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Corresponding author(s): Drs. Alejandro Chavez, Samira Kiani, and George M. Church

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No methods were used to predetermine sample size. Sample sizes were chosen due to being able to show reproducibility and statistical significance.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the analyses.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Yes, all attempts at replication were successful. Methods and materials used in our experiments were described in the manuscripts to allow reliable replication of our studies.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No randomization was used for samples as samples with particular genetic constituents were needed for the experiments.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not relevant to the studies as samples with particular genetic constituents were needed for the experiments. Labeling of samples was used to prevent mixed up of experimental samples.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	FlowJo (version 7) was used to analyze data generated from flow-cytometry experiments. MAGeCK (0.5.7) was used to analyze single gRNA library screens to determine gene essentiality. All custom scripts are available upon request.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Unique plasmids such as dCas9-KRAB and dCas9-KRAB-MeCP2 expression plasmids are deposited with Addgene (plasmid # 110820-110824) and are described in the Methods section. All other vectors are available upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	HEK293T cells were gift from P. Mali, University of California, San Diego. HAP1 cells were purchased from commercial company Horizon Discovery. SH-SY5Y cells were purchased from commercial company ATCC.
b. Describe the method of cell line authentication used.	The method of cell line authentication was defined by commercial company from which the cell line was purchased. Horizon Discovery validated all HAP1 cell lines by PCR amplification and Sanger Sequencing to confirm the mutation at the genomic level. ATCC uses methods including an assay to detect species specific variants of the cytochrome C oxidase I gene (COI analysis) to rule out inter-species contamination and short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination.
c. Report whether the cell lines were tested for mycoplasma contamination.	Yes, cells were tested every 3 months for mycoplasma contamination and consistently tested negative.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	No animals were used in our studies.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	No human research participants were used in our studies

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☐ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☐ 2. 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).

☐ 3. All plots are contour plots with outliers or pseudocolor plots.

☐ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Cell cultures were treated with trypsin and diluted in complete media or PBS for flow cytometry experiments.
6. Identify the instrument used for data collection.	BD LSRFortessa™ was used for data collection.
7. Describe the software used to collect and analyze the flow cytometry data.	All cytometry data were analyzed by FlowJo (version 7).
8. Describe the abundance of the relevant cell populations within post-sort fractions.	No cell sorting was performed.
9. Describe the gating strategy used.	>80% of viable and intact cells were gated from FSC/SSC for analysis. Within the population, >50% were transfected cells that were selected for downstream analysis by gating cells expressing > 10^3 arbitrary units of EBFP2 (transfection marker).

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