nature portfolio

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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.

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	We provide information in the manuscript on the versions of commercial software used to capture and process images (Micromanager (version 1.4.23), Adobe Photoshop (version 22.0.0), and Fiji/ImageLab (version 2.1.0/1.53s).
Data analysis	We provide information in the manuscript on the versions of commercial software used for statistical analysis and preparation of graphs: Excel (version 16.54), Prism (10.0.2).

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

All data supporting the findings of this study are available within the paper and its supplementary information files. Source data are provided with this paper. Biological material can be obtained from the corresponding author upon request.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	*Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used.* *Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.* *Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.*
Reporting on race, ethnicity, or other socially relevant groupings	*Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).* *Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)* *Please provide details about how you controlled for confounding variables in your analyses.*
Population characteristics	*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*
Recruitment	*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*
Ethics oversight	*Identify the organization(s) that approved the study protocol.*

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

For a reference copy of the document with all sections, see 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	Sample size was chosen based on how many samples would allow meaningful statistical differences while taking into account the difficulty and time required to obtain particular types of data.
Data exclusions	As we describe in the methods section, we excluded calcium transients for the HSN neuron that were undergoing defibrillation-like excessive numbers of transients in defined periods of time. The data exclusion criteria was not pre-planned because we did not know that defibrillation-like events would occur until we observed them in our experiments. As described, these defibrillation events occurred in only a small percentage of animals in all genotypes at roughly equal numbers.
Replication	Data for experiments were collected on multiple days and biological replicates were undertaken. Most experiments were replicated by different people at different times.
Randomization	Animals were selected randomly for the experimental and control groups.
Blinding	Experiments were not performed blind because the same experimentalist had to freshly prepare unstable folates immediately prior to experiments, and different genotypes could be inferred by visualization, and so could not be performed blind.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	anti-5HT (Serotonin) rabbit polyclonal antibody (Neuromics, catalog number RA20080; lot number 403732); anti-rabbit IgG alpaca nanoantibody AlexaFluor 488 (Chromotek, catalog number srbAF568-1-100); anti-FLAG M2 mouse monoclonal antibody (Sigma, catalog number F3165); anti-FLAG M2 Affinity Gel (Sigma, catalog number A2220, lot SLCG5835); anti-HA Affinity Gel (Sigma, catalog number E6779, lot SLCF6754); anti-HA polyclonal rabbit antibody (Invitrogen, catalog number 71-550, lot XD345766); anti-rabbit IgG (H+L) HRP-conjugated polyclonal goat antibody (Invitrogen, catalog number 32460, lot WC3229462); anti-mouse IgG (H+L) HRP-conjugated polyclonal goat antibody (Invitrogen, catalog number 31432, lot WC3216884).
Validation	We validated the anti-5HT (Serotonin) antibody by showing that it did not detect signal in the NSM and HSN neurons of tph-1 mutants, which are defective for the synthesis of serotonin (see Supplementary Fig. 10). We validated the anti-FLAG and anti-HA primary antibodies and anti-rabbit and anti-mouse secondary antibodies by detecting multiple different FLAG- and HA-tagged proteins on western blots (see Fig. 3c).

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK 293T cells were originally obtained from the ATCC (catalog number CRL-3216).
Authentication	The cells were not authenticated. We only used the HEK293T cells for expression of ectopic proteins for protein-protein interaction studies, and are not studying the properties of the HEK 293T cells.
Mycoplasma contamination	The cells were tested for mycoplasma contamination years ago, but not recently. The cells divide rapidly suggesting that they are not mycoplasma contaminated, and such contamination would not be expected to alter the use of the cells for ectopic protein-protein interaction studies.
Commonly misidentified lines (See ICLAC register)	No cells in the ICLAC register were used in this study.

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	Caenorhabditis elegans (a nematode roundworm). The use of C. elegans does not require ethical or animal use approval.
Wild animals	Not applicable
Reporting on sex	Not applicable
Field-collected samples	Not applicable
Ethics oversight	Not applicable

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

Plants

Seed stocks	Not applicable
Novel plant genotypes	Not applicable
Authentication	Not applicable