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.

- 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
- 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 | Software source code is available in: https://github.com/AdrianA-T/Guidance-Model and was written in C# using Unity Engine |
|-----------------|------------------------------------------------------------------------------------------------------------------|
| Data analysis   | Data were quantified using Fiji 2.6.0 and analysis were performed using Matlab2015a, further details are in Materials and Methods and upon request. |

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

Source data generated in this study is provided with this paper in Source Data file and more supplementary information from the corresponding authors upon request.
Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| Reporting on sex and gender | N/A since not human research was performed. |
|----------------------------|--------------------------------------------|
| Population characteristics | N/A since not human research was performed. |
| Recruitment                | N/A since not human research was performed. |
| Ethics oversight           | N/A since not human research was performed. |

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

Report on sex and gender N/A since not human research was performed.

Population characteristics N/A since not human research was performed.

Recruitment N/A since not human research was performed.

Ethics oversight N/A since not human research was performed.

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

The sample size were calculated for each study according to the regular sample size determined in other Drosophila studies (see the references) and taking into account the confidence range for Wilcoxon rank sum test. The exact sample size per study is also detailed in the figure legends of the manuscript, the material and methods and in the source data file.

Data exclusions

No data were excluded.

Replication

All experiments were reproduced to reliably support the conclusions stated in the manuscript. The exact sample size per study is included and detailed in the figure legends of the manuscript, the material and methods and in the source data file.

Randomization

N/A. The fly samples were selected evenly according to the genotype of study.

Blinding

Administration of samples was carried out using coded labels that did not contain the genotype nomenclature. Hence, the acquisition of the image samples in the microscope were a blinded experiment.

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

Antibodies

Antibodies used

The mouse monoclonal α-Dlp primary antibody from (Lum L, Yao S, Mozer B, et al. Identification of Hedgehog pathway components by RNAi in Drosophila cultured cells. Science (80-. ). 2003;299(5615):2039-2045. doi:10.1126/science.1081403) at 1:30 dilution.

The rat monoclonal α-Ci primary antibody from (Motzny CK, Holmgren R. The Drosophila cubitus interruptus protein and its role in the wingless and hedgehog signal transduction pathways. Mech Dev. 1995;52(1):137-150. doi:10.1016/0925-4773(95)00397-J) at

Methods

Involved in the study

ChiP-seq

Flow cytometry

MRI-based neuroimaging
1:20 dilution.
α-GFP (rabbit anti GFP polyclonal antibody from Chromotek, ref: PABG1) at 1:1000 dilution
anti-mouse Pacific Blue from ThermoFischer (ref: P-31582) at 1:400 dilution
anti-rabbit Alexa 647 from ThermoFischer (ref: A-31573) at 1:400 dilution

Validation

The validation of the antibodies were performed by: Primary: α-Dlp: doi:10.1126/science.1081403; α-GFP: Chromotek; α-Ci: doi:10.1016/0925-4773(95)00397-J, Secondary: anti-mouse Pacific Blue: ThermoFischer; anti-rabbit Alexa 647: ThermoFischer.

Since our studied proteins have a specific pattern in the tissue, we use that unique distribution to validate that the antibody was working properly in our samples.

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
Drosophila melanogaster strains (mutants and transgenes) are described in the manuscript. The fly sample were collected at third instar larvae (L3) which correspond to three-five days from the egg fertilization.

Wild animals
No wild animals were used in the study.

Reporting on sex
Not applicable

Field-collected samples
No field collected samples were used in the study.

Ethics oversight
The study did not require an ethical approval.

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