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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Nucleic acids samples were visualized using Qiaxcel ScreenGel Software (v1.4 and v1.5). Sequencing data was acquired using an Illumina MiSeq machine.
Data analysis	Nucleic acids samples were analyzed using Qiaxcel ScreenGel Software (v1.4 and v1.5). Data visualization was performed using GraphPad Prism 7.0c. GUIDE-seq data was analyzed with guideseq software v1.1 (https://github.com/aryeelab/guideseq), and amplicon sequencing data was analyzed with CRISPResso (https://github.com/lucapinello/CRISPResso). Custom scripts to analyze PAM depletion data and perform targeting range calculations are available upon request.

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 upon request. 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

Data sets from GUIDE-seq and high-throughput sequencing experiments have been deposited with the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under BioProject ID PRJNA508751 (http://www.ncbi.nlm.nih.gov/bioproject/508751). Otherwise, data sets generated and analyzed as part of this study will be available upon request upon publication.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-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. Final data sets are comprised of at least triplicate independent samples for all conditions for each data set.
Data exclusions	No data were excluded.
Replication	Experimental data are expressed as the mean and s.e.m. of at least three independent replicates, with replicate experiments performed on separate days when possible.
Randomization	No randomization was performed in our study as appropriate control samples were used to establish baseline/reference activities.
Blinding	Blinding was not performed during data analysis. Raw data not already included in the manuscript will be made available upon request.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☒	☐ Unique materials
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human U2OS (from Toni Cathomen, Freiburg), HEK293 cells (Invitrogen), HEK293T cells (ATCC), and primary human T cells (MGH Blood Transfusion Service)
Authentication	Cell line identities were confirmed by STR profiling (ATCC)
Mycoplasma contamination	Media supernatant was analyzed biweekly for the presence of Mycoplasma and all tests were negative for contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ Magnetic resonance imaging