

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 [Editorial Policies](#) and the [Editorial Policy Checklist](#).

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
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ | 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
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ | 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 micro-manager (Version 1.4.23, Build 20190321) and Zeiss Zen 2012 SP5 FP2 (Version 14.0.0.0) were used to capture microscopy data.

Data analysis Custom MATLAB codes are provided at Mendeley Data (<http://dx.doi.org/10.17632/jnx89bmv4s.1>) as indicated in the data availability section in Methods. The codes were run on MATLAB 2018b. The gene expression module analysis uses the R library "dynamicTreeCut" (v1.63.1) ran in R v4.0.0.

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 [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

Target area sequencing data (Fig. 4) are deposited at SRA (SRR12247894, SRR12245390, and SRR12245389). Single-cell RNAseq data are deposited at SRA (SRR13716225). Raw in situ sequencing images (Fig. 2-4) are deposited at Brain Image Library (<https://download.brainimaginglibrary.org/06/35/0635a0b3b0954c7e/>). Other data and processed sequencing data are deposited at Mendeley Data (<http://dx.doi.org/10.17632/jnx89bmv4s.1>).

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 [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.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	No sample-size calculation was performed. Because this manuscript focuses on reporting the development of the BARseq2 technique, we performed BARseq2 on three biological replicates to ensure the reproducibility of the technique.
Data exclusions	No data was excluded
Replication	The main BARseq2 experiments were performed on three animals and multiple findings from BARseq2 are validated using other techniques. The main new findings were validated using FISH and retrograde labeling. This validation experiments was replicated successfully across two animals for each experiment.
Randomization	Randomization was not applicable because the findings did not rely on comparison across different biological samples, but among different neurons. The scoring of neuronal properties (i.e. gene expression and projections) was automated and did not involve human scoring.
Blinding	Blinding was not relevant to the main dataset because the findings did not rely on comparison across different biological samples, but among different neurons. The scoring of neuronal properties (i.e. gene expression and projections) was automated and did not involve human scoring. Other experiments (i.e. comparison across experimental conditions) were also quantified without human input.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Male C57BL/6J mice of age 8-10 wks were used. The animals were housed at maximum of 5 in a cage on a 12 hrs on/12 hrs off light cycle. The temperature in the facility was kept at 22 C with a range not exceeding 20.5 C to 26 C. Humidity was maintained at around 45-55% not exceeding a range of 30-70%. A list of animals used is provided in Supp. Table S1.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected in the field.
Ethics oversight	All animal procedures were carried out in accordance with the Institutional Animal Care and Use Committee protocol 19-16-10-07-03-00-4 at Cold Spring Harbor Laboratory.

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