nature portfolio

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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	The amplicons with different barcodes were subjected to deep sequencing on an Illumina HiSeq X Ten platform (2 × 150 PE) by Annoroad Gene Technology (Beijing, China).
Data analysis	The web portal of OPED is accessible at http://oped.bioinfotech.org/. The source codes of OPED are available at https://github.com/wenjiegroup/OPED. Deep sequencing data were analyzed with CRISPResso2 version 2.2.9 with prime editing mode (https://github.com/pinellolab/CRISPResso2) and GATK4 version 4.2.6.1 (https://github.com/broadinstitute/gatk/).

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

The deep sequencing data from this study have been submitted to the National Center for Biotechnology Information Sequence Read Archive database under accession number PRJNA882795.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	This study does not involve animal or human participants.
Population characteristics	This study does not involve animal or human participants.
Recruitment	This study does not involve animal or human participants.
Ethics oversight	This study does not involve animal or human participants.

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 previous reports on prime editing experiments and sufficient for the following test. Citation: Anzalone, A. V. et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 576, 149-157 (2019) Kim, H. K. et al. Predicting the efficiency of prime editing guide RNAs in human cells. Nature Biotechnology 39, 198-206, doi:10.1038/s41587-020-0677-y (2021). Hsu, J. Y. et al. PrimeDesign software for rapid and simplified design of prime editing guide RNAs. Nature Communications 12, 1034, doi:10.1038/s41467-021-21337-7 (2021).
Data exclusions	No data were excluced.
Replication	Independent biological replicates (n = 3) were performed in human HEK293T cells. All attempts at replication were performed successfully.
Randomization	For the development of OPED, we selected the datasets published by Kim et al. which were generated by stratified random sampling. Human cells in this study were grown under identical conditions, and no randomization was used.
Blinding	We were not blinded to group allocation.

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

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)	Source: ATCC; cell line: HEK293T (ATCC CRL-3216).
Authentication	STR profiling by ATCC.
Mycoplasma contamination	Not tested.
Commonly misidentified lines (See ICLAC register)	HEK293T is not listed in the ICLAC register.