nature portfolio

Corresponding author(s):	Denise J Montell
Last updated by author(s):	Dec 20, 2021

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>.

~ .					
Λt	. 그	t١	121	П	\sim

For	all statistical ar	halyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a	Confirmed					
	The exact	exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
	A stateme	tatement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly				
	The statis Only comm	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 descript	tion of all covariates tested				
	A descript	tion 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 coefficien AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)					
	For null h	ypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted see as exact values whenever suitable.				
\boxtimes	For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
\boxtimes	For hierar	chical and complex designs, identification of the appropriate level for tests and full reporting of outcomes				
\boxtimes	Estimates	of 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.				
So	ftware an	d code				
Poli	cy information	about <u>availability of computer code</u>				
Da	ata collection	Zeiss ZEN 2012 SP5 FP3 (black) release version 14 and ZEN 2.5 (blue edition) of microscope software.				
Da	ata analysis	Image J 1.53c-f, Imaris v8.3.0, Graphpad Prism 9, SigmaPlot 10, Adobe Photoshop CC, Adobe Illustrator CC				
For m	nanuscripts utilizing	g 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				

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:

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.

- 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

Complete data is available in the main article, supplementary materials and in the source data files. All the raw data supporting the findings of this study are available from the corresponding authors upon reasonable request.

Field-spe	ecific	reporting			
Please select the or	ne 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 t	the docume	ent with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>			
Life scier	nces	study design			
All studies must dis	sclose on	these points even when the disclosure is negative.			
Sample size	experime reproduc	uple size was not statistically determined but all experiments were carried out using at least 10 animals per condition. Also the eriments were conducted and followed for different time periods as well as in multiple replicates with minimum of 3 times for roducibility which from experience is sufficient to give true representation. Variability between the replicates were not dramatically need with in each group and time period analyzed.			
Data exclusions	No data	a were excluded from the analyses.			
Replication	Experime	riments were repeated at least 3 times for reproducibility and were successful.			
Randomization		nental groups were formed based on the genotype or based on treatments in case of same genotypes. samples prepared, imaged or analyzed were randomly selected without any bias.			
Blinding	_	gators were not blinded since the experiments were carried out over progressing aging conditions and different treatments with le controls. Also the different temperature set up is easily detectable and blinding is not standard in the field.			
		r specific materials, systems and methods uthors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material,			
'		vant 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			
Antibodies		ChIP-seq			
Eukaryotic cell lines					
Palaeontology and archaeology MRI-based neuroimaging					
Animals and other organisms					
Human research participants Clinical data					
Dual use research of concern					
Antibodies					
Antibodies used		The antibodies used in this study are rat anti-Vasa (anti-vasa; DSHB; 1:20), mouse anti-Hts(1B1; DSHB; 1:20), mouse anti-H2AvD (UNC93-5.2.1; DSHB; 1:20); guinea pig anti-traffic jam (Tj) (gift from Dorothea Godt; 1:5000); rabbit anti-pH3(Cell Signaling Tech,			

The antibodies used in this study are rat anti-Vasa (anti-vasa; DSHB; 1:20), mouse anti-Hts(1B1; DSHB; 1:20), mouse anti-H2AvD (UNC93-5.2.1; DSHB; 1:20); guinea pig anti-traffic jam (Tj) (gift from Dorothea Godt; 1:5000); rabbit anti-pH3(Cell Signaling Tech, #9701, Lot. 16; 1:200); rabbit anti-pMAD/anti-Smad (Abcam, ab52903, Lot. GR3194559-4; 1:400); mouse anti-Dpp (R&D SYSTEMS, MAB159, Lot. FRW022006A; 1:200); rabbit anti-dAnillin8-8(Gift from Christine Field; 1:500) and rabbit anti-ACTIVE Caspase-3 (Promega, G748A, Lot. 0000237765; 1:500).

Validation

anti-Vasa - please see Yan Song and Bingwei Lu, Genes Dev 2011 and https://dshb.biology.uiowa.edu/anti-vasa anti-Hts - please see Rachel T Cox and Allan C Spradling, Development 2003 and https://dshb.biology.uiowa.edu/1B1 anti-H2AvD - please see Cathleen M Lake et al., G3(Bethesda) 2013 and https://dshb.biology.uiowa.edu/UNC93-5-2-1 anti-traffic jam - please see Trupti Panchal et al., PLoS Genet 2017

 $anti-pH3 - please see \ Natalie \ A. \ Dye et al., Development \ 2017 \ and \ https://www.cellsignal.com/products/primary-antibodies/phospho-histone-h3-ser 10-antibody/9701$

 $anti-pMAD/anti-Smad-please\ see\ https://www.abcam.com/smad3-phospho-s423--s425-antibody-ep823y-ab52903.html?\ productWallTab=ShowAll$

 $anti-Dpp-please\ see\ Yu-Han\ Su\ et\ al.,\ Development\ 2018\ and\ https://www.rndsystems.com/products/drosophila-decapentaplegic-dpp-antibody-146609_mab159$

anti-dAnillin - please see Christine M Field and Bruce M Alberts, J Cell Biol 1995

 $anti-ACTIVE\ Caspase-3-please\ see\ https://www.promega.com/-/media/files/resources/protocols/product-information-sheets/g/seeds-files/files/resources/protocols/product-information-sheets/g/seeds-files/files/files/resources/protocols/product-information-sheets/g/seeds-files/files$

Animals and other organisms

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

Laboratory animals

Study involved Drosophila melanogaster, Drosophila virilis (gift from Giovanni Bosco) and Leptopilina heterotoma Lh14 strain (gift from Giovanni Bosco). The Drosophila melanogater strains were from Bloomington Drosophila Stock Center (BDSC) - Canton-S (CS) (#64349), Ubi-FRT-STOP-FRT-nEGFP (#32251), hs-FLP (#55815), Aug21-GAL4 (#30137), UAS-HA-NiPp1 (#23711); p53[5A-1-4] (#6815); Bam∆86 (#5427); bam deficiency (#27401); tub-mito-roGFP2-Orp1 (#67673); UASp-PAGFP-alphaTub84B (#32075); nos-GAL4 vp16 (gift from Jecelyn McDonald); lok-KD and lok-P30 (gift from Pamela Geyer); P53R-GFP NLS biosensor (gift from John M Abrams). Female virgin flies are used in experiments with different age/time period as mentioned for different experiments.

Wild animals

Study did not involve wild animals.

Field-collected samples

Study did not involve samples collected from the field.

Ethics oversight

Drosophila and wasp strains were obtained and reared according to standard protocols and institutional regulations from the University of California Santa Barbara.

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