routinely examined to be free of mycoplasma using the MycoAlert mycoplasma detection kit (Lonza, LT07-318).
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.