

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	Cell viability and luminescence was collected with BioTek Gen5 software. To analyze fluorescent protein expression, cells were analyzed by flow cytometry on a BD LSR Fortessa. For CD81 expression and ABA-inducible repression, cells were analyzed on an Intellicyt iQue3. Clonal lines were expanded from single cells sorted by BD FACS Melody.
Data analysis	Flow cytometry data was analyzed using FlowJo v10.6. MAGECK (0.5.7) was used to map guide RNAs and BAGEL (0.91) was used to determine gene essentiality. RNA-seq reads were aligned to the genome with STAR v2.7.3a and edgeR package 3.30.3. All custom scripts used 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 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

RNA sequencing data generated in this study have been deposited in NCBI SRA under BioProject PRJNA640683. Raw counts for CRISPRi screen are available in associated supplementary files. The authors declare that source data for all figures are available upon request.

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	Sample sizes were not predetermined. We performed three replicates for most experiments to ensure data reproducibility and robustness.
Data exclusions	No data were excluded from analysis.
Replication	All replicates are included in the manuscript. All attempts at replication were successful. Most experiments were performed in three replicates unless otherwise noted in the manuscript.
Randomization	Samples were not randomized as it was not deemed necessary with the experimental setup. Head-to-head comparisons of dCas9 fusions were tested at the same time to minimize variation between sample conditions.
Blinding	Samples were not blinded as it was not deemed necessary with the experimental setup.

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	APC conjugated anti-human CD81 (TAPA-1) antibody (BioLegend, cat# 349510), monoclonal anti-FLAG M2 antibody (Sigma, F1804), monoclonal anti-FLAG M2-HRP antibody (Sigma, A5892), rabbit polyclonal TRIM28 (KAP1) antibody (Abcam, ab10483), mouse monoclonal Cas9 antibody (Diagenode, cat# 15200203)
Validation	Antibodies were purchased based on the information available on the supplier's website and on their suitability for each application (flow cytometry, western blotting). All bands in western blots were observed at expected sizes, and thus the antibodies were not further validated for the study.

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293T cells with stably integrated SV40-EGFP were a gift from Lei Stanley Qi lab (Stanford University). K562 reporter cells containing insertion of hPGK-EGFP.TetO7 cassette into intron 1 of AAVS1 locus were a gift from Angelo Lombardo's lab (IRCCS San Raffaele Scientific Institute). Parental HEK293T, K562 and A375 were purchased from ATCC.
Authentication	Cell lines were not authenticated.
Mycoplasma contamination	All cell lines were routinely tested for mycoplasma contamination. No tests were positive.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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 washed once in 1xPBS, adherent cells were dissociated using dissociated buffer (1 mM EDTA, 150 mM NaCl, 5 mM NaHCO₃, 0.1% glucose and 10 mM KCl) and resuspended in FACS buffer (Ca²⁺/Mg²⁺-free 1xPBS, 5 mM EDTA, 25 mM HEPES pH 7.0 and 1% BSA). For APC-anti-CD81, approximately 5E5 cells were incubated in 1:100 antibody in the dark at 4C for 30 min, washed twice with 1xPBS and resuspended in FACS buffer.

Instrument

To analyze fluorescent protein expression, cells were analyzed by flow cytometry on a BD LSR Fortessa. For CD81 expression and ABA-inducible repression, cells were analyzed on an Intellicyt iQue3. Clonal lines were expanded from single cells sorted by BD FACS Melody.

Software

Flow cytometry data was analyzed using FlowJo v10.6.

Cell population abundance

Post sort clonal reporter lines expressing gRNA tagged with EBFP2 had >95% BFP+ cells at each stage.

Gating strategy

For SV40-EGFP cells, single cells with high EBFP2, surrogate for gRNA were gated. For K562 reporter, in addition to EBFP2 positive cells, dsRed+ cells were gated since a C-terminal P2A-dsRed was added to dCas9.

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