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Reporting Summary

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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	none
Data analysis	Code used in sequence analysis is described in the methods, where links to the Github repository are provided (http://github.com/TiongSun/iSeq). These links are publicly available. This code was made available to reviewers and editors during review. Publicly available software bowtie2 2.3.2, samtools 1.3.1, iSeq (http://github.com/TiongSun/iSeq), Integrative Genomics Viewer 2.4, and ueye cockpit software 4.91.1 were used for data analysis.

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/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 [data availability statement](#). 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

The sequences and design details used in this study are available as accession numbers (all deposited online at GenBank) as well as Supplementary Data: Helper plasmid (contains lambda-red recombination components, and Cas9 with tracrRNA, accession number MN927219, available from the authors); Spacer plasmid (contains spacer arrays, template sequence in accession number MK809152.1, available from the authors); Plasmid pJF146 (optional for experiments involving conjugation, accession number MK809154.1, available from the authors); Template for BAC vector (contains BAC replication and segregation components, an origin of transfer oriT and a URA3 selectable marker, accession number MK809150.1, available from authors); pSC101_YAC-ori template plasmids bearing sacB negative marker, accession number MN927220, available from authors); pSC101_YAC-ori template plasmids bearing rpsL negative marker and rpsL-KanR double selection cassette, accession number MN927221, available from authors); all primers used in this study, available in Supplementary Data 1.

Field-specific reporting

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☒ Life sciences   ☐ Behavioural & social sciences   ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

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

Sample size	No sample-size calculation was performed. Because the variation in the assays used is small and we are interested in large effects, the sample sizes used, as indicated in the manuscript, were deemed appropriate.
Data exclu...	
Replicatio...	The exact number of replicates is stated in the relevant legend. All attempts at replication were successful.
Randomiz...	
Blinding	

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

- Antibodies
- Eukaryotic cell lines
- Palaeontology
- Animals and other organisms
- Human research participants
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Methods

- ChIP-seq
- Flow cytometry
- MRI-based neuroimaging