

## 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

Illumina MiniSeq control softwares (v2.2.1) were used to collect next-generation sequencing data.  
Biorad ProSort software v1.6 (S3e Cell Sorter) was used for cell sorting and collection  
Western blot imaging was done with Image Studio (v 6.1)

#### Data analysis

Illumina Local Run Manager Generate FASTQ analysis module (v2.0) was used to deduplicate and prepare NGS data  
CRISPResso2 (version 2.2.14)–<https://github.com/pinellolab/CRISPResso2> was used to identify percent of edited sequences  
PRISM GraphPad 9 was used for statistical analysis and graph preparation  
FlowJo (v9) was for gating and analysis of flow cytometry data  
Molecular graphics and analyses were performed with UCSF ChimeraX (v1.9)  
RNA-Seq analysis utilized the following pipeline: FastQC (v0.12.1) for quality control check, FastP (v1.0.1) for adapter trimming, STAR (2.7.11b +galaxy0) for splice-aware alignment, samtools (v2.0.1) for deduplication, and REDItools3 (v3.4) for variant calling. Determination of significantly edited sites was done with a custom Python script, available in Supplementary Note 1. Preparation of RNA-seq figures was done using Python (v3.9) and Seaborn (0.13.2) or Logomaker (0.8.7).

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 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

Next-generation sequencing data will be deposited in the NCBI Sequencing Read Archive database under Accession Number PRJNA1247975 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1247975>).

## 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://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 statistical methods were used to predetermine sample size. Sample sizes were determined (n = 3 to 6) based on literature precedence for genome editing experiments (e.g. Gaudelli et al. 2020, <a href="https://www.nature.com/articles/s41587-020-0491-6">https://www.nature.com/articles/s41587-020-0491-6</a> ).                                                                                                                                                                                                                 |
| Data exclusions | Observation of unexpected serial dilution patterns for bacteria plated on control (kanamycin only) plates from same-day/same-sample different multichannel dilution lanes suggested colony count was impacted by instrumental variability. A ROUT 1% outlier test run on survival rate calculated from n = 9 biological replicate +/- chloramphenicol plating pairs was used to account for outliers that resulted from dilution variability. Exclusion criteria were not prestablished. All other assays included all data collected. |
| Replication     | All experiments were performed as three or more independent biological replicates, as specified in each figure caption. In most cases, there were at least three biological replicates with one performed by an independent researcher. All replications were successful.                                                                                                                                                                                                                                                              |
| Randomization   | No randomization was used; human cells were treated identically throughout experiments.                                                                                                                                                                                                                                                                                                                                                                                                                                                |
| Blinding        | No blinding was used; human cells were treated identically throughout experiments.                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

## 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                                     |
|-------------------------------------|-----------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies            |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Animals and other organisms      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                    |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern     |

### Methods

| n/a                                 | Involved in the study                              |
|-------------------------------------|----------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | <p>mouse IgG1, <math>\kappa</math> anti-Cas9 primary antibody (BioLegend, catalog number 698301, clone: 6G12-H11, Lot number: B276385) was used in 1:1000 dilution.</p> <p>rabbit IgG anti-mCherry primary antibody (AbCam, catalog number ab213511, Lot number:1124480-1) was used in 1:2000 dilution</p> <p>rat IgG2a, <math>\kappa</math> anti-<math>\beta</math>-Actin primary antibody (BioLegend, catalog number 664801, Lot number: B329540) was used in 1:5000 dilution</p> <p>Goat-anti-mouse Licor IRDye 800CW secondary antibody (Licor, catalog number 926-32210) was used in 1:10,000 dilution</p> <p>Goat-anti-rabbit Licor IRDye 800CW secondary antibody (Licor, catalog number 926-32211) was used in a 1:10,000 dilution</p> <p>Goat anti-rat Licor IRDye 680RD secondary antibody (Licor, catalog number 926-68076) was used in a 1:10,000 dilution</p> |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## Validation

