

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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 data was collected with BD CSampler Plus software.
Data analysis	Final analysis of flow cytometry data was performed with FlowJo. Microsoft Excel, Python pandas, and Graphpad Prism were used in data analysis and visualization. Statistical tests were performed in Graphpad Prism. Full details and code for the editing quantification and RNAseq data analysis are provided in Supplementary Discussions 3 and 4 and at https://github.com/rflusi/ALTER-code .

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](#)

All data supporting these findings are available in the Article and the associated materials. Plasmid sequences for key constructs have been uploaded to Benchling and are publicly available (see Supplementary Table 4). High-throughput sequencing data from RNAseq experiments have been deposited in the National Center for

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](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	All flow experiments were carried out in at least three biological replicates in accordance with standard cell biology procedures. RNAseq runs were carried out on three biological replicates. Three biological replicates is standard practice in the field.
Data exclusions	In EGFP(Y66H) editing experiments, fluorescence and percent positive values reflect cells which were positive for an iRFP transfection marker.
Replication	All data were measured in at least 3 biological replicates as indicated in Figure legends and the Methods section.
Randomization	No formal randomization was performed, as is standard in cell model studies. Internal controls were used in quantitative experiments.
Blinding	Experimental requirements precluded blinding. Additionally, measurements of physical and chemical properties are usually unaffected by knowledge of the sample and standardized protocols were employed to reduce the potential for biasing results.

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 obtained from the American Tissue Culture Collection (ATCC)
Authentication	Cell lines were authenticated by the supplier according to their standard protocols.
Mycoplasma contamination	Cells were checked for mycoplasma routinely and immediately upon suspicion. No cultures used in this study tested positive for mycoplasma at any time.
Commonly misidentified lines (See ICLAC register)	n/a

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 cultured in accordance with standard techniques and, where relevant, transfected with JetPrime according to the manufacturer's protocol. Prior to analysis by flow cytometry cell culture media was aspirated away and cells were either suspended in PBS with gentle pressure, or lifted by trypsin treatment, quenched with complete media, pelleted, then resuspended in PBS.
Instrument	BD Accuri C6+
Software	BD CSampler Plus
Cell population abundance	n/a
Gating strategy	Cells were identified by FSC and SSC area, followed by doublet removal based on FSC-H vs FSC-A. Cells transiently transfected with plasmids containing an iRFP transfection marker were gated for transfection, with transfected cells defined as those with fluorescence area >99% of untransfected cells. In cases where the percentage of cells positive for GFP was measured, gating was performed similarly.

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