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Manuscript Number: NN-A57523B

Manuscript Type: Article

# Main Figures: 8

# Supplementary Figures: 12

# Supplementary Tables: 1

# Supplementary Videos: 4

## Reporting Checklist for Nature Neuroscience

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. For more information, please read [Reporting Life Sciences Research](#).

Please note that in the event of publication, it is mandatory that authors include all relevant methodological and statistical information in the manuscript.

### ► Statistics reporting, by figure

- Please specify the following information for each panel reporting quantitative data, and where each item is reported (section, e.g. Results, & paragraph number).
- Each figure legend should ideally contain an exact sample size (n) for each experimental group/condition, where n is an exact number and not a range, a clear definition of how n is defined (for example x cells from x slices from x animals from x litters, collected over x days), a description of the statistical test used, the results of the tests, any descriptive statistics and clearly defined error bars if applicable.
- For any experiments using custom statistics, please indicate the test used and stats obtained for each experiment.
- Each figure legend should include a statement of how many times the experiment shown was replicated in the lab; the details of sample collection should be sufficiently clear so that the replicability of the experiment is obvious to the reader.
- For experiments reported in the text but not in the figures, please use the paragraph number instead of the figure number.

**Note:** Mean and standard deviation are not appropriate on small samples, and plotting independent data points is usually more informative. When technical replicates are reported, error and significance measures reflect the experimental variability and not the variability of the biological process; it is misleading not to state this clearly.

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #	
example 1a	one-way ANOVA	Fig. legend	9, 9, 10, 15	mice from at least 3 litters/group	Methods para 8	error bars are mean +/- SEM	Fig. legend	p = 0.044	Fig. legend	F(3, 36) = 2.97	Fig. legend	
example results, para 6	unpaired t-test	Results para 6	15	slices from 10 mice	Results para 6	error bars are mean +/- SEM	Results para 6	p = 0.0006	Results para 6	t(28) = 2.808	Results para 6	

		TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
FIGURE NUMBER	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #	
+ -	2d	Chi-square for linear trend	Methods, Statistics section	15, 44, 20	clones labeled at 3 different embryonic stages	Fig. legend	N/A	Fig. legend	0.0005932	Fig. legend	Chi squared=11.8, df=1	not included
+ -	2e	linear regression analysis	Methods, Statistics section	23, 13, 9	clones labeled at 3 different embryonic stages	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	(****) p=2.5e-05	Fig. legend	t=-4.72, df=43	not included
+ -	3c	Unpaired t test with Welch's correction	Methods, Statistics section	77, 100	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	(****) p=1e-15	Fig. legend	t=15.47, df=3706	not included
+ -	3e	Unpaired t test with Welch's correction	Methods, Statistics section	11, 11, 11	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	RedGreen/RedGreen v.s. RedGreen/Yellow (****) p=1e-15; Yellow/Yellow v.s. RedGreen/Yellow (****) p=1e-15	Fig. legend	RedGreen/RedGreen v.s. RedGreen/Yellow (****) t=22.88, df=3615 Yellow/Yellow v.s. RedGreen/Yellow (****) t=27.43, df=3455	not included
+ -	4e	linear regression analysis	Methods, Statistics section	77, 77	individual clones	Fig. legend	N/A		NA		R-squared =0.0004	not included
+ -	4f	Unpaired t test with Welch's correction	Methods, Statistics section	25, 100	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	(****) p=1e-15	Fig. legend	t=21.08, df=2738	not included
+ -	5b	NA	NA	77	individual clones	Fig. legend	mean +/- SEM	Fig. legend	NA		NA	
+ -	6b	Mann Whitney test	Methods, Statistics section	9, 33	individual clones	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	(**) p=0.0083	Fig. legend	Mann-Whitney U=64, df=40	not included
+ -	6d	Mann Whitney test	Methods, Statistics section	5, 20	individual clones	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	(*) p=0.0122	Fig. legend	Mann-Whitney U=14, df=23	not included

