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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	No software was used.
Data analysis	FACS - flowjo (v10); Pooled screen - https://doi.org/10.7554/eLife.19760.001; Amplicon sequencing - CRISPResso (v1.010); ChIP-Seq - MACS2 (v2.1.1); DAVID (v6.7)

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 from the CRISPRi screens are publically available through the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) project PRJNA473287.

ChIP-seq data are publically available through the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) project PRJNA473287.

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 ☐ Ecological, evolutionary & environmental 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 calculations were performed. Sample size was determined to be adequate based on the magnitude and consistency of measurable differences between groups.
Data exclusions	Data were only excluded for failed experiments, reasons for which included suboptimal activation and microbial contamination.
Replication	ata were only excluded for failed experiments, reasons for which included suboptimal activation and microbial contamination.
Randomization	No randomization was performed.
Blinding	Experimenters were not blinded. This is unnecessary as data are not subjective.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	FANCD2: Abcam 108928; FANCA: Bethyl A301-980A; HSP90: SCBT 69703; CD90: BioLegend 5E10; HA: SCBT Y-11
Validation	No further validation was performed on commercial antibody stocks.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	ATCC or Berkeley Cell Culture
Authentication	STR Profiling.
Mycoplasma contamination	All lines tested negative and are routinely retested.
Commonly misidentified lines (See ICLAC register)	None of the cell lines in this study are listed in the ICLAC database.

ChIP-seq

Data deposition

☒ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) project PRJNA473287
Files in database submission	There are 94 files.
Genome browser session (e.g. UCSC)	no longer applicable

Methodology

Replicates	Independent experiments
Sequencing depth	>5e6 reads/ replicate.
Antibodies	Abcam 108928
Peak calling parameters	macs2 callpeak -t [Input] -c [Control] --broad --broad-cutoff 0.1 -f BAM -g hs -B -n
Data quality	Peaks identified by MACS2 (p<0.01) were cross-referenced with Guide-seq peaks. Peaks overlapping with centromeric sequences were manually censored.
Software	Reads were trimmed to remove adaptor sequences and aligned to hg19 using BWA. The resulting SAM files were sorted, converted to .BAM format, marked for duplicates, and converted to .tdf format. Data presented in the text was generated by visualizing .tdf files using IGViewer. Statistical identification of peaks was perfomed using MACS2

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	Cells were minimally processed (resuspended in standard media)
Instrument	Attune NxT
Software	FlowJo v10
Cell population abundance	Analytical flow had >5000 cells. Pooled screen had >7,000,000 total cells and is reproduced in Extended Data Figure 2.
Gating strategy	Cells were gated on FSC/SSC to identify live cell populations. All subsequent analysis was performed on the live cell population.

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