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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	GraphPad Prism 10 for macOS (v10.3.1)
Data analysis	GraphPad Prism 10 for macOS (v10.3.1), SnapGene (v8.0b2), CRISPResso2 (v2.3.3), EditR (v1.0.10), CRISPOR (v5.2), Illumina DRAGEN Bio-IT Platform (v07.031.779.4.4.7), bcftools (v1.21), bam-readcount (v1.0.1), Dorado (v0.7.4), minimap2 (v2.28-r1209), samtools (v1.21), ApE (v3.1.7), Numbers (v14.4), Microsoft Excel for Mac (v16.109.3), gSUITE (v2.3.0), cellSens (v1.9), ZEN 2 (blue edition). Custom scripts were used to calculate base conversion frequencies (https://github.com/BaeLab/ABE-conversion-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

DATA AVAILABILITY
SNP array data are available at Gene Expression Omnibus (GEO) under accession GSE290961 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE290961).
WGA and deep sequencing data are available at the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) under accession PRJNA1480961 (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1480961).
RNA-seq data are available at the NCBI SRA under accession PRJNA1454036 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1454036).
Data used for the preparation of graphs in Figures 1–6 and Extended Data Figures 1–10 are provided in Supplementary Tables 1–10. Uncropped images are in Supplementary Figure 1. All other relevant data are available from the corresponding author upon request. Data sharing was approved by the Institutional Review Board.
Published HUES64 hESCs WGS data (NCBI, accession SRX347298) were used (https://www.ncbi.nlm.nih.gov/sra/SRX347298).
The UCSC Genome Browser (http://genome.ucsc.edu) was used to visualize and compare genomic data.
The genome assembly GRCh37 was used for human SNP arrays and chromosome breakage analysis (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/001/405/GCF_000001405.13_GRCh37/).
The genome assembly GRCh38 was used for off-target analyses and RNA-seq reads alignment (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/001/405/GCF_000001405.40_GRCh38.p14/).
The genome assembly GRCm38 was used for mouse genomic coordinates (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/001/635/GCF_000001635.20_GRCm38/).
The schematic in Extended Data Fig. 2g contains two vector images from NIAID Visual & Medical Arts (10/7/2024): Lab Mouse, NIAID NIH BIOART source (bioart.niaid.nih.gov/bioart/279), and Syringe, NIAID NIH BIOART source (bioart.niaid.nih.gov/bioart/505).

CODE AVAILABILITY
The custom code (available at https://github.com/BaeLab/ABE-conversion-analysis) is central to our analysis. It calculates A→G conversion rates from targeted deep-sequencing data using Digenome-seq peak positions rather than spacer positions, which was specifically needed for our study.
Genomic Prediction, Inc. provides preimplantation embryo sample analysis service using gSUITE software (v2.3.0).

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	The sex of analyzed human embryos was determined only after WGA and karyotyping. Base editing efficiency depends on multiple factors, including the number of targets, chromatin accessibility, and kinetics, while DNA repair depends on cell type, cell cycle, and developmental stage. The findings reported in this study apply to both sexes.
Reporting on race, ethnicity, or other socially relevant groupings	All sample donors were anonymous, and the population characteristics of the human research participants (e.g., age, gender, genotypic information, past and current diagnoses) were therefore unknown to the research team.
Population characteristics	All sample donors were anonymous, and all embryos were de-identified prior to use in research.
Recruitment	No specific recruitment was conducted for the study. All sample donors were anonymous, and donation to research was chosen in lieu of destruction.
Ethics oversight	The work with human gametes, embryos, and embryonic stem cells was reviewed and approved by the Columbia University Human Embryonic and Human Pluripotent Stem Cell Research Committee and the Institutional Review Board.

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 the experience and work of this and other groups in the field, which led to reproducible results when performing similar experiments (e.g., references 1, 2, and 12). Ethical considerations apply to work with human embryos. We analyzed more than 100 single-cell samples collected from base-edited human embryos so rare events would be no greater than 1% and all frequent and common DNA repair events in the embryos would be captured.
Data exclusions	One ABE experiment targeting HBG and involving six embryos showed a complete failure of editing. We suspect a problem during RNP assembly.
Replication	Reproducibility was ensured by performing experiments in replicates. Each embryo is a biological replicate of the other. Genotyping analyses and mouse embryo experiments were reproduced by different team members, and all attempts at replication were successful.
Randomization	Human embryo cell samples were barcoded at the time of collection. Samples were not randomized, as randomization of samples is not relevant to this study.
Blinding	Although individual embryo samples were barcoded at the time of collection, data analyses were performed following automated processes. Team members performing repeated genotyping experiments were blinded to the original identity of used samples.

