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

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	Cor	firmed					
	\boxtimes	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement					
	\square	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly					
\boxtimes		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.					
\boxtimes		A description of all covariates tested					
\boxtimes		A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons					
	\boxtimes	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)					
\boxtimes		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.					
\ge		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings					
\boxtimes		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
\boxtimes		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.							

Software and code

Policy information about availability of computer code								
Data collection	No custom code was used in the collection of data for this work.							
Data analysis	Specific analyses were performed with code provided at the Github repository and the R statistical software.							

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 Research guidelines for submitting code & software for further information.

Data

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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data and codes are publicly available or available upon request.

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must dis	sclose on these points even when the disclosure is negative.
Sample size	Sample sizes are detailed in Supplementary Table 2. The number of samples were chosen to ensure each hgRNA is observed in at least 2 mice in at least 2 embryonic stages.
Data exclusions	Low-frequency mutant spacer alleles (below 0.5% for timecourse analyses and below 0.25% for brain analysis in Figure 5) were removed because at lower frequencies PCR and sequencing error as well as index hopping errors cannot be distinguished from true mutant alleles.
Replication	For timecourse of hgRNA activation, each hgRNA was replicated in at least two different mice. For brain analysis, duplicate samples were taken from each brain region and each sample was amplified in three technical replicates.
Randomization	Samples were grouped based on date of collection. All samples collected on the same day were processed together.
Blinding	Investigators were not blinded, however investigator does not know the hgRNA composition of each sample at the time of collection.

Reporting for specific materials, systems and methods

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	n/a	Involved in the study
\boxtimes	Antibodies	\boxtimes	ChIP-seq
\boxtimes	Eukaryotic cell lines	\boxtimes	Flow cytometry
\boxtimes	Palaeontology and archaeology	\boxtimes	MRI-based neuroimaging
	Animals and other organisms		
\boxtimes	Human research participants		
\boxtimes	Clinical data		
\boxtimes	Dual use research of concern		

Animals and other organisms

 Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

 Laboratory animals
 Mus musculus; a variety of age and genders on a B6 background

 Wild animals
 No wild animals were used in this study.

 Field-collected samples
 No field-collected samples were used in this study.

 Ethics oversight
 All animal protocols were approved and overseen by the Harvard Medical Area Standing Committee on Animals.

Note that full information on the approval of the study protocol must also be provided in the manuscript.