Corresponding Author: Gwyneth Card

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Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work we publish. This form is published with all life science papers and is intended to promote consistency and transparency in reporting. All life sciences submissions use this form; while some list items might not apply to an individual manuscript, all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

   Describe how sample size was determined.

   No statistical methods were used to predetermine sample size. At least 6 flies were imaged for every experimental condition.

2. Data exclusions

   Describe any data exclusions.

   No data was excluded from analysis. For our anatomical analyses of LPLC2 morphologies, we focused on visually-isolatable neurons with more central positions (as described in the Methods).

3. Replication

   Describe whether the experimental findings were reliably reproduced.

   Experiments are highly consistent and reproducible. Every fly imaged is included and individual fly and neuron data are shown throughout.

4. Randomization

   Describe how samples/organisms/participants were allocated into experimental groups.

   Flies were group housed, and individual flies were selected randomly from housing vials for imaging and behavior.

5. Blinding

   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

   Experimenter was not blind to fly genotypes.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly.
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *p* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A summary of the descriptive statistics, including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Custom MATLAB code was used to analyze calcium imaging data. 3D ROI for calcium data were generated with Fluorender and ImageJ. Statistics and plotting were done with Prism and Origin. Anatomical characterizations were done using Fluorender, Fiji, and Vaa3D.

For all studies, we encourage code deposition in a community repository (e.g. GitHub). Authors must make computer code available to editors and reviewers upon request. The *Nature Methods* guidance for providing algorithms and software for publication may be useful for any submission.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	Drosophila strains are available from the Janelia split-GAL4 website: http://splitgal4.janelia.org

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Antibodies used for histology were validated previously. Please see Aso Y. et al. 2014 eLife (doi:10.7554/eLife.04577). Other antibodies used are described in Methods: Primary antibodies used were mouse anti-GFP (mAb 3E6, #A-11120, ThermoFisher Scientific, 1:100 dilution), rat anti-CadN (mAb DN-Ex #8, Developmental Studies Hybridoma Bank, 1:20 dilution) and rabbit anti-DsRed (#632496, Clontech, 1:1000 dilution). Secondary antibodies used were DyLight 488-AffiniPure Donkey Anti-Mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. #715-485-151, 1:500 dilution), DyLight 594 AffiniPure Donkey anti Rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. , #711-515-152, 1:300 dilution) and Alexa Fluor® 647 AffiniPure Donkey Anti-Rat IgG (H+L) (Jackson ImmunoResearch Laboratories, Inc. , #712-605-153, 1:300 dilution).

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No cell lines were used.
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used.
d. If any of the cell lines used in the paper are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Fly stocks used in this study were split-GAL4 constructs targeting LPLC2, T4/T5, LPi4-3, GF, and control (empty nervous system) cell types. These were crossed with combinations of effectors for optogenetic activation (CsChrimson), calcium imaging (Gcamp6f, OpGCamp6f, OpGcamp6fs), and visualization (myrGFP, MCFO-1, MCFO-7). Full genotypes are provided in Supplementary Tables 1-2, and split-GAL4 expression patterns are available at http://splitgal4.janelia.org.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.