+ -	6f	Mann Whitney test	Methods, Statistics section	9, 33	individual clones	Fig. legend	mean +/- SEM	Fig. legend	s/m-related, cyan (****): p=5e-05  Cognition- related, purple (****): p=2.5e-05  Unclear function, gray (n.s.): p=0.9735	Fig. legend	s/m-related, cyan : U=29, df=40  Cognition- related, purple (****): U=28, df=40  Unclear function, gray (n.s.): U=146.5, df=40	not included
+ -	7f	Chi-square test	Methods, Statistics section	order: 25, 8, 4  modality: 9,4,24	individual clones	Fig. legend	N/A	Fig. legend	100% cells in one group (****): p=0.00019  >80% cells in one group: p=0.21  Other (****): p=2e-06	Fig. legend	100% cells in one group: chi square=13.9294, df=1  >80% cells in one group: chi square=1.59. df=1  other: chi square=22.98 df=1	not included
+ -	8b	Mann Whitney test	Methods, Statistics section	9, 4; 33, 20	individual clones	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	anterior, white (*): p=0.0196  medial ventral/ posterior, black (n.s.): p=0.2176	Fig. legend	anterior, white (*): U=3, df=11  medial ventral/ posterior, black (n.s.): U=262, df=51	not included
+ -	S3a	Mann Whitney test	Methods, Statistics section	23, 23	individual clones	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	(*): p=0.0305	Fig. legend	U=166.5, df=44	not included

+ -	S3d	Unpaired t test with Welch's correction	Methods, Statistics section	18, 28, 19, 11, 100	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	E9 v.s. random (****): p=1e-15; E10 v.s. random (****): p=1e-15; E11 v.s. random (****): p=1e-15; E12 v.s. random (****): p=1e-15; E9 v.s. E10 (n.s.): p=0.6370; E10 v.s. E11 (n.s.): p=0.2656; E11 v.s. E12 (n.s.): p=0.5464 E9 v.s. E11 (n.s.): p=0.4961; E10 v.s. E12 (n.s.): p=0.0718; E9 v.s. E12 (n.s.): p=0.1746	Fig. legend	E9 v.s. random, red (****): t=15.45, df=3636; E10 v.s. random, red (****): t=15.70, df=3685; E11 v.s. random, red (****): t=14.13, df=3858; E12 v.s. random, red (****): t=14.05, df=3728 E9 v.s. E10 (n.s.): t=0.4720, df=3995; E10 v.s. E11 (n.s.): t=1.113, df=3953; E11 v.s. E12 (n.s.): t=0.6032, df=3971 E9 v.s. E11 (n.s.): t=0.6808, df=3929; E10 v.s. E12 (n.s.): t=1.801, df=3996; E9 v.s. E12 (n.s.): t=1.358, df=3988	not included
+ -	S5b	N/A	Methods, Statistics section	45	individual brains	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	N/A	N/A	N/A	N/A
+ -	S5c	N/A	Methods, Statistics section	45	individual brains	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	N/A	N/A	N/A	N/A
+ -	S5e	N/A	Methods, Statistics section	45	individual brains	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	N/A	N/A	N/A	N/A
+ -	S6c	Mann Whitney test	Methods, Statistics section	33, 9, 18, 17	clones in 4 different clusters	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	'mvp' v.s. 'a', (**): p=0.0051 'mvp' v.s. 'GABAergic', (**): p=0.0011	Fig. legend	'mvp' v.s. 'a', (**): U=60, df=40 'mvp' v.s. 'GABAergic', (**): U=125.5, df=49	not included

+ -	S6d	Mann Whitney test	Methods, Statistics section	33, 9, 18, 17	clones in 4 different clusters	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	'mvp' v.s. 'GABAergic', (****): p=3.7e-09  'a' v.s. 'GABAergic', (****): p=6.7e-05  'md' v.s. 'GABAergic', (****): p=8.8e-07  'mvp' v.s. 'a', (n.s.): p=0.3544  'md' v.s. 'a', (n.s.): p=0.9032  'mvp' v.s. 'md', (n.s.): p=0.2467	Fig. legend	'mvp' v.s. 'GABAergic', (****): U=31, df=49  'a' v.s. 'GABAergic', (****): U=13, df=24  'md' v.s. 'GABAergic', (****): U=21.5, df=33  'mvp' v.s. 'a', (n.s.): U=118, df=40  'md' v.s. 'a', (n.s.): U=78.5, df=25  'mvp' v.s. 'md', (n.s.): U=238.5, df=49	not included
+ -	S7	Silhouette coefficients	Methods, Statistics section	60	individual clones	Fig. legend	N/A	N/A	N/A	N/A	N/A	N/A
+ -	S11e	Unpaired t test with Welch's correction	Methods, Statistics section	31, 100	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	(****) p<1e-15	Fig. legend	t=16.38, df=3613	not included
+ -	S11b	Mann Whitney test	Methods, Statistics section		clones labeled at 3 different embryonic stages	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	E10-P21 (n.s.): p=0.8489; E11-P21 (n.s.): p=0.5715; E12-P21 (n.s.): p=0.1455	Fig. legend	E10-P21 (n.s.): U=121, df=32; E11-P21 (n.s.): U=55.5, df=21; E12-P21 (n.s.): U=17.5, df=11	not included
+ -	S11c	Mann Whitney test	Methods, Statistics section	anterior: 9, 5; medial dorsal: 18, 6; medial ventral posterior : 33, 21	clones located in 3 different regions	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	anterior (n.s.): p=0.9261; medial dorsal (n.s.): p=0.4215; medial ventral posterior (n.s.): p=0.1857	Fig. legend	anterior (n.s.): U=21.5, df=12; medial dorsal (n.s.): U=41.5, df=22; medial ventral posterior (n.s.): U=271.5, df=52	not included
+ -	S12a	Mann Whitney test	Methods, Statistics section	34, 24	individual clones	Fig. legend	median with interquartile range, whiskers are the minimum and maximum	Fig. legend	(n.s.): p=0.7622	Fig. legend	U=388.5, df=56	not included
+ -	S12b	Chi-square test	Methods, Statistics section	ctrl: 43, 36; SmoM2: 8,24	individual clones	Fig. legend	none	Fig. legend	(**)p=0.0033	Fig. legend	Chi squared=8.638, df=1	not included
+ -	S12c	unpaired t test with Welch's correction	Methods, Statistics section	14, 100	individual clones and 100 random simulations	Fig. legend	mean +/- SEM	Fig. legend	(****): p=1e-15	Fig. legend	t=24.74, df=2719	not included

## ► Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?

If so, what figure(s)?

All images are representative and most of them are accompanied by quantitative analysis. The following figures contain only representative images:

Main figures: Fig 1c, Fig 2a-c

Supplementary figures: Fig 1a,d, Fig2, Fig3b, Fig 6b, Fig 11d, Fig 8b, Fig 10, Fig 11d

2. For each representative image, is there a clear statement of how many times this experiment was successfully repeated and a discussion of any limitations in repeatability?

If so, where is this reported (section, paragraph #)?

No, but all were representative of at least three experimental replicates.

## ► Statistics and general methods

1. Is there a justification of the sample size?

If so, how was it justified?

Where (section, paragraph #)?

Even if no sample size calculation was performed, authors should report why the sample size is adequate to measure their effect size.

No statistical methods were used to predetermine sample sizes but our sample sizes are similar to those reported in previous publications, as stated in the Statistics section in the Methods.

2. Are statistical tests justified as appropriate for every figure?

Where (section, paragraph #)?

Yes.  
See details in Methods.

- a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Yes.

- b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?

Where is this described (section, paragraph #)?

Yes. All meet criteria of  $p < 0.05$  in all tests.

- c. Is there any estimate of variance within each group of data? Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

Yes. Most graphs are represented as median with interquartile range with whiskers representing the minimum and maximum. Some graphs were represented as mean  $\pm$  s.e.m, accompanied by the individual values. Details are described in the corresponding Figure legends and methods.

- d. Are tests specified as one- or two-sided?

Two-sided

- e. Are there adjustments for multiple comparisons?

No

3. To promote transparency, *Nature Neuroscience* has stopped allowing bar graphs to report statistics in the papers it publishes. If you have bar graphs in your paper, please make sure to switch them to dot-plots (with central and dispersion statistics displayed) or to box-and-whisker plots to show data distributions.
- Box-and-whisker plots were used in most figures reporting statistics.
- Bar graphs in Fig. 6f, 7b were all accompanied by the value of individual samples in Fig. 6e, 7a respectively.
4. Are criteria for excluding data points reported?  
Was this criterion established prior to data collection?  
Where is this described (section, paragraph #)?
- No data points were excluded.
5. Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.  
If no randomization was used, state so.  
Where does this appear (section, paragraph #)?
- No randomization was used.
6. Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?  
If no blinding was done, state so.  
Where (section, paragraph #)?
- No blinding experiment was done, because all reported differences amongst groups were clear.
7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?  
Where (section, paragraph #)?
- Yes, in Methods section, paragraph 1.
8. Is the species of the animals used reported?  
Where (section, paragraph #)?
- Yes, in Methods section, paragraph 1.
9. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?  
Where (section, paragraph #)?
- Yes, in Methods section, paragraph 1.
10. Is the sex of the animals/subjects used reported?  
Where (section, paragraph #)?
- No. Both male and female mice were used without distinction.
11. Is the age of the animals/subjects reported?  
Where (section, paragraph #)?
- Yes, in Methods section and Figures, Figure legends for all figures.
12. For animals housed in a vivarium, is the light/dark cycle reported?  
Where (section, paragraph #)?
- No
13. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?  
Where (section, paragraph #)?
- No

14. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?

Where (section, paragraph #)?

