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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics

For	all st	atistical 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.				
X		A description of all covariates tested				
x		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.				
X		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

Data collection	Illumina HiSeq were carried out by HaploX Genomics Center and sequenced on Illumina HiSeq.
D	Sequences displayed in this study was Sanger sequenced by Sangon.
Data analysis	SnapGene (V4.3.6)
	GraphPad Prism (v.9.0.0),
	CRISPResso2 (https://github.com/pinellolab/CRISPResso2)
	COBALT (https://blast.ncbi.nlm.nih.gov/Blast.cgi)

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.

Policy information about availability of data

All manuscripts must include a <u>data availability statement</u>. 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 data used in this study can be found in the manuscript and/or in the supplementary information. All Illumina sequencing data has been deposited to NCBI under accession code PRJNA996576.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation),</u> and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	NA
Reporting on race, ethnicity, or other socially relevant groupings	ΝΑ
Population characteristics	NA
Recruitment	NA
Ethics oversight	NA

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 science
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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	All clones on the plate, about 100-1000 colonies were randomly picked, and the genome was extracted and send for Illumina sequencing.
Data exclusions	No data was excluded.
Replication	Three or more biological and technical replicates were evaluated with positive for related experiments.
Randomization	Clones for sanger sequencing were randomly picked.
Blinding	Blinding was not performed in this study, because the data collection or analysis are not susceptible to biased interpretation.

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.

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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