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Corresponding author(s): Nicolas H. Thoma and Benjamin L. Ebert

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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	Proteins were identified and quantified using Proteome Discoverer 2.2 (Thermo Fisher Scientific): RRID:SCR_014477 QPCR signal was quantified with CFX96 Real Time system (Bio-Rad). Western blot data were imaged by Odyssey Imaging System, Image Studio (Li-Cor). The luminescent BRET signal was acquired with FilterMax F5 plate reader (Molecular Devices) or EnVision Multilabel Plate Reader (Perkin Elmer). The luminescent CTG signal was acquired with EnVision Multilabel Plate Reader (Perkin Elmer) or CLARIOstar Plus, MARS 3.4 (BMG LabTech). Flow data were collected with BD FACSDiva 8.0 (BD Biosciences) or CytExpert Software (Beckman Coulter Life Sciences). TR-FRET data collection was performed using a Pherastar FS (BMG).
Data analysis	Crystallographic data processing and refinement was done using XDS, DIALS (2.0), AIMLESS (CCP4 suite 7.0), STARANISO, PHASER, COOT (0.9), phenix (1.17.1-3660), BUSTER, eLBOW, PyMOL (2.3.2), MOLPROBITY/PDB-REDO. Fluorescence polarization fits were done using Prism7 (Graphpad). Bioinformatic, CRISPR screen data analyses and data visualization were done using R programming 3.5.1 and RStudio (1.1.453) with the following packages: tidyverse (1.2.1), ggrepel (0.8.1), GGally (1.4.0), dr4pl (1.1.11), ShortReads (Bioconductor) and Statistical Analysis Limma Package (Bioconductor). Custom R scripts were used to analyze the data, which are attached as Supplementary Code. Flow data were analyzed with FlowJo 10 (BD).

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Structural data is deposited in the PDB under the accession code 6TD3. Gene expression data and drug sensitivity data are available through the DepMap portal (https://depmap.org). Drug sensitivity data are also archived via Figshare (doi:10.6084/m9.figshare.9393293). For the manuscript analysis, we used a provisional data set, however, all the findings can be reproduced from the final release of the data. Proteome quantification data are available in the PRIDE repository (PXD016187 and PXD016188). Uncropped western blot gels can be found in the Supplementary Information.

Figures that have associated raw data:

Extended Data Fig. 1e is associated with Primary data for validation of 96 E3 gene-compound pairs. Fig. 1c and Extended Data Fig. 1f, g is associated with Supplementary Data: Proteome quantification using tandem mass tag spectrometry data. Fig1 f,g, Extended Data Fig. 2b,i is associated with Supplementary Data: Functional genomics data.

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 performed. The sample size for the number of replicates (n) for each experiment is provided in the figure captions.
Data exclusions	No data was excluded from the analysis. For functional genomics experiments, data processing, which includes filtering, is described in Methods section and can be re-analyzed with attached R-programming scripts.
Replication	Data presented in the figure represent technical replicates, however drug treatment or perturbation was performed independently for each replicate. Replications were consistent across multiple experiments performed on different days.
Randomization	No randomization was performed since no animal-based experiments were performed.
Blinding	Investigators were not blinded during data collection or analysis. However, controls and samples were analyzed in exactly the same way using the same computational pipeline.

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	anti-cycK (Bethyl Laboratories, A301-939A for full length cycK, 1:2,000, Lot#6), anti-cycK (abcam, ab251652, for cycK1-267, 1:2000), anti-beta-actin (Cell Signaling, #3700, 1:5000, lot#17), anti-CRBN (Sigma prestige, HPA045910, 1:1000, lot#Q103829), anti-mouse 800CW (LI-COR Biosciences, 926-32211, 1:10 000, lot#C90917-25), anti-rabbit 680LT (LI-COR Biosciences, 925-68021, 1:10 000, lot#C90501-05), and anti-rabbit IgG antibodies (abcam, ab6721) were used in this study.

Validation	All antibodies are commercially available. Anti-cycK (Bethyl Laboratories), anti-beta-actin, and anti-CRBN were validated for immunoblotting by their manufacturer. Anti-cycK (Abcam) were validated only for immunocytochemistry and immunofluorescence by their manufacturer.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The human HEK293T cell lines were provided by the Genetic Perturbation Platform, Broad Institute and K562-Cas9 , THP1-Cas9, P31FUJ-Cas9 cell lines were provided by Zuzana Tothova (Broad Institute) and HEK293T-Cas9 and MM1S-Cas9 were previously published (see Methods section). Sf9 (purchased from Thermo Fischer Scientific Cat# 11496-015) and Hi5 cells (Tni cells, purchased from Expression Systems Cat# 94-002F) were also used in this study. MOLT-4 were purchased from ATCC.
Authentication	HEK293T, K562-Cas9, THP1-Cas9, P31FUJ-Cas9, HEK293T-Cas9,MM1S-Cas9, and MOLT-4 cell lines were authenticated by STR profiling. Sf9 and Hi5 cells were authenticated by the vendor.
Mycoplasma contamination	Mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	None of commonly misidentified lines were used in this study.

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	Adherent cells were trypsinized, collected, and the cell pellets resuspended in PBS. Suspension cells were washed with PBS or directly subjected to analysis without fixation.
Instrument	Cytoflex LX (Beckman), MA900 Cell Sorter (Sony), Fortessa FACS (BD Biosciences), FACSCanto (BD Biosciences).
Software	BD FACSDiva 8.0 (BD Biosciences), CytExpert Software (Beckman), FlowJo 10 (BD).
Cell population abundance	Round cells (population with forward and side scatter properties consistent with the alive, non-treated cell line) were usually > 50% in most measurements (rarely lower due to drug toxicity), singlets were > 90%. For reporter assays, mCherry positive cells > 50%.
Gating strategy	Cells were first gated for live cells based on forward and side scatter. Single cells were discriminated based on the area vs. height of the side scatter. Finally, reporter positive cells were gated based on the mCherry expression.

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