

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](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

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	No data were collected for this manuscript.
Data analysis	The CAST guide RNA design tool and associated documentation are available online via GitHub (https://github.com/sternberglab/CAST-guide-

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](#)

NGS data used for Fig. 6 and Fig. 7 are available in the NCBI Sequence Read Archive (BioProject accession code PRJNA668381). Published genomes used for Tn-seq analyses were obtained from the NCBI (accessions code CP001509.3).

Human research participants

Policy information about [studies involving human research participants](#) and [Sex and Gender in Research](#).

Reporting on sex and gender	NA
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 sciences

Life sciences study design

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

Sample size	NA
Data exclusions	NA
Replication	NA
Randomization	NA
Blinding	NA

Behavioural & social sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

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

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing and spatial scale	
Data exclusions	
Reproducibility	
Randomization	

Blinding

Did the study involve field work? Yes No

Field work, collection and transport

Field conditions

Location

Access & import/export

Disturbance

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

Validation

Eukaryotic cell lines

Policy information about [cell lines](#) and [Sex and Gender in Research](#)

Cell line source(s)

Authentication

Mycoplasma contamination

Commonly misidentified lines
(See [ICLAC](#) register)

Palaeontology and Archaeology

Specimen provenance

Specimen deposition

Dating methods

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight

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

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	
Wild animals	
Reporting on sex	
Field-collected samples	
Ethics oversight	

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about [dual use research of concern](#)

Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

No	Yes
<input type="radio"/>	<input type="radio"/> Public health
<input type="radio"/>	<input type="radio"/> National security
<input type="radio"/>	<input type="radio"/> Crops and/or livestock
<input type="radio"/>	<input type="radio"/> Ecosystems
<input type="radio"/>	<input type="radio"/> Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
<input type="radio"/>	<input type="radio"/> Demonstrate how to render a vaccine ineffective
<input type="radio"/>	<input type="radio"/> Confer resistance to therapeutically useful antibiotics or antiviral agents
<input type="radio"/>	<input type="radio"/> Enhance the virulence of a pathogen or render a nonpathogen virulent
<input type="radio"/>	<input type="radio"/> Increase transmissibility of a pathogen
<input type="radio"/>	<input type="radio"/> Alter the host range of a pathogen
<input type="radio"/>	<input type="radio"/> Enable evasion of diagnostic/detection modalities
<input type="radio"/>	<input type="radio"/> Enable the weaponization of a biological agent or toxin
<input type="radio"/>	<input type="radio"/> Any other potentially harmful combination of experiments and agents

ChIP-seq

Data deposition

Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links <i>May remain private before publication</i>	
Files in database submission	
Genome browser session (e.g. UCSC)	

Methodology

Replicates	<input type="text"/>
Sequencing depth	<input type="text"/>
Antibodies	<input type="text"/>
Peak calling parameters	<input type="text"/>
Data quality	<input type="text"/>
Software	<input type="text"/>

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	<input type="text"/>
Instrument	<input type="text"/>
Software	<input type="text"/>
Cell population abundance	<input type="text"/>
Gating strategy	<input type="text"/>

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

Magnetic resonance imaging

Experimental design

Design type	<input type="text"/>
Design specifications	<input type="text"/>
Behavioral performance measures	<input type="text"/>

Acquisition

Imaging type(s)	<input type="text"/>
Field strength	<input type="text"/>
Sequence & imaging parameters	<input type="text"/>
Area of acquisition	<input type="text"/>
Diffusion MRI	<input type="radio"/> Used <input type="radio"/> Not used

Preprocessing

Preprocessing software	<input type="text"/>
Normalization	<input type="text"/>
Normalization template	<input type="text"/>
Noise and artifact removal	<input type="text"/>
Volume censoring	<input type="text"/>

Statistical modeling & inference

Model type and settings	<input type="text"/>
Effect(s) tested	<input type="text"/>
Specify type of analysis:	<input type="radio"/> Whole brain <input type="radio"/> ROI-based <input type="radio"/> Both
Statistic type for inference (See Eklund et al. 2016)	<input type="text"/>
Correction	<input type="text"/>

Models & analysis

n/a

Involvement in the study

- Functional and/or effective connectivity
- Graph analysis
- Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis



