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Last updated by author(s): Jul 6, 2026

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
<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

No software was used.

Data analysis

Behavioral videos were processed to .hdf5 files using custom python script: <https://github.com/anthonysego77/behavioralVideoProcessing>
These behavioral data were analyzed via Ilastik v.1.4.0 for Windows. RNAseq data were analyzed via CLC Genomics Workbench 24.0.1. Image analysis was conducted manually through visualization in Fiji (ImageJ v.1.54p). Statistical analyses were performed with Prism v. 9.0 and v. 10.0.

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 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](#)

RNA-sequencing data that support the findings of this study have been deposited in NCBI Sequence Read Archive (BioProject PRJNA1249448).

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 sizes followed previously published work by our lab, and prior power analyses showing that with a modest effect size, a sample size of 8 should be sufficient. Our experiments relied on 10-12 animals for increased power.
Data exclusions	Animals with pieces of tissue missing are excluded from data analysis where quantification requires a cell number to body ratio, as this is not determinable without an intact animal.
Replication	All attempts at replication have been successful and are included in the major conclusions of the manuscript. Main conclusions of genes affecting TH cell number have been replicated in independent experiments a minimum of 3 times. Expression patterns show the representative image of at least 2 independent experiments of 6 animals each.
Randomization	Animals of the same size were randomly allocated into each RNAi condition prior to the onset of feeding. Animals in a given experiment were all from the same source container.
Blinding	All data were blinded either by a colleague or using the Blind Analysis Tools package in Fiji prior to analysis.

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-Digoxigenin-AP, Fab fragments (Roche, Catalog # 11093274910, sheep polyclonal, Lot #65752121, used at 1:2000); anti-Digoxigenin-POD, Fab fragments (Roche, Catalog #11207733910, sheep polyclonal, Lot #74946000, used at 1:2000); anti-Fluorescein-POD, Fab fragments (Roche, Catalog #11426346910, sheep polyclonal, Lot #57545020, used at 1:2000); anti-Dinitrophenyl-HRP
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(Vector Laboratories Catalog #MB-0603-0.5-1, rabbit polyclonal, Lot #ZJ0509, used at 1:1000); anti-Oregon Green-HRP (ThermoFisher, Catalog #A21253, rabbit polyclonal, Lot #3219292, used at 1.5:1000)

Validation

Validated for in situ hybridization (anti-DIG-AP PMID: 24318810), (anti-DIG-POD and anti-FITC-POD PMID:25232110, PMID:24797636), anti-DNP-HRP PMID: 35041722, PMID: 31549962). Validated for use in IF labeling in tandem with F-ara-EdU (anti-Oregon Green-HRP, PMID: 34156924)

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

Schmidtea mediterranea lab strain CIW4 (asexual)

Wild animals

The study did not involve wild animals.

Reporting on sex

As the animals are asexual, no sex-based reporting is included.

Field-collected samples

The study did not involve samples collected from the field.

Ethics oversight

No ethical guidance or approval was required because the animals are invertebrates.

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

Plants

Seed stocks

N/A

Novel plant genotypes

N/A

Authentication

N/A