Cas9 and  $\beta$ -Actin primary antibodies were validated and quality control tested by Western blotting - per manufacturer's website mCherry primary antibody was "validated in IHC on an automated Leica Biosystems BOND RX™ stainer" and "was first used in a scientific publication in 2017 and has been cited over 20 times in peer-reviewed journals" - per manufacturer's website

## Eukaryotic cell lines

### Policy information about cell lines

|                                                                   |                                                                                                                                                                                                |
|-------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                               | HEK293T (CRL-3216) and HeLa (CCL-2) cells from American Type Cell Culture Collection (ATCC)                                                                                                    |
| Authentication                                                    | All cells were authenticated by ATCC prior to purchase. Cells were maintained at low passage (less than 29 sub-passages from frozen stock). No additional authentication was performed in lab. |
| Mycoplasma contamination                                          | Cells were tested both by ATCC and our lab for mycoplasma contamination, and all tests were negative for contamination.                                                                        |
| Commonly misidentified lines (See <a href="#">ICLAC</a> register) | No commonly misidentified cell lines were used in this study.                                                                                                                                  |

## 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        | All flow cytometry and FACS were performed on a BioRad S3e cell sorter equipped with 488 nm, 561 nm and 640 nm lasers. HeLa cells were plated in 6-well plates in penicillin-streptomycin free media at a density of 350,000 cells/well 24 hours prior to transfection, HEK293T cells were plated in 48-well plates in penicillin-streptomycin free media at a density of 35,000 cells/well 24 hours prior to transfection. Cells were harvested and prepared for sorting 48 hours (stability studies) or 72 hours (fluorescence turn-on and HeLa editing activity) post-transfection. Media was removed and cells were gently washed with PBS which was then removed to allow for disassociation with Accumax (ThermoFisher, 00-4666-56) – 50 $\mu$ L in 48-well plate setting, 500 $\mu$ L in 6-well plates. Cells were resuspended to approximately 1 x 10 <sup>6</sup> cells/mL in resuspension buffer (PBS, 2% FBS, 1mM EDTA), before passing through a 0.35 $\mu$ M filter cap round bottomed test tube (Fisher, 352235) and stored on ice until analyzed. A minimum of 50,000 cells passing scatter gating were analyzed for all flow cytometry experiments. For cell sorting, approximately 10,000 mCherry+ cells were sorted for further analysis. |
| Instrument                | Biorad S3e cell sorter                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Software                  | Biorad ProSort Software (v1.6)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| Cell population abundance | In the HeLa editing activity fluorescent enrichment experiments, approximately 3-6% of BE-treated cells were mCherry+ and were collected. Cells were sorted with the "Enrich" sort mode on the Biorad ProSort software. For episomal fluorescent reporter assays, approximately 10-50% of cells were considered double color (EGFP+ and mCherry+), while approximately 50-70% were considered transfected (mCherry+).                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| Gating strategy           | The instruments were calibrated and quality control checked before each FACS experiment. Scatter gating (FSC-A v SSC-A > FSC-A vs FSC-H > SSC-W > SSC-H > FSC-W > FSC-H) was first performed to isolate live, singlet cells (see Supplemental Figure 1E). For dual-color fluorescence experiments, single color controls helped establish color gates. mCherry was detected with FL3 channel (602–627 nm), while EGFP used FL1 (510-540 nm). Single-color and no color controls were established with cell samples transfected with plasmids matching the experimental samples with inactivating mutations in either or both fluorophores: for mCherry, the Y72H deactivating mutation was introduced, and for EGFP the A111V or H182Y mutation was used as appropriate for reporter being tested. For cell sorting, similar scatter gating protocols were used to establish a population of live, singlet HeLa cells. An ABE0.1-P2A-dmCherry (Y72H) transfected sample was used to establish the single color mCherry+ gate.                                                                                                                                                                                                                               |

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