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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	Standard manufacturer's software on the relevant scientific instruments was used to collect all data.
Data analysis	Custom software was used for analysis of all Illumina sequencing data. Sequence analysis also utilized fastq_quality_filter from the FASTX toolkit, Illumina bcl2fastq software, SeqPrep, Bowtie2, and Biopython pairwise2 global aligner. Flow cytometry data was analyzed with FlowJo (v10.4, FlowJo LLC). OriginPro (v 7.5 OriginLab) was used for bar plots, scatter plots, non-linear curve fits, and standard statistical tests. Microsoft excel was used for 3D bar plots, pie charts, calculating simple statistics, and generating tables. PyMol v2.2.0 (Shrodinger LLC) was used to generate the structural plot in Figure 1d.

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

Illumina sequencing data for all key experiments has been deposited to the SRA under accession PRJNA540312. Custom computer scripts used to perform the standard indel analysis and increased sensitivity indel analysis can be found in Supplementary Methods. Custom computer scripts used to automate more standard portions of the data analysis pipeline are available upon request.

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 ☐ Ecological, evolutionary & environmental 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 power calculations were performed to determine sample sizes. When practical, samples were processed in triplicate or quadruplicate and in most cases this was sufficient to observe clear differences between samples. For the increased sensitivity indel assay, the sample size was increased until the desired level of sensitivity was achieved.
Data exclusions	A small number of individual replicates were excluded from analysis due to failed PCR reactions. Some samples were also excluded and listed as "ND" for failing one or more quality metrics described in Supplementary Methods. One sample from the experiment shown in Figure 1c was completely excluded due to a failed mRNA prep. One data point from one replicate was also excluded from Supplementary Figure 16 due to some type of expeirmental failure- this is mentioned in the figure legend and shown in the gel image.
Replication	All of the experiments in Table 1 and figures 1, 2, and 3 of the main text are better controlled and/or better executed versions of preliminary experiments. In all cases the results from the unreported preliminary experiments and the reported final experiments are consistent. The experiment shown in figure 4 is the result of pooling two separate T-cell transfections. Similar behavior was observed with two different ZFN pairs, knockout values obtained with sequencing and flow cytometry agreed remarkably well, and results were consistent with earlier experiments using the same ZFN pairs performed in a different cell type.
Randomization	Randomization was not necessary for any of these experiments because all experiments involved aliquots of cells from a homogeneous cell culture.
Blinding	Samples were not blinded because there was no subjective component in the data processing or sample handling

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Mobilized peripheral blood (for purification of CD34+ stem cells) and peripheral blood CD3+ pan T-cells were obtained from AllCells. Catalog number of CD3+ pan T-cells provided in materials and methods.

Antibodies

Antibodies used	APC Mouse Anti-Human CD3 antibody was obtained from BD biosciences. Catalog number provided in materials and methods.
Validation	Validated by manufacturer

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	K562 cells obtained from ATCC
Authentication	Cell lines obtained from ATCC were not internally authenticated
Mycoplasma contamination	Cells were not tested for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	K562 cells are not commonly misidentified

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	Please refer to materials and methods
Instrument	Attune NXT Acoustic Focussing Cytometer, Model: AFC2 (Thermo Fisher)
Software	FlowJo (v10.4, FlowJo LLC)
Cell population abundance	We analyzed 100,000 cells per sample
Gating strategy	Gating strategy is shown in Supplementary Methods

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