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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Prepared DNA libraries were sequenced on an Illumina MiSeq instrument. MiSeq-generated images were converted into nucleotide sequences and base quality scores in the bcl format using Illumina RTA software (v1.18.54).
Data analysis	Data were analyzed using custom-built pipelines. Bcl file data were demultiplexed (Picard tools v2.9; https://github.com/broadinstitute/picard); forward and reverse reads were merged into extended amplicons (flash v1.2.11); reads were aligned against the GRCh38 genomic reference (bwa v0.7.15), and were assigned to targets in the multiplex primer pool (bedtools v2.25). Reads with any base quality score <10 were filtered out. Editing was calculated as the percentage of total reads containing an INDEL or SNP within a 10bp window of the predicted cut site.

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/reviewers upon request. 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

The authors declare that the data supporting the findings of this study are available within the paper. The sequencing data is available at NCBI under SRA accession: SRP150376.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size tests were performed before initiating experiments. Instead, experiments were performed in at least triplicate to determine reproducibility and statistical variance.
Data exclusions	There were no datasets excluded from analysis in this work.
Replication	Experiments were performed in triplicate (or greater) to establish reproducibility. Altogether, the level of reproducibility in these studies were very high.
Randomization	There were no live animals or human participants in this study for randomization. For in vitro experiments, the manuscript data were generated by two entirely different research groups with vastly different procedures and methods arriving to the same overall conclusion. This gives us a high level of confidence in our interpretations.
Blinding	There were no live animals or human participants in this study for blinding. For in vitro studies, researchers were not blinded except for Figures where Cas9 mutants were tested in HSPCs.

Materials & experimental systems

Policy information about availability of materials

n/a	Involved in the study
☐	☒ Unique materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Research animals
☒	☐ Human research participants

Unique materials

Obtaining unique materials	The eSpCas9(1.1), SpCas9-HF1, HypaCas9, and EvoCas9 proteins are unique items that were first described in previous publications that were appropriately cited in this manuscript. The HiFi Cas9 is available for purchase from Integrated DNA Technologies, a commercial entity.

Antibodies

Antibodies used	The following antibodies were validated and then used in these studies: Anti-CD3 (clone OKT3, Tonbo Biosciences), soluble anti-CD28 (clone CD28.2, Tonbo Biosciences), Anti-CD3-PE (clone UCHT1, BioLegend, San Diego, CA, USA), Anti-CD271 (NGFR)-APC (clone ME20.4, BioLegend), hCD45 V450 (HI30, BD Biosciences), CD34 FITC (8G12, BD Biosciences), CD71 PE-Cy7 (OKT9, Affymetrix), CD235a PE (GPA) (GA-R2, BD Biosciences), CD49d BV421 (9F10, BD Biosciences), and Band3 APC (a kind gift from Dr. Anumpama Narla and Dr. Mohan

Narla). The Anti-beta-actin (Cat# ab8226) and Anti-SpCas9 (Cat# ab191468) were both obtained from Abcam. For Cas9, reactive bands in a Western Blot were confirmed to be of the correct molecular weight, and were dependent on the presence of the corresponding gene.

Validation

The antibodies used herein for flow cytometry were validated by using positive (antigen positive cells) and negative (isotype) controls with the recommended antibody concentrations from the manufacturers.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	The HEK293 cell line was obtained from ATCC.
Authentication	Cell line was authenticated by ATCC. They use morphology, karyotyping and PCR.
Mycoplasma contamination	Batch of HEK293 cells was certified Mycoplasma-free by ATCC.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Method-specific reporting

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ Magnetic resonance imaging

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 spun down, washed, and analyzed for GFP, or were stained with CD19 or NGFR (CD271).
Instrument	Cells were analyzed for viability and GFP expression using either the Accuri C6 flow cytometer (BD Biosciences) or a FACS Aria II SORP (BD Biosciences)
Software	FlowJo software was used.
Cell population abundance	N/A
Gating strategy	Cells were gated base on FSC/SSC, doublet discrimination, live/dead, and then by GFP, CD19, or NGFR.

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