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Supplementary Figures: 5

Supplementary Tables: 2

Supplementary Videos: 1

Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read Reporting Life Sciences Research.

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

Note: Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
example	1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend
example	results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ -	7i	unpaired two-tailed Student's t-test, type 3	Figure legend	9	optic lobes	Figure panel Methods, para 5	error bars are average +/- 95% confidence intervals	Figure legend	p=0.0007	Figure legend	Welch-corrected not assuming equal SD: df=15.96 t=4.16	Figure legend
+ -	7p	unpaired two-tailed Student's t-test, type 3	Figure legend	15	optical sections [3 per sample (5)]	Figure panel, Methods para 5	error bars are average +/- 95% confidence intervals	Figure legend	p=1.18×10-12 p=1.07×10-12 p=1.95×10-13	Figure legend	Welch-corrected not assuming equal SD: 1: df=23.32 t=13.71 2: df=27.12 t=12.41 3: df=24.68 t=14.29	Figure legend
+ -	7q	unpaired two-tailed Student's t-test, type 3	Figure legend	24 15	optical sections [3 per sample (8)] optical sections [3 per sample (5)]	Figure panel, Methods para 5	error bars are average +/- 95% confidence intervals	Figure legend	p=2.88×10-17 p=0.020 p=2.11×10-11 p=0.0007	Figure legend	Welch-corrected not assuming equal SD: 1: df=43.17 t=13.65 2: df=35.00 t=2.44 3: df=28 t=10.70 4: df=27.51 t=3.84	Figure legend
+ -	3d	Present. of percentages	Figure legend	15 23	percentages of samples with 1, 2,3 or 4 cell streams presented in a 100% stacked column chart	Figure panel	-		-		-	

Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

   If so, what figure(s)?

Figures 1-8 and Supplementary Figures 1-4 each contain several panels with representative images.

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

   If so, where is this reported (section, paragraph #)?

Sample numbers have been provided for each panel of each figure in Supplementary Tables 1 and 2. Each figure legend contains a reference that information on sample numbers can be found in these tables. The Online Methods (paragraph 5) and Supplementary Tables each include a note explaining that - if not otherwise indicated - the penetrance of observed phenotypes is 100%.
All experiments rely on the analysis of progeny from Drosophila strains or crosses with defined genotypes, and therefore are not limited in repeatability. Information on strains and genetic crosses is provided in the Online Methods. Genotypes are listed in Supplementary Tables 1 and 2. A comment on repeatability is provided in the Statistics section.

Statistics and general methods

1. Is there a justification of the sample size?

   If so, how was it justified?

   Where (section, paragraph #)?

   Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

Sample sizes are based on the standard used in the field. This information is provided in the Online Methods, paragraph 6.

2. Are statistical tests justified as appropriate for every figure?

   Where (section, paragraph #)?

Statistical tests have been conducted for data described in the Results and in Figure panels 7i,p and q.

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Quantification and statistical methods are described in the Online Methods, paragraphs 5 and 6. Statistical analyses use the Student's t-test. This is also stated in Figure legend 7.

   b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

      Where is this described (section, paragraph #)?

Data sets were subjected to the Shapiro-Wilk and D'Agostino-Pearson omnibus normality tests, and met the assumption for normality in at least one of these. This justifies the use of a parametric test, such as the Student's t-test. The information concerning these tests has been provided in the Online Methods, paragraph 6.

   c. Is there any estimate of variance within each group of data?

      Is the variance similar between groups that are being statistically compared?

      Where is this described (section, paragraph #)?

Student's t-tests, type 3, were performed not assuming equal variance between control and experimental data sets. This is described in the Method section, paragraph 6.

   d. Are tests specified as one- or two-sided?

Student's t-tests are unpaired, two-tailed, not assuming equal variance (type 3); this information is provided in the Online Methods section, paragraph 6.

   e. Are there adjustments for multiple comparisons?

No adjustments have been made, as all comparisons were between pairs of defined control and experimental genotypes.

3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	As no data points were excluded in this study, this is currently not mentioned.
4. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data. If no randomization was used, state so. Where does this appear (section, paragraph #)?	All data were obtained from samples with unambiguously defined genotypes (described in Online Methods, paragraph 6). In a pool of control or experimental animals, specimens of the correct stage were selected randomly and independently from vials. A statement regarding this point is provided in the Statistics Methods section (paragraph 6).
5. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included? If no blinding was done, state so. Where (section, paragraph #)?	All data were obtained from samples with defined genotypes (Online Methods, paragraph 6); an additional statement that tests therefore were not performed blindly has been added to this paragraph.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	As our study solely uses Drosophila melanogaster, a statement of compliance is not required.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Drosophila is used throughout the manuscript. "melanogaster" has been added on page 3 in the Introduction and in the Online Methods section (paragraph 1).
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	All genotypes of Drosophila melanogaster strains used for analysis and crosses are listed in the Methods section, paragraph 1. Precise genotypes of each experiment are listed in Supplementary Tables 1 and 2.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	This study used either females or males; or solely males depending on the genetic cross. This is reported in Supplementary Tables 1 and 2.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Larval brains were generally examined at the late wandering third instar larval stage (Results section), if not stated otherwise. The size of the lamina was used to ensure that control and experimental samples of the same age are compared. This information has been included in the Online Methods section (paragraph 2).
11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	As flies have been raised using standard conditions and not under a specific light/dark cycle, no details have been provided.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	The number of adult flies used per strain, cross and vial varied to ensure that a sufficient number of larvae of desired genotypes could be retrieved for each experiment. Generally, crosses involved about 5 males and 7 unfertilized females. To avoid overcrowding, parents were transferred to fresh food containing vials every or every second day. This information has been included in the Online Methods section (paragraph 1).

