

## 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](#).

### 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** A custom software was used to capture and synchronize video (using two cameras (FLIR BFS-U3-200S6M-C, Edmund optics #11-521) with 50mm lens (Edmund optics #63-248)) and audio recordings (using a custom 96-channel recording apparatus). SLEAP (v1.2.0a6) was used to for behavioral tracking. The custom Matlab software Tempo (<https://github.com/JaneliaSciComp/tempo>) was used to annotate male songs, and when applicable, female WS in response to song.

**Data analysis** Data analysis was performed in Python (v3.8.13) with the following packages: h5py (v3.6.0), numpy (v1.23.5), scipy (v1.8.0), and pandas (v1.4.2), and R (v4.2.2) with the following packages: tidyverse (v1.3.2), lme4 (v1.1-31), emmeans (v1.8.3), lmerTest (v3.1-3), ape (v5.7.1), and BayesTraits (v4.1.2).

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 supporting the findings of this study and scripts used in data analysis are available in Supplementary Information. Source data are provided as a Source Data file.

## Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 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	We do not have a priori knowledge of population statistics such as standard deviation or expected effect size to inform calculations of sample sizes beforehand. Instead we used sample sizes of around 10-20 for each condition, which is typical of our field for capturing variability while maintaining experimental feasibility.
Data exclusions	Recordings of poor quality were excluded from analysis. In addition, flies with damaged wings and decapitated females that were unable to stand still during the activation experiments were excluded. These criteria were pre-established.
Replication	Replicates used were included in the statistical analyses and significance was confirmed using the appropriate statistical tests.
Randomization	Flies were raised in cohorts that developed and were collected at the same time. Flies used in experiments were randomly selected. Females were randomly assigned to treatment vs. control groups after eclosion in the antennae cut, arista cut, and wing cut experiments. For comparison between females grown at different development temperatures, the parental generation was randomly distributed into two groups, and their offspring were treated as described in Fig. 5 to generate the two groups.
Blinding	Behaviors were scored blind to the flies' treatment group to the extent possible (e.g. male songs were annotated blind to the female's treatment, female activation behaviors were annotated blind to their developmental temperature treatment, but videos of wing cut vs. intact females could not be blinded).

## 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 &amp; experimental systems

n/a	Involvement 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 and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

## Methods

n/a	Involvement 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	<p>Primary antibodies used were: chicken-anti-GFP (1:600, ab13970, Abcam), mouse-anti-nc82 (1:30, AB 2314866, DHSB). Secondary antibodies used were: goat-anti-chicken/AF488 (1:500, A-11039, Thermo Fisher), goat-anti-mouse/AF568 (1:500, A-11031, Thermo Fisher).</p>
Validation	<p>The primary and secondary antibodies used in this study were widely and extensively used for IHC in previously published research. Specificity information for chicken-anti-GFP (<a href="https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970#tab=datasheet">https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970#tab=datasheet</a>), mouse-anti-nc82 (<a href="https://dshb.biology.uiowa.edu/nc82">https://dshb.biology.uiowa.edu/nc82</a>), goat-anti-chicken/AF488 (<a href="https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&amp;productsubtype=antibody_secondary&amp;productId=A-11039&amp;version=Local">https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&amp;productsubtype=antibody_secondary&amp;productId=A-11039&amp;version=Local</a>) and goat-anti-mouse/AF568 (<a href="https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&amp;productsubtype=antibody_secondary&amp;productId=A-11031&amp;version=Local">https://www.thermofisher.com/order/genome-database/dataSheetPdf?producttype=antibody&amp;productsubtype=antibody_secondary&amp;productId=A-11031&amp;version=Local</a>) are available on the manufacturers' websites.</p>

## Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	Flies were maintained on cornmeal-agar-yeast medium (Fly Food B, Bloomington Recipe, Lab Express) at 23°C and 50% humidity on a 12 hour light/dark cycle, unless otherwise specified. All fly stocks used in this study are listed in Supplementary Table 1. We also generated Otd-Flp lines in <i>D. santomea</i> and <i>D. yakuba</i> . The methods used to generate the transgenic fly lines are described in more detail in the Materials and Methods. All injections were performed at Rainbow Transgenic Flies using a standard protocol.
Wild animals	The study did not involve wild animals.
Reporting on sex	Sex was determined based on external morphology. Both female and male <i>Drosophila</i> are used in the study, and the sex where each piece of data was collected from (e.g. wing angle, abdomen length, and probability of wing spreading in females, and courtship song features in males) was clearly stated in the text and figures.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	No ethical approval or guidance was required for <i>Drosophila</i> studies.

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

## Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A