nature portfolio

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Last updated by author(s): Feb 20, 2025

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	Zeiss "Zen Blue v3.1" for image acquisition
Data analysis	FIJI/ImageJ v1.53 GraphPad Prism v10 Mathworks MATLAB 2021b, v9.11 FlyPAD v2.0 (http://www.flypad.pt/) FLIC v1.0.0beta (https://github.com/PletcherLab/FLIC_R_Code). The script used for image analysis is posted at https://doi.org/10.5281/zenodo.6641933 Analysis of sleep data using a custom scripts in MATLAB (The MathWorks), available at https://zenodo.org/record/5772445

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 this study's findings are included in the article and its supplementary materials. The raw numerical data for charts and graphs are available in the Source Data files provided with this paper. Additional inquiries for resources and reagents should be directed to the corresponding author, K.R., and will be fulfilled 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	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 size was chosen based on similar previously published studies of Drosophila and mouse behavior and metabolism (e.g., https://doi.org/10.1038/s41467-022-28268-x, https://doi.org/10.1038/s42255-022-00672-z, https://doi.org/10.1038/s41467-021-25146-w, https://doi.org/10.1038/s41467-024-54927-2). No sample-size calculations were performed. The numbers of samples are large enough to capture normal variation while maintaining feasibility for preparation and are similar to or larger than those used in other published studies in the field. qPCR used 5-6 samples, each containing several tissues or animals, the standard in our lab; starvation and metabolic assays used approximately 10 replicates containing multiple animals. Image analyses made use of multiple tissues per genotype or condition, as described in the appropriate figure legends or methods.
Data exclusions	No data were excluded.
Replication	Representative images were chosen from multiple options, generally at least 5. All experiments producing numerical data include at least 5 replicates. All attempts at replication were successful.
Randomization	Animals were randomly grouped into batches as indicated in the text.
Blinding	Researchers were not blinded during the study because this is not generally done in fly studies. With limited staff with expertise in these particular studies, the person handling sample prep must usually also be the one performing the assay.

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

Rabbit anti-AKH, non-commercial, polyclonal, obtained from Jan Veenstra (University of Bordeaux), 1:1000. (Generated in Isabel et al., 2005; Comparative and Evolutionary Physiology 288, 531-538.)

Rabbit anti-Tk, non-commercial, polyclonal, obtained from Jan Veenstra (University of Bordeaux), 1:1000. (Generated in Veenstra, Agricola, and Sellami, 2008. Cell Tissue Res 334, 499-516.)

Mouse anti-GFP, ThermoFisher #A11120, 1:500.
Chicken anti-GFP, Abcam #ab13970, 1:2000.
Mouse anti-Substance P, Abcam #ab14184, 1:1500.
Rat anti-mCherry, ThermoFisher #M11217, 1:1000.

Alexa Fluor 488 goat anti-mouse, ThermoFisher #A32723, 1:500.
Alexa Fluor 488 goat anti-chicken, Abcam #ab150169, 1:500.
Alexa Fluor 555 goat anti-rat, ThermoFisher #A21434, 1:500.
Alexa Fluor 594 goat anti.rabbit, ThermoFisher #A11012, 1:500.
Alexa Fluor 647 goat anti-mouse, ThermoFisher #A21240, 1:2000.
Alexa Fluor 647 goat anti-rabbit, ThermoFisher #A32733, 1:500.

Undisclosed anti-Substance P antibody component of commercial ELISA kit (ThermoFisher #EEL013).

Alexa Fluor 647-conjugated phalloidin, ThermoFisher #A30107, 1:100.

Validation

Rabit anti-AKH validated in Guillaume Isabel, Jean-Rene Martin, Saad Chidami, Jan A. Veenstra, Philippe Rosay, (2005). AKH-producing neuroendocrine cell ablation decreases trehalose and induces behavioral changes in Drosophila. Comparative and Evolutionary Physiology 288, 531-538.

