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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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

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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.
\boxtimes	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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
\times	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\boxtimes	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 2009 was used for two-photon calcium imaging and PA-GFP labelling/imaging. Single sensillum recordings were performed with AutoSpike. Patch clamp recordings were performed with MATLAB (2023a). The tethered fly assay was run by Python 3.7.3. For more details refer to the respective sections of the Methods.

Data analysis

Data were analysed and plotted using Excel, R (v3.2.3; R Foundation for Statistical Computing, Vienna, Austria, 2005; R-project-org), MATLAB (2023a), GraphPad Prism (10.1.1) and Python (v3.11).

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 relevant data and code supporting the findings of this study are included as Source Data or available from the corresponding authors upon request. All unique biological materials generated in this study are available from the corresponding authors upon request.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender

Use the terms sex (biological attribute) and gender (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.

Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status).

Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)

Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

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 bel	ow that is the best fit for your research	If you are not sure, read the appropriate sections before making your selection.	
X Life sciences	Behavioural & social sciences	Ecological, evolutionary & environmental sciences	

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

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

Sample size

Preliminary experiments were used to assess variance and determine adequate sample sizes in advance of conducting the experiment. Generally, these were $n = ^30$ animals in tethered fly assays, 5-10 animals for wind tunnel assays, and 5-10 animals in physiological experiments. We used similar sample sizes for all experiments where a single variable (e.g., genotype, species or stimulus) was being compared.

Data exclusions

For the PA-GFP experiment, samples with non-specific labelling were excluded from the analysis. For the patch clamp experiment, a sample where two cells were coupled was excluded from voltage and input resistance analysis but included in the spike analysis. For the wind tunnel assay, we excluded flies that simply transited the wind tunnel along the ceiling, far away from the plume. For the calcium imaging experiment, animals with high solvent response or no response to diagnostic odour were excluded. For the tethered fly assay, animals that stopped flying during stimulus presentation, or when the optic fibre was displaced at the end of the experiment, were excluded.

Replication

All attempts at repelication were successful. Several experiments were carried out repeatedly due to the fact that they served as controls for different experimental manipulations. In particular, we ran wild-type control experiments in parallel with mutant analyses in behavioural experiments and replicated them multiple times. For electrophysiological recordings and calcium imaging, data were collected from multiple

	flies on several distinct experimental days, interleaving wild-type and mutant genotypes. In all cases the results were reliable and robust over the course of the many years it took to complete this study.
omization	For all experiments, we interleaved genotypes and stimuli when applicable and randomised if possible their order. To control for potential

Rando variations in experimental conditions across days, we were careful to collect, if possible, a similar sample size for each variable every day the experiment was conducted and performed each experiment on multiple days.

Blinding

The experimenter was blinded to the genotype for quantification of OSN numbers and SPARC2-CsChrimson and SPARC2-TNT tethered fly assays, but not for other behavioural or physiological experiments.

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Reporting fo	r specific materials, systems and methods
'	buthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, want 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 & experime n/a Involved in the study Antibodies Eukaryotic cell lines Palaeontology and a Animals and other cology Clinical data	n/a Involved in the study ChIP-seq Flow cytometry MRI-based neuroimaging
Dual use research o Plants Antibodies	f concern
Antibodies used	The following antibodies were used: guinea pig anti-Ir75b 1:500 (RRID:AB_263109322), mouse monoclonal antibody nc82 1:10 (Developmental Studies Hybridoma Bank), rabbit anti-GFP 1:500 (Invitrogen) and rat anti-HA 1:500 (Roche). Alexa488-, Cy3- and Cy5-conjugated goat anti-guinea pig, goat anti-mouse, goat anti-rabbit and goat anti-rat IgG secondary antibodies (Molecular Probes; Jackson Immunoresearch) were used at 1:500.
Validation	All primary antibodies have been validated in previous studies.
Animals and othe	r research organisms
Policy information about <u>st</u> <u>Research</u>	udies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in
Laboratory animals	Ages of the flies (days-old post eclosion) are as follows: 5-8 for calcium imaging, 5-9 for PA-GFP labelling, 1-2 for patch clamp recordings, 5-7 for single sensillum recordings, 5-9 for tethered fly assay (except for SPARC-TNT, in which 0-1), and 3-7 for wind tunnel assays. Wild-type, mutant and transgenic Drosophila lines used in this study are listed in Supplementary Table 1, and genotypes are provided in the corresponding figure legends.
Wild animals	This study did not involve wild animals.
Reporting on sex	All experiments (except for Fig. 1e and Supplementary Fig. 2j) were performed with female flies to minimise variation.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	All experiments were conducted in accordance with ethical guidelines from the University of Lausanne.

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.