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

Antibodies

Antibodies used	For immunostaining, the following primary antibodies were used: mouse monoclonal anti-OCT4 (Santa Cruz Biotechnology; Clone C-10: Cat. no. sc-5279; Lot: A1821) and rabbit monoclonal anti-SOX2 (Cell Signaling Technology; Cat. no. 23064; Lot: 4). Secondary antibodies Alexa Fluor 488 donkey anti-mouse (Thermo Fisher Scientific; Cat. no. A-21202; Lot: 2266877) and Alexa Fluor 555 donkey anti-rabbit (Thermo Fisher Scientific; Cat. no. A-31572; Lot: 3282894) were used. For Western blot, primary antibodies for PCSK9 (Invitrogen; Clone 2F1: Cat. no. MA5-32843; Lot: ZD4295831A) and GAPDH (Proteintech; Cat. no. 10494-1-AP) were used. HRP-conjugated secondary antibodies (Cytiva; Cat. no. NA934 and NXA931) were used.
Validation	All antibodies were validated by the suppliers with indicated suitability for the application used in the study. The antibodies were also validated in previous studies, and selected references are provided below. OCT4 (Cat. no. sc-5279): Web: https://www.scbt.com/p/oct-3-4-antibody-c-10?srsltid=AfmBOopc4jWtE07DsAX7B0GAhNMrQAnsrcbxT2by86BJjpGgjY4ouiQ7 References: 1) PMID: 10742100, 2) PMID: 1408763. SOX2 (Cat. no. 23064): Web: https://www.cellsignal.com/products/primary-antibodies/sox2-d9b8n-rabbit-mab/23064?srsltid=AfmBOopkoCDfj4v3XpATx88nkis0Gm71KDhnqq9fdBxwWVV7gKpSRnm_ References: 1) PMID: 15010323, 2) PMID: 11463946. In our study, we detected OCT4 and SOX2 in nine hESC lines (ESbe1-9) derived from day six blastocysts. PCSK9 (Cat. no. MA5-32843): Web: https://www.thermofisher.com/antibody/product/PCSK9-Antibody-clone-2F1-Monoclonal/MA5-32843 References: 1) PMID: 36708718, 2) PMID: 38191764, 3) PMID: 34352405. All three studies validated the MA5-32843 antibody, performing Western blots and using mouse or human cells. GAPDH (Cat. no. 10494-1-AP): Web: https://www.ptglab.com/products/GAPDH-Antibody-10494-1-AP.htm?srsltid=AfmBOorGEhGbCxw8acd8r-X4wXWFHGovhk3-8CaazBmythI5XyETaaSV References: 1. PMID: 39013469, 2) PMID: 38297130.

In our study, we detected PCSK9 protein in the HUES64 hESC line, in which PCSK9 genes have never been targeted or base-edited. Thus, the expression of a functional protein is expected from intact PCSK9 alleles in this cell line. In contrast, we have not detected PCSK9 protein in six hESC lines derived from the ICM of ABE-edited embryos at blastocyst stage on day six of development. These hESC lines show homozygous A→G editing at the splice donor site between exon 1 and intron 1 of the PCSK9 gene. The lack of detectable protein in these edited lines is in accordance with a previous study reporting that ABE editing at this position results in read-through and premature termination of translation (PMID: 34012082). GAPDH was detected in all cell lines.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Nine base-edited hESC lines (ESbe1-9) were derived in house from ABE-injected embryos during the study. The HUES64 hESC line was previously derived at Harvard University by the lead author, Dieter Egli. HEK293T cells were obtained from Peter Quinn's laboratory at Columbia University.
Authentication	Authentication of hESC lines was performed using SNP array and PCR-based genotyping. HEK293T cells were not authenticated.
Mycoplasma contamination	All cell lines were tested for mycoplasma contamination and confirmed mycoplasma-free.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

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	B6D2F1/J female mice were obtained from The Jackson Laboratory (Strain #100006) and housed at the Russ Berrie Medical Science Pavilion mouse facility, managed by the Columbia University Institute for Comparative Medicine. Mice were maintained on a 12-hour light–12-hour dark cycle at a constant temperature of 21–24°C and humidity range of 45–65%. They were fed a standard mouse diet (ad libitum water and food access). The mice were used for experiments at the age of 5-7 weeks.
Wild animals	No wild animals were used in the study.
Reporting on sex	Oocytes from superovulated female mice were used for experimental work.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	The Columbia University Institutional Animal Care and Use Committee and the Institutional Review Board approved animal protocols required for this work.

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