

## 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

LAS X software, Leica Microsystems, [www.leica-microsystems.com](http://www.leica-microsystems.com)  
Inspector Software, Max Planck Innovation GmbH, v1.0  
MESc v3.5 Software, Femtonics Ltd., version 3.5  
Femto2D-Resonant, Femtonics Ltd.  
Clampex, Molecular Devices, Version 10.5

Data analysis

MATLAB, MathWorks R2010b/R2016a  
GraphPad Prism, Graphpad Software, Version 5.01/6.01  
ImageJ, NIH, Version 1.48q/1.50g; <http://rsbweb.nih.gov/ij/>  
LCS AF, Leica Microsystems, [www.leica-microsystems.com](http://www.leica-microsystems.com)  
Clampfit, Molecular Devices, Version 10.5  
Custom MATLAB and ImageJ scripts available from Alexander M. Walter 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/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

The data that support the findings of this study are available from the corresponding authors upon 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

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.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	Sample sizes were chosen in accordance with previous publications and are in line with those generally employed in the field.
Data exclusions	For electrophysiological recordings, cells were excluded from analysis as mentioned in the methods section for reasons such as poor membrane potentials or resistances. This was performed irrespective of treatment or genotype.
Replication	Electrophysiological recordings and immunohistochemistry experiments were performed in multiple animals in each genotype/treatment group over a number of days. For exact numbers, please see supplementary table 1.
Randomization	No randomization was used. However, for immunostainings, all genotypes were prepped in one session, stained in one cup, mounted on one slide, scanned in one session (different larvae of genotypes/treatments in an alternating manner) and strictly analyzed in an unbiased manner. Electrophysiological recordings were performed in an alternating fashion in regards to genotype and treatment, aiming to gather equal data from each genotype/ treatment group in any particular day and were also strictly analyzed in an unbiased manner.
Blinding	No blinding was performed. As stated above immunostainings and electrophysiological recordings were performed in an unbiased manner.

## Behavioural & social sciences study design

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

Study description	Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).
Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

## Ecological, evolutionary & environmental sciences study design

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

Study description	Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.
Research sample	Describe the research sample (e.g. a group of tagged <i>Passer domesticus</i> , all <i>Stenocereus thurberi</i> within Organ Pipe Cactus National

Research sample	<i>Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.</i>
Sampling strategy	<i>Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.</i>
Data collection	<i>Describe the data collection procedure, including who recorded the data and how.</i>
Timing and spatial scale	<i>Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken</i>
Data exclusions	<i>If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.</i>
Reproducibility	<i>Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.</i>
Randomization	<i>Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.</i>
Blinding	<i>Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.</i>
Did the study involve field work?	<input type="checkbox"/> Yes <input type="checkbox"/> No

## Field work, collection and transport

Field conditions	<i>Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).</i>
Location	<i>State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).</i>
Access and import/export	<i>Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).</i>
Disturbance	<i>Describe any disturbance caused by the study and how it was minimized.</i>

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used	Guinea-pig Unc13A, Bohme et al., 2016 Mouse monoclonal Syx-1A 8C3, Developmental Studies Hybridoma Bank, Cat# 8c3, RRID:AB_528484 Mouse Rop 4F8, Developmental Studies Hybridoma Bank, Cat# Rop 4F8, RRID:AB_1157869 Mouse monoclonal GFP 3E6, Thermo Fisher, Cat# A-11120, RRID:AB_221568 Mouse monoclonal NC82, Developmental Studies Hybridoma Bank, Cat# nc82, RRID:AB_2314865 Rabbit BRPLast200, Ullrich et al., 2015 Rabbit RBPC-term, Liu et al., 2011 Guinea-pig vGlut, Chen et al., 2017 Mouse Anti-alpha-Tubulin, Sigma-Aldrich, Cat# T9026, RRID:AB_477593 Goat anti-HRP-Cy5, Jackson ImmunoResearch Goat anti-HRP-647, Jackson ImmunoResearch, Cat# 111-165-144, RRID:AB_2338967
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Goat anti-rabbit-Cy3, Jackson ImmunoResearch, Cat# 111-165-144, RRID:AB\_2338006  
 Goat anti-mouse-Cy3, Jackson ImmunoResearch, Cat# 115-165-146, RRID:AB\_2338690  
 Goat anti-mouse Alexa-Fluor-488, Life Technologies, Cat# A11001, RRID:AB\_2534069  
 Goat anti guinea-pig Alexa-Fluor-488, Life Technologies, Cat# A11073, AB\_2534117  
 Goat anti-mouse Atto590, ATTO-TEC; Dianova, Atto590: Cat#AD 590-31  
 Goat anti-rabbit Atto590, ATTO-TEC; Dianova, Atto590: Cat#AD 590-31  
 Goat anti-mouse Alexa594, Thermo Fisher, Cat# A-11032, RRID:AB\_2534091  
 Goat anti-rabbit Alexa594, Thermo Fisher, Cat# A-11037, RRID:AB\_2534095  
 Goat anti-guinea pig star635, Abberior; Dianova, Star635: Cat#1-0101002-1  
 Goat anti-rabbit star635, Abberior; Dianova, Star635: Cat#1-0101002-1

Validation

Primary antibodies were validated for use in Drosophila for the relevant applications as noted by the commercial supplier and/or had been used in previous published studies.

## Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

*State the source of each cell line used.*

Authentication

*Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.*

Mycoplasma contamination

*Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.*

Commonly misidentified lines  
(See [ICLAC](#) register)

*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

## Palaeontology

Specimen provenance

*Provide provenance information for specimens and describe permits that were obtained for the work (including the name of the issuing authority, the date of issue, and any identifying information).*

Specimen deposition

*Indicate where the specimens have been deposited to permit free access by other researchers.*

Dating methods

*If new dates are provided, describe how they were obtained (e.g. collection, storage, sample pretreatment and measurement), where they were obtained (i.e. lab name), the calibration program and the protocol for quality assurance OR state that no new dates are provided.*

Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Male or female third instar larvae and adult flies (*Drosophila melanogaster*) were used for all experiments, except for Supplementary Fig.1B where only male w1118 and AD9, df(2L)clh4 /II third instar larvae were used.

The following genotypes were used:

D. melanogaster: Wild type: w1118  
 D. melanogaster: Ok6-GAL4/II  
 D. melanogaster: A22/WIM  
 D. melanogaster: AD9, df(2L)clh4 /II  
 D. melanogaster: UAS-Unc13A-GFP/Tm6b  
 D. melanogaster: Elav-Gal4/I  
 D. melanogaster: GluRIIB-GFP/III  
 D. melanogaster: UAS-C-term-GFP/III  
 D. melanogaster: rbpStop1/TM6b  
 D. melanogaster: rbp2.01/TM6b  
 D. melanogaster: rimex1.103/III  
 D. melanogaster: rimex1.103,fife1027/Tm6b  
 D. melanogaster: liprin- $\alpha$ F3ex15/Cyo,act-GFP  
 D. melanogaster: liprin- $\alpha$ R60/Cyo,act-GFP  
 D. melanogaster: syd-11.2/TM6c  
 D. melanogaster: syd-13.4/TM6c  
 D. melanogaster: UAS-Syd-1-GFP/Tm3,sb  
 D. melanogaster: UAS-Unc13A-RNAi/III  
 D. melanogaster: brp $\Delta$ 6.1/WIM  
 D. melanogaster: brp69/WIM  
 D. melanogaster: EMS7.5/CiD  
 D. melanogaster: UAS-BRP-D3-Straw/II  
 D. melanogaster: aplip-1ex213/TM6c

D. melanogaster: aplip-1ek4/III  
 D. melanogaster: srpk79Datc/III  
 D. melanogaster: Ok107-Gal4/IV  
 D. melanogaster: MB247-Gal4/III  
 D. melanogaster: Unc13null: ry506 ; P{ry11}unc-13P84200 / ciD  
 D. melanogaster: aplip-1ek4/III  
 D. melanogaster: Df(3L)BSC799/Tm6b  
 D. melanogaster: atg1ey07351/Tm6c  
 D. melanogaster: Df(3L)BSC10/Tm6b  
 D. melanogaster: Df(3L)BSC10/Tm6b  
 D. melanogaster: UAS-Aplip-1-RNAi/III  
 D. melanogaster: UAS-srpK79D-RNAi/III  
 D. melanogaster: VT1211-LexA, LexAop-GCaMP6f/CyO; sb/Tm3Ser

## Wild animals

Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.

## Field-collected samples

For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.

## Ethics oversight

Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.

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

## Human research participants

Policy information about [studies involving human research participants](#)

## Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, 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.

## Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

## Clinical trial registration

Provide the trial registration number from [ClinicalTrials.gov](#) or an equivalent agency.

## Study protocol

Note where the full trial protocol can be accessed OR if not available, explain why.

## Data collection

Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.

## Outcomes

Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.

## ChIP-seq

### Data deposition

- Confirm that both raw and final processed data have been deposited in a public database such as [GEO](#).  
 Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

## Data access links

May remain private before publication.

For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.

## Files in database submission

Provide a list of all files available in the database submission.

Genome browser session  
(e.g. [UCSC](#))

Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.

## Methodology

Replicates	<i>Describe the experimental replicates, specifying number, type and replicate agreement.</i>
Sequencing depth	<i>Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.</i>
Antibodies	<i>Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.</i>
Peak calling parameters	<i>Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.</i>
Data quality	<i>Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.</i>
Software	<i>Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.</i>

## Flow Cytometry

### Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation	<i>Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.</i>
Instrument	<i>Identify the instrument used for data collection, specifying make and model number.</i>
Software	<i>Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.</i>
Cell population abundance	<i>Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.</i>
Gating strategy	<i>Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.</i>

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

## Magnetic resonance imaging

### Experimental design

Design type	<i>Indicate task or resting state; event-related or block design.</i>
Design specifications	<i>Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.</i>
Behavioral performance measures	<i>State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).</i>

## Acquisition

Imaging type(s)

Field strength

Sequence & imaging parameters

Area of acquisition

Diffusion MRI  Used  Not used

## Preprocessing

Preprocessing software

Normalization

Normalization template

Noise and artifact removal

Volume censoring

## Statistical modeling & inference

Model type and settings

Effect(s) tested

Specify type of analysis:  Whole brain  ROI-based  Both

Statistic type for inference   
(See [Eklund et al. 2016](#))

Correction

## Models & analysis

n/a | Involved in the study

Functional and/or effective connectivity

Graph analysis

Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Graph analysis

Multivariate modeling and predictive analysis