13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

    Where (section, paragraph #)?

N/A

14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

    Where (section, paragraph #)?

N/A

    a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

       Where (section, paragraph #)?

N/A

15. If any animals/subjects were excluded from analysis, is this reported?

    Where (section, paragraph #)?

No animals were excluded from the analysis.

    a. How were the criteria for exclusion defined?

       Where is this described (section, paragraph #)?

N/A

    b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

       Where is this described (section, paragraph #)?

N/A

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

This study does not use new antibodies, but relies on reagents which have previously been generated and validated. This includes antibodies generated by other laboratories, including those deposited into Developmental Studies Hybridoma Bank (DSHB) and four widely used commercially available antibodies.

    a. Is antibody catalog number given?

       Where does this appear (section, paragraph #)?

Primary antibodies obtained from DSHB are generally listed with antibody and antigen names. For the commercially available antibodies, information about the company, from which the reagents were purchased, has been provided and catalog numbers have been added. Finally, for antibodies, kindly provided by colleagues, generally, the name of the PI, a reference or both have been provided. All information is listed in the Online Methods section (paragraph 2).

    b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

       Where does this appear (section, paragraph #)?

All antibodies used in this study have been validated in previous studies, and references are provided for most of these. DSHB antibody validations are found on linked on-line Data sheets. All information is listed in the Methods section, paragraph 2.

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

    Where (section, paragraph #)?

This study does not involve cell-based assays.

a. Were they recently authenticated?

Where is this information reported (section, paragraph #)?

N/A

Data deposition

Data deposition in a public repository is mandatory for:

a. Protein, DNA and RNA sequences
b. Macromolecular structures
c. Crystallographic data for small molecules
d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available here. We encourage the provision of other source data in supplementary information or in unstructured repositories such as Figshare and Dryad.

1. Are accession codes for deposit dates provided?

   Where (section, paragraph #)?

This study does not include any data sets requiring deposition in a public repository.

Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

This study does not rely on custom software.

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

This study relies on software that is commercially available or has been deposited into the public domain.

Human subjects

1. Which IRB approved the protocol?

   Where is this stated (section, paragraph #)?

N/A

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

N/A

3. Is the number of human subjects, their age and sex clearly defined?

   Where (section, paragraph #)?

N/A

4. Are the inclusion and exclusion criteria (if any) clearly specified?

   Where (section, paragraph #)?

N/A

5. How well were the groups matched?

   Where is this information described (section, paragraph #)?

   N/A

6. Is a statement included confirming that informed consent was obtained from all subjects?

   Where (section, paragraph #)?

   N/A

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

   Where (section, paragraph #)?

   N/A

fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

   N/A

   a. If yes, is the number rejected and reasons for rejection described?

      Where (section, paragraph #)?

      N/A

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

   Where (section, paragraph #)?

   N/A

3. Is the length of each trial and interval between trials specified?

   N/A

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

   N/A

5. Is the task design clearly described?

   Where (section, paragraph #)?

   N/A

6. How was behavioral performance measured?

   N/A

7. Is an ANOVA or factorial design being used?

   N/A

8. For data acquisition, is a whole brain scan used?

   If not, state area of acquisition.

   N/A

   a. How was this region determined?

      N/A

9. Is the field strength (in Tesla) of the MRI system stated?	N/A
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	N/A
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/ flip angle clearly stated?	N/A
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	N/A
11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?	N/A
12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?	N/A
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	N/A
14. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
15. Is the contrast construction clearly defined?	N/A
16. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	N/A
17. Were repeated measures used (multiple measurements per subject)?	N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	N/A
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	N/A
19. Are statistical inferences corrected for multiple comparisons?	N/A
a. If not, is this labeled as uncorrected?	N/A

20. Are the results based on an ROI (region of interest) analysis?	N/A
a. If so, is the rationale clearly described?	N/A
b. How were the ROI's defined (functional vs anatomical localization)?	N/A
21. Is there correction for multiple comparisons within each voxel?	N/A
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	N/A

Additional comments

Additional Comments	Genetic tools, including driver stocks and UAS-RNA interference (UAS-RNAi) transgenes used for knockdown of specific genes have been validated whenever possible by testing two UAS-RNAi lines as well as antibody staining. Validations are shown in Supplementary Figures.