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Corresponding author(s): Feng, GP; Fu, ZY; Levin J

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒ An indication of 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 statistics 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
☐	☒ Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Sequencing data was collected on NextSeq with NextSeq Software System Suite 2.0.2. Flow cytometry (operating software Summit 6.3.1) was used only for sorting nuclei out of dissociated tissue. No flow cytometry analysis is used in this study.
Data analysis	Graphpad Prism 7 was used for statistical analysis in this study. Single nuclei data was mapped to the mm10 reference with TopHat 2.0.10 for QC purposes, and independently quantified with RSEM v1.2.8 and Bowtie2. Analysis of the TRN data was performed with biSNE software (https://github.com/yinqingl/nucseq_analysis), implemented in Matlab (R2017a). All other single cell/ single nuclei data was analyzed with Seurat (v2.2.0) in R (v3.3). Differential expression was performed with MAST (version 1.05). Data was visualized using ggplot (version 3.1.1).

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 upon request. 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

Sequencing data for this study is available through the Gene Expression Omnibus and Bioproject with accession code GSE145273. Single nuclei expression data will also be placed on the Broad's single cell portal. All additional data including raw data and plasmids are available from the authors upon reasonable request.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	No statistical calculations were applied to pre-determine sample size, but our sample sizes are similar to those reported in previous publications (reference Habib et al., Science 2016 and Cadwell et al., Nat Biotechnol 2016).
Data exclusions	No data point from any behavioral assays was excluded for all results reported in this study. Single nuclei RNA sequencing data exclusion criteria for filtering low quality cells was described in the method section, following an established procedure (Habib et al., Science 2016).
Replication	All experimental findings were reliably reproduced among in all experiments. The number of replicates were described in the legends of the corresponding figures.
Randomization	Randomization of animals and groups were applied throughout in vivo sleep experiments in the study.
Blinding	All EEG scoring, data analysis in this study were carried out by trained researchers. Researchers were blinded to group allocation during data collection and analysis. For the retrograde fluorescent beads tracing quantification, researchers were blinded to the retrobeads injection conditions. For in vivo CRISPR/Cas9 screen experiment, electrophysiologists were blinded to the viral pool # during data analysis.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	We utilized Anti-NeuN antibody conjugated with Alexa Fluor®488 (EMD Millipore, #MAB377x). The antibody was purchased from Millipore Sigma, with the catalog number MAB377X, Clone A60, lot 2736529, and was used at 1:500 dilution of 1 mg/ml stock.
Validation	The antibody utilized in this study was previously validated in the reference Krishnaswami et al. Nat Protoc 2016. "a nuclear staining gate using Hoescht and NeuN labeling to isolate single neuronal nuclei (Fig. 3d,e)." Additional references can be found in the manufacturer's website, "Vertebrate neuron-specific nuclear protein called NeuN (Neuronal Nuclei). MAB377X reacts with most neuronal cell types throughout the nervous system of mice including cerebellum, cerebral cortex, hippocampus, thalamus, spinal cord and neurons in the peripheral nervous system including dorsal root ganglia, sympathetic chain ganglia and enteric

ganglia. The immunohistochemical staining is primarily in the nucleus of the neurons with lighter staining in the cytoplasm. The few cell types not reactive with MAB377X include Purkinje, mitral and photoreceptor cells.".

Animals and other organisms

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

Laboratory animals	Juvenile (21-28 days) and adult (94-114 days) laboratory mice (Mus musculus) of both genders were utilized in this study.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from fields.

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	Isolating nuclei from mouse brain tissue using the sucrose gradient method. Using Vybrant® DyeCycle™ Violet Stain for nuclei in general and using Anti-NeuN Antibody for neuron nuclei selection.
Instrument	MoFlo Astrios EQ cell sorter, Beckman Coulter
Software	Summit 6.3.1, operating software for the flow cytometer
Cell population abundance	Tissue dissociation was loaded on the flow cytometer. Out of all detected events, nuclei, 78.75% (gate1), singlet, 54.58% (gate2), GFP+ 0.97% (gate3).
Gating strategy	We set fluorescence activated cell sorting (FACS) gating on forward scatter plot, side scatter plot (gate1) and on fluorescent channels to include only Violet+ (gate2) or Violet+ NeuN+ (gate3) (for neuronal nuclei), as shown in Extended Data Figure 1.

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