

## Life Sciences Reporting Summary

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For further information on the points included in this form, see [Reporting Life Sciences Research](#). For further information on Nature Research policies, including our [data availability policy](#), see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### ▶ Experimental design

#### 1. Sample size

Describe how sample size was determined.

We predetermined sample sizes not using any statistical methods but based on effect sizes and sample-by-sample variability observed in pilot experiments.

#### 2. Data exclusions

Describe any data exclusions.

We excluded the data when animals failed to complete a predetermined number of trials in behavioral, behavioral genetics, and calcium imaging experiments. Criteria were established prior to data collection as described in "Behavioral experiments" and "Calcium imaging" in Methods.

#### 3. Replication

Describe whether the experimental findings were reliably reproduced.

The experimental findings were reliably reproduced as shown in the figures where we showed the entire distribution of data where appropriate. We showed only example data for the results of immunostaining experiments because the high repeatability of the method (Gal4-UAS based fluorescent protein expression) has been widely recognized in the field.

#### 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No randomization was used in sample allocation. Rather, we controlled the effects of covariates by systematically allocate samples into groups (e.g., sample #1 for group 1, sample #2 for group 2, sample #3 for group 1, sample #4 for group 2, and so on).

#### 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not relevant in all but behavioral genetics experiments where no blinding was used because of the limitation of human resource.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

## 6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- n/a Confirmed
- The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
  - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
  - A statement indicating how many times each experiment was replicated
  - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
  - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
  - The test results (e.g.  $P$  values) given as exact values whenever possible and with confidence intervals noted
  - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
  - Clearly defined error bars

See the web collection on [statistics for biologists](#) for further resources and guidance.

## ► Software

Policy information about [availability of computer code](#)

### 7. Software

Describe the software used to analyze the data in this study.

Data were analyzed using custom code written in MATLAB (MathWorks, 2012a, 2013a, 2015b).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). [Nature Methods guidance for providing algorithms and software for publication](#) provides further information on this topic.

## ► Materials and reagents

Policy information about [availability of materials](#)

### 8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

N/A

### 9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

rat anti-GFP (nacalai tesque Cat# 04404-84, RRID:AB\_10013361), mouse nc82 (DSHB Cat# nc82, RRID:AB\_528108), rabbit anti-RFP (Abcam Cat# ab62341, RRID:AB\_945213), Goat Anti-Rat CF488A (Biotium inc Cat#20023, RRID:AB\_10557403), Goat Anti-Mouse CF633 (Biotium inc Cat#20120, RRID:AB\_10556971), Goat Anti-Rabbit CF633 (Biotium inc Cat#20122, RRID:AB\_10559042). All antibodies are widely used in the field.

### 10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

N/A

b. Describe the method of cell line authentication used.

N/A

c. Report whether the cell lines were tested for mycoplasma contamination.

N/A

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

N/A

## ► Animals and human research participants

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Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

### 11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

Drosophila melanogaster, females, 2 to 3 days after eclosion.

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

### 12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

N/A