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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 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	iBright FL1500 Imager(ThermoFisher Scientific A44241) Plate Reader M1000Pro (Tecan) Illumina Nextseq500, Novaseq 6000 (https://www.illumina.com/)
Data analysis	ImageJ 1.46 (https://imagej.nih.gov/ij/) R 3.5 & 3.6 (various functions; https://www.r-project.org/) Illustrator 25.2.3 (Adobe)

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 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

All raw data is available upon request. There is no restriction on data availability.

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 calculations were performed and sample sizes were arbitrarily chosen according to convention in the field. For small-scale experiments, the number of replicates exceeds at least 3 biological replicates (= independent experiments) and/or at least 3 technical replicates (= repeated measurements of the same original sample). For screens, the initial mutagenized cell pool was split into 3 replicates post-selection and processed independently in all downstream steps.
Data exclusions	No data were excluded from any experiments and figures shown.
Replication	We present no experimental results that were not reproducible.
Randomization	Screen samples were processed and sequenced in a randomized manner and labelled with numbers instead of sample names.
Blinding	No sample allocation to groups was performed, so blinding was not relevant to 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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	anti-Cas9 (Diagenode C15200229) anti-Myc (Sigma M4439) anti-Beta Tubulin (ProteinTech 10094-1-AP)
Validation	All antibodies are commercially available, were validated by the manufacturers and are routinely used in scientific studies and publications. Furthermore, positive and negative controls built into our experimental design further confirmed specificity of the Cas9 and Myc antibodies.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HAP1 cells were obtained from Horizon (clone C631, sex: male with lost Y chromosome, RRID: CVCL_Y019). RPE1 (CRL-4000) and HEK 293T cells were obtained from ATCC.
Authentication	Cell lines were not authenticated for this study. However, HAP1 and RPE1 cells were previously authenticated in our previous study (Gonatopoulos-Pournatzis et al., Nature Biotechnology, 2020) by STR profiling at the Centre for Applied Genomics (TCAG) at the Hospital for Sick Children (SickKids) in Toronto. HAP1 cells were also whole-genome sequenced.
Mycoplasma contamination	All cell lines were routinely tested and confirmed negative for mycoplasma contamination.

Commonly misidentified lines (See ICLAC register)	None of the cell lines used in this study is listed as commonly misidentified.