Rabbit anti-Tk: validated in Veenstra, J.A., Agricola, H.J., and Sellami, A. (2008). Regulatory peptides in fruit fly midgut. Cell Tissue Res 334, 499-516.

Mouse anti-GFP validated for staining by manufacturer (https://www.thermofisher.com/antibody/product/GFP-Antibody-clone-3E6-Monoclonal/A-11120).

Rat anti-mCherry validated for staining by manufacturer (https://www.thermofisher.com/antibody/product/mCherry-Antibody-clone-16D7-Monoclonal/M11217).

Chicken anti-GFP validated by the manufacturer (https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970)

Mouse anti-Substance P validated by the manufacturer (https://www.abcam.com/en-us/products/primary-antibodies/substance-p-antibody-sp-de4-21-ab14184)

Goat anti-mouse, Alexa Fluor 488, validated by manufacturer (https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG-H-L-Highly-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32723).

Goat anti-mouse, Alexa Fluor 647, validated by manufacturer (https://www.thermofisher.com/antibody/product/Goat-anti-Mouse-IgG1-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21240)

Goat anti-rabbit, Alexa Fluor 647 , validated by manufacturer (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A32733).

Goat anti-rabbit, Alexa Fluor 594, validated by manufacturer (https://www.thermofisher.com/antibody/product/Goat-anti-Rabbit-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11012)

Goat anti-rat, Alexa Fluor 555, validated by manufacturer (https://www.thermofisher.com/antibody/product/Goat-anti-Rat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21434).

Goat anti-chicken IgY, Alexa Fluor 488, validated by manufacturer (https://www.abcam.com/en-us/products/secondary-antibodies/goat-chicken-igy-h-l-alexa-fluor-488-ab150169)

Anti-Substance P ELISA validated for use in mice by the manufacturer (https://assets.thermofisher.com/TFS-Assets%2FBID%2Fmanuals%2FEEL013-manual.pdf)

Alexa Fluor 647-conjugated phalloidin, validated by the manufacturer (https://www.thermofisher.com/order/catalog/product/A30107)

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	This study used adult female Drosophila melanogaster, aged 5+ days post-eclosion, and female mice (C57BL/6J, 10 weeks old, for feeding experiments, from Taconic) and transgenic Tph1-CPH mice from Jackson Labs (#028366), 15-32 weeks old. Mice were housed at a temperature of 22 °C and a relative humidity of 55% in the University of Copenhagen mouse facility, adhering to normal standards of care, feeding, and environmental enrichment.
Wild animals	No wild animals were used in this study.
Reporting on sex	Our study identifies a hormonal pathway that functions in Drosophila to modulate their appetite for sugar versus protein-rich food. Most characterization was performed in mated female flies (this is noted throughout), but we also conducted a few experiments in males, which suggest that the basic principle operates in both sexes (Extended Data Fig. 1a and 6c-e). Additionally, both organoid studies and dietary intervention experiments were conducted in female mice, supporting the Drosophila results and speaking to the broad conservation of the system.
Field-collected samples	No field-collected animals were used in this study.
Ethics oversight	No ethics approval or oversight is required for Drosophila studies. Mouse procedures complied with European Union Directive 63/2010/EU ('European Convention for the Protection of Vertebrate Animals Used for Scientific Purposes') and ETS 123 guidelines, with regular health monitoring by animal care technicians and a veterinarian. All animal procedures were conducted in accordance with institutional and national ethical guidelines and were approved by the Danish Animal Experiments Inspectorate. The dietary intervention studies in female mice were conducted under permit number 2020-15-0201-00628, and the intestinal organoid studies using female mice were approved under permit number 2023-15-0202-00197. This study also complies with the European Convention on the Protection of Animals Used for Scientific Purposes. (This information is also noted in the manuscript.)

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

Plants

Seed stocks	No plant stocks or seeds were used in this study.
Novel plant genotypes	No plant stocks or seeds were used in this study.
Authentication	No plant stocks or seeds were used in this study.