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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 Authors & Referees 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	LCS (Leica); LAS X (Leica); Axiovision (Zeiss)
Data analysis	Photoshop 2020 (Adobe); ImageJ 1.52q (NeuronJ plugin, NIH); ICY (http://icy.bioimageanalysis.org/); Excel v.16 (Microsoft); Prism 9 (GraphPad)

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. 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

All data supporting the findings of this study are available from the corresponding authors on reasonable request.

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

Life sciences study design

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

Sample size	Drosophila numbers are not limiting, and thus pre-determining sample size is not necessary. Sample sizes were determined empirically, based on the expected variations upon different genotypes and treatments, and the processing time required for each experiment. In each experimental condition, similar sample sizes were used to make comparisons.
Data exclusions	No data were excluded from the analyses.
Replication	All experiments were repeated 2-3 times with the same results. Representative experiments are shown in the manuscript. Key points of the study were also verified using different genotypes (e.g. Gal4 drivers with the same tissue specificity or independent RNAi lines targeting the same gene).
Randomization	The allocation of experimental organisms (flies) into groups was random. For example, from pools of animals of the same genotype, age and sex, we generated different experimental groups, which were subsequently subjected to various treatments.
Blinding	Blinding was performed for some experiments quantifying tracheal phenotypes (e.g. Fig. 1k, Fig. 7a,c). For all others, the experimenter was not blind to the fly genotypes.

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
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	All antibodies used in the study are commercially available: rabbit-anti-pH3 (#06-570, Millipore 1:4000); rabbit anti-cleaved-Caspase-3 (#9661, Cell Signaling Technology 1:400); mouse-anti-Prospero (MR1A, DSHB 1:100); mouse anti-β-gal (Z3781, Promega 1:500); chicken-anti-GFP (A10262, Invitrogen 1:1000); rabbit anti-GFP (A11122, 1:3000; Invitrogen). Secondary antibodies against mouse, rabbit or chicken conjugated to Alexa Fluor 488 and 555 (A31572, A11039, A21206, A31570, Invitrogen) were used at 1:1000.
Validation	All antibodies used in the study were previously validated to work specifically in the Drosophila midgut: rabbit-anti-pH3, and mouse-anti-Prospero (Micchelli and Perrimon, 2006); mouse anti-β-gal, rabbit anti-cleaved-Caspase-3, chicken-anti-GFP, and rabbit anti-GFP (Apidianakis et al., 2009).

Animals and other organisms

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

Laboratory animals	The study involved the use of Drosophila melanogaster. Mature (3-5 days old) mated females were used for all experiments. Details of the time line for each experiment are provided in the methods section of the paper.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The use of Drosophila does not require ethical approval.

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