# nature portfolio

Corresponding author(s):	Gilad Barnea
Last updated by author(s):	Jul 29, 2022

# **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.

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section

<u> </u>				
St	at	715	ŤΠ	$\cap \subseteq$

	To all statistical analyses, committed the following teems are present in the figure regend, table regend, main test, of 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				
x	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>				
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
x	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 <i>d</i> , Pearson's <i>r</i> ), 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 software version 2.1 was used to collect confocal data.

Data analysis Zeiss Zen software version 2.1 was used for image processing. PRISM 9 from GraphPad was used to analyse photophobia data

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 data are available in the main text or the supplementary materials. Source data are provided as a Source Data file.

## ife sciences study design

All studies must d	isclose on these points even when the disclosure is negative.
Sample size	Behavioural assays were run in a similar manner to Mazzoni et al. 2005 and Dettman et al. 2001. Our sample sizes (12-15 animals for each of the 12-15 replicas for each group) exceed both of these articles.
Data exclusions	No data were excluded from the analysis
Replication	All behavioral experiments were run over a three day period with 4-6 replica per day to account for reproducibility concerns. All attempts for replication were successful.
Randomization	Animals from each genotype/treatment were randomly selected from a group of larvae.

Blinding

The experimenter was blind to the genotype of the animals for inhibition experiments. For activation experiments, blinding could not be performed since the experimenter had to avoid chromosomal balancers and the presence of certain balancers indicated which genotype was being used.

### 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		Methods	
n/a	Involved in the study	n/a	Involved in the study
	x Antibodies	×	ChIP-seq
x	Eukaryotic cell lines	×	Flow cytometry
x	Palaeontology and archaeology	x	MRI-based neuroimaging
	🗶 Animals and other organisms		
x	Human research participants		
x	Clinical data		
X	Dual use research of concern		

### **Antibodies**

Antibodies used

The antibodies used in this study are: anti-PDF rabbit, anti-PTTH guinea pig, anti-PER mouse, anti-GFP rabbit (Thermo Fisher Scientific, A11122), anti-HA rat (Roche, 11867423001), anti-Brp mouse (nc82; DSHB), donkey anti-rabbit Alexa Fluor 488 (Thermo Fisher Scientific, A-21206), goat anti-rat Alexa Fluor 555 (Invitrogen, A21434), donkey anti-mouse Alexa Fluor 647 (Thermo Fisher Scientific, A-31571)

Validation

anti-PDF: This antibody was developed and first used by Dircksen, H. et al., Cell and Tissue Research 1987

anti-PTTH: This antibody was developed and first used by Yamanaka, N. et al., Science 2013

anti-PER: This antibody was developed and first used by Lamaze et al., Curr Biol 2018

anti-GFP: This commercial antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated. https://www.thermofisher.com/antibody/product/GFP-Antibody-Polyclonal/A-11122

anti-HA The commercially available Anti-HA-Biotin; High Affinity is function tested by Western blot analysis of a HA-tagged fusion protein. https://www.sigmaaldrich.com/US/en/product/roche/roahaha

anti-Brp: This antibody was developed by Wagh, et al. 2006 Neuron it is distributed by the DSHB.

donkey anti-rabbit Alexa Fluor 488: https://www.thermofisher.com/order/genome-database/generatePdf?productName=Rabbit% 20lgG%20(H+L)&assayType=PRANT&productId=A-21206&detailed=true

goat anti-rat Alexa Fluor 555: https://tools.thermofisher.com/content/sfs/manuals/mp02794.pdf

donkey anti-mouse Alexa Fluor 647: https://www.thermofisher.com/order/genome-database/generatePdf?productName=Mouse% 20lgG%20(H+L)&assayType=PRANT&productId=A-31571&detailed=true

#### Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Drosophila melanogaster, adult males and early third instar stage male and female larvae were used for all experiments. The genotypes of the flies used is included in the methods section of the article.

Wild animals

No wild animal was used

Field-collected samples

No field-collected samples were used.

Ethics oversight

Use of fruit flies in this study did not require ethical approval.

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