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>.

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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			
	extstyle ext			
	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> 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 <u>statistics for biologists</u> contains articles on many of the points above.			
Soft	ware and code			

Policy information about <u>availability of computer code</u>

Data collection EPU, Illumina MiSeq

Data analysis cryoSPARC v3.3.1, Nautilus, Buccaneer, DeepEMhancer, COOT, PHENIX, UCSF ChimeraX, CueMol2, CRISPResso2, GraphPad Prism

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 $\underline{\text{policy}}$

The structural model has been deposited in the Protein Data Bank under the accession code 7XHT. The EM density map has been deposited in the Electron Microscopy Data Bank under the accession code EMD-33198.

Human research participants						
Policy information a	about <u>studies i</u>	nvolving 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 informa	ation on the appr	oval of the study protocol must also be provided in the manuscript.				
Field-spe	ecific re	porting				
<u>.</u>		s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.				
X Life sciences	В	ehavioural & social sciences				
For a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>				
Lite scier	ices stu	udy design				
All studies must dis	close on these	points even when the disclosure is negative.				
Sample size	No statistical m	No statistical methods were used to predetermine the sample size.				
Data exclusions	No data exclusi	on was performed.				
The state of the s		periments were repeated at least three times. Mammalian genome editing assays were repeated four times, and data are \pm s.e.m. (n = 4).				
Randomization No random		ation was performed.				
Blinding	No blinding was	s performed.				
Reportin	g for sp	pecific materials, systems and methods				
'		about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.				
Materials & exp	perimental s	ystems Methods				
n/a Involved in th		n/a Involved in the study				
	Antibodies ChIP-seq					
	Eukaryotic cell lines					
	— —					
Clinical dat						
=1=	esearch of concer	n				
Eukaryotic cell lines						
	olicy information about <u>cell lines and Sex and Gender in Research</u>					
	Cell line source(s) HEK293FT line (American Type Culture Collection (ATCC))					
Authentication The used cell lines were authenticated.						
Mycoplasma contamination The cell lines were not tested for mycoplasma contaminations.						

Commonly misidentified lines (See <u>ICLAC</u> register)

No misidentified cell lines were used in this study.

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