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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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

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For all statistical analys	es, 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 of	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. <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					
For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
Estimates of e	effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated				
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.				
Software and c	ode				
Policy information abo	ut availability of computer code				
Data collection	Two-photon imaging: Scanimage Video tracking system for sleep analysis: custom code in LabVIEW Electrophysiology: custom protocols in Nclamp/Neuromatic				
Data analysis	Two-photon imaging: custom script in MATLAB Video tracking system for sleep analysis: custom code in LabVIEW Electrophysiology: custom protocols in Neuromatic and Igor Pro Statistics: Prism				

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 <u>data availability statement</u>. 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 datasets generated during this study are available from the corresponding author upon reasonable request.

Field-specific reporting				
Please select the o	e one below that is the best fit for your research. If you are not sure, read the appropriate sections before makir	g your selection.		
\times Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences			
For a reference copy of	y of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	ences study design			
All studies must dis	disclose on these points even when the disclosure is negative.			
Sample size	Sample sizes in behavioural experiments (typically n=32 flies per genotype) were chosen to detect 2-h differences in daily sleep with a power of 0.9.			
Data exclusions	Immobile flies (< 2 beam breaks per 24 h) in standard sleep assays in the Trikinetics Drosophila Activity Monitor system Flies sleeping before the onset of illumination in video-tracking analyses of sleep after light-induced ROS generation Flies with a confirmed waking time <30 s in video-tracking analyses of waking movements after light-induced ROS generation Poorly constrained fits of slow inactivation time constants in voltage-clamp measurements of A-type currents			
Replication	All behavioural experiments were run at least three times, on different days and with different batches of flies. The figures show representative examples.			
Randomization	Female flies of a given genotype, as indicated in Methods and figure legends, were randomly selected for analysis.			
Blinding	The investigators were blind to group allocation in MitoTimer imaging experiments but not otherwise.			
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.				
		ciccing a response.		
n/a Involved in th	experimental systems n the study Methods n/a Involved in the study			
Antibodies				
	otic cell lines Flow cytometry			
Palaeontol				
Animals ar	s and other organisms			

Animals and other organisms

Human research participants

Clinical data

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

Laboratory animals	Female Drosophila melanogaster strains, aged 2–6 days after eclosion, carrying transgenes, transgene combinations, or genomic mutations as indicated in Methods and figure legends.
Wild animals	n/a
Field-collected samples	n/a
Ethics oversight	No ethical approval was required for research on Drosophila melanogaster.

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