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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 bioinformatic codes for the design of predicted potent crRNA is available at https://github.com/david-ma/cas13. crRNA design web-server described in this study is available at: https://cas13b.github.io/.

Data analysis	The design of in silico prediction code is based on the experimental data presented in this article. Briefly, the algorithm generates single-nucleotide tiled spacer sequences for any input DNA or RNA sequence using HTML5, CSS and JavaScript libraries. The program then removes all spacer sequences that possess more than three consecutive T bases (>3T) that are predicted to act as a transcription termination signal and yield premature crRNAs. The algorithm scores the remaining spacer sequences based on their nucleotide composition and position. Spacers with a G nucleotide at the first or second positions receive a maximum score of +20 each. In contrast, a C nucleotide at spacer position 1, 2, 3, or 4 receive a penalty score of -20 each. Additionally, C bases at positions 11, 12, 15, 16 and 17 receive a -5 score each. All other nucleotides or spacer positions that did not show any distribution bias in the potent and ineffective crRNA receive a score of 0. The algorithm then calculates the cumulative score for each spacer and ranks them accordingly. As a result, the top spacers with high scores are enriched with G bases at 1st and 2nd positions, and depleted from C bases at positions 1, 2, 3, 4, 11, 12, 15, 16, and 17, and are predicted to yield potent silencing. Conversely, the lowest scoring spacers at the bottom of the list are enriched with C bases at positions 1, 2, 3, 4, 11, 12, 15, 16, and 17 and are predicted to yield ineffective silencing. The prediction accuracy of the algorithm is supported by in silico analysis and functional validation data in Figure 1, 2, and Extended Data Figure 2, 3, 4, & 7. This PspCas13b crRNA design tool is open source and available to the wider scientific community at https://cas13b.github.io/. The predicted RNA secondary structures and minimum free energy were generated using the RNAfold program (ViennaRNA webservices; Lorenz, R. et al. ViennaRNA Package 2.0. Algorithms Mol. Biol. (2011) doi:10.1186/1748-7188-6-26.).

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 the raw data supporting the findings are available in the source Data file submitted with this manuscript.

Human research participants

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

Reporting on sex and gender	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

Life sciences study design

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

Sample size	The sample sizes were determined to match the standards in comparable studies available in the literature (Chunlong Xu et at, Nat Methods, 2021).
Data exclusions	Experiments and protocols were optimized in pilot assays before generating high-quality publication data. No data was excluded from the analysis.
Replication	All experiments were repeated at least 3 times as biological replicates with the following exceptions: - As mentioned in the figure legend, Data in Fig 1d (screening 61 single-nucleotide resolution crRNAs targeting mCherry mRNA) was performed in two biological replicates due to the large size of crRNAs screened. - As indicated in the figure legend, western blot analysis in Figure 4i is presented in two biological replicates (N=2). After the initial optimization of the experimental conditions, all experiments were reproducible in independent experiments. RNA targeting with CRISPR-Cas13 is well-established and similar experiments have been reported by independent researchers.

Randomization	No randomization was used in this study. Due to the small sample, randomization was not relevant for this study. Covariates were controlled for by running controls in parallel whenever is applicable. Appropriate controls (e.g. non targeting crRNAs, loading controls in WB, and crRNA dose-dependent silencing) were used throughout the study.
Blinding	No blinding was used in this study. Blinding is not relevant to this study as RNA targeting with Cas13 is well-established in the field by independent groups using assays that do not require blinding (Chunlong Xu et at, Nat Methods, 2021). Most our experiments were performed, analysed, and confirmed by independent co-authors in our labs.

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

Antibodies

Antibodies used	β actin monoclonal antibody (source: mouse application: 1:2000) Sigma-Aldrich A2228 α-Tubulin Antibody (source: rabbit, application: 1:2000) Sigma-Aldrich SAB4500087 Phospho-Stat5 (Tyr694) (D47E7) (source: rabbit, application 1:2000) Cell signalling technology 4322 Stat5 (D2O6Y) (source: rabbit, application 1:2000) Cell signalling technology 94205 P44/42 MAPK (Erk1/2) (source: rabbit, application 1:1000) Cell signalling technology 9102 p44/42 MAPK (Erk1/2) P Thr202/Tyr204 (source: rabbit, application 1:1000) Cell signalling technology 9101 (Horseradish peroxidase) HRP conjugated goat anti-mouse IgG secondary Antibody (application: 1:10,000) Abcam ab97023 HRP conjugated goat anti-rabbit IgG secondary Antibody (application: 1:2000) Abcam ab205718 IRDye® 680RD Goat anti-Rabbit IgG Secondary Antibody (0.1mg) (application: 1:10,000) Li-cor 92668071 IRDye® 800CW Donkey anti-Mouse IgG Secondary Antibody (0.1mg) (application: 1:10,000) Li-cor 92632212
Validation	We used commercial antibodies validated by the suppliers. We confirmed the validation as we used untrafected cells and other appropriate controls to validate the specificity of various antibodies we used in this study.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK 293T (ATCC CRL-3216);
Authentication	Cell lines were authenticated by the supplier ATCC. We did not perform any additional authentication upon reception. We made a bulk stocks for each cell line after recovering from the original frozen vials. We discard the cells after ~20 passages, and thaw new cells from the liquid nitrogen stocks. Cell morphology was monitored at each passage by microscope.
Mycoplasma contamination	Cells were routinely tested (QPCR based test and microscopy) and were mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this manuscript.