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Reporting Summary

Nature Portfolio 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 Portfolio 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	Flow cytometry: ACEA Biosciences Novocyte 3000, Software: NovaExpress. Flow analysis: FlowJo 10.10.0. Next-generation sequencing was performed by Novogene (Sacramento, CA, USA) using Illumina NovaSeq 6000 and NovaSeq X platforms, PE150 reads. Libraries were prepared following the manufacturer's protocol.
Data analysis	CRISPResso2 (v2.3.2) was used to align the raw reads and perform adapter trimming from the high-throughput sequencing data. Custom python scripts were used to quantify the editing efficiency and generate data plots. Image analysis was performed using Fiji (Fiji Is Just ImageJ, version 2.14.0, based on ImageJ 1.54f; March 2024 build). Adobe Illustrator (Version 29.7.1) was used for assembling and editing figures. Python version (Version 3.9.19) was used to to generate graphs and perform data analysis. Custom Python scripts for automated CRISPResso2 batch processing and CRISPR editing outcome classification are freely available at https://github.com/finkelsteinlab/retron-editing-analysis.

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 Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

High-throughput sequencing data has been deposited in the NCBI database under a BioProject accession number PRJNA1308753.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

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	Sample sizes were determined based on precedent in the genome editing literature and are consistent with similar studies in the field.
Data exclusions	Data were excluded only in cases of experimental failure, such as reagent degradation or sample contamination, as pre-defined prior to analysis.
Replication	Each data point represents an independent biological replicate, as indicated in the figure captions.
Randomization	Mammalian cells and fish embryos used in this study were grown under identical conditions; no randomization was used.
Blinding	Mammalian cells and fish embryos used in this study were maintained under identical conditions; randomization was not performed. Blinding was not performed, as it was not applicable to the study design.

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
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293T (ATCC), U2OS (ATCC)
Authentication	Cell lines were authenticated by the supplier using STR analysis.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	None used.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Zebra fish (Danio rerio).
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex is not determined in zebrafish during the stage of development used for genome editing analysis.
Field-collected samples	This study did not use field collect samples.
Ethics oversight	All experiments were performed according to University of Texas at Austin IACUC standards.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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 trypsinized and re-suspending in fresh culturing media.
Instrument	ACEA Biosciences Novocyte 3000.
Software	Measurement: NovaExpress. Analysis: FlowJo 10.10.0
Cell population abundance	Cells were first gated using FSC/SSC. All subsequent analysis was done on live cells. Cells were analyzed for EGFP and TurboRFP fluorescence.
Gating strategy	Cells were first gated using FSC/SSC. All subsequent analysis was done on live cells. Cell populations were analyzed for EGFP and TurboRFP+. An example of the gating strategy is outlined in Supplementary Figure S10.

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