No

15. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

Where (section, paragraph #)?

No

a. If multiple behavioral tests were conducted in the same group of animals, is this reported?

Where (section, paragraph #)?

No behavioral tests in this study.

16. If any animals/subjects were excluded from analysis, is this reported?

Where (section, paragraph #)?

No animals were excluded.

a. How were the criteria for exclusion defined?

Where is this described (section, paragraph #)?

N/A

b. Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.

Where is this described (section, paragraph #)?

N/A

## ► Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?

Yes, by the previous publications and the vendors, as reported in the Methods section, paragraph 2.

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

Yes, in the Methods section, paragraph 2.

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

Yes, in the Methods section, paragraph 2.

2. Cell line identity

a. Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by [ICLAC](#) and [NCBI Biosample](#)?

Where (section, paragraph #)?

No cell lines were used.

b. If yes, include in the Methods section a scientific justification of their use--indicate here in which section and paragraph the justification can be found.

- c. For each cell line, include in the Methods section a statement that specifies:
- the source of the cell lines
  - have the cell lines been authenticated? If so, by which method?
  - have the cell lines been tested for mycoplasma contamination?

Where (section, paragraph #)?

## ► Data availability

Provide a Data availability statement in the Methods section under "Data availability", which should include, where applicable:

- Accession codes for deposited data
- Other unique identifiers (such as DOIs and hyperlinks for any other datasets)
- At a minimum, a statement confirming that all relevant data are available from the authors
- Formal citations of datasets that are assigned DOIs
- A statement regarding data available in the manuscript as source data
- A statement regarding data available with restrictions

See our [data availability and data citations policy page](#) for more information.

Data deposition in a public repository is mandatory for:

- a. Protein, DNA and RNA sequences
- b. Macromolecular structures
- c. Crystallographic data for small molecules
- d. Microarray data

Deposition is strongly recommended for many other datasets for which structured public repositories exist; more details on our data policy are available [here](#). We encourage the provision of other source data in supplementary information or in unstructured repositories such as [Figshare](#) and [Dryad](#).

We encourage publication of Data Descriptors (see [Scientific Data](#)) to maximize data reuse.

Where is the Data Availability statement provided (section, paragraph #)?

The data that support the findings of this study are available from the corresponding author upon request.

## ► Computer code/software

Any custom algorithm/software that is central to the methods must be supplied by the authors in a usable and readable form for readers at the time of publication. However, referees may ask for this information at any time during the review process.

1. Identify all custom software or scripts that were required to conduct the study and where in the procedures each was used.

As stated in the Methods section.

As stated in the Methods section.

2. If computer code was used to generate results that are central to the paper's conclusions, include a statement in the Methods section under "**Code availability**" to indicate whether and how the code can be accessed. Include version information as necessary and any restrictions on availability.

Code availability was stated in the "Data and code availability" section in the Methods: "the scripts and data that support the findings of this study are available from the corresponding author upon request."

## ▶ Human subjects

1. Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

No human subjects in this study.

2. Is demographic information on all subjects provided?

Where (section, paragraph #)?

3. Is the number of human subjects, their age and sex clearly defined?

Where (section, paragraph #)?

4. Are the inclusion and exclusion criteria (if any) clearly specified?

Where (section, paragraph #)?

5. How well were the groups matched?

Where is this information described (section, paragraph #)?

6. Is a statement included confirming that informed consent was obtained from all subjects?

Where (section, paragraph #)?

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?

Where (section, paragraph #)?

## ▶ fMRI studies

For papers reporting functional imaging (fMRI) results please ensure that these minimal reporting guidelines are met and that all this information is clearly provided in the methods:

1. Were any subjects scanned but then rejected for the analysis after the data was collected?

No.

- a. If yes, is the number rejected and reasons for rejection described?

Where (section, paragraph #)?

2. Is the number of blocks, trials or experimental units per session and/or subjects specified?

Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified?

4. Is a blocked, event-related, or mixed design being used? If applicable, please specify the block length or how the event-related or mixed design was optimized.

5. Is the task design clearly described?

Where (section, paragraph #)?

6. How was behavioral performance measured?

7. Is an ANOVA or factorial design being used?

8. For data acquisition, is a whole brain scan used?

If not, state area of acquisition.

- a. How was this region determined?

9. Is the field strength (in Tesla) of the MRI system stated?

- a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?

- b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?

10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?

11. Is the coordinate space for the anatomical/functional imaging data clearly defined as subject/native space or standardized stereotaxic space, e.g., original Talairach, MNI305, ICBM152, etc? Where (section, paragraph #)?

12. If there was data normalization/standardization to a specific space template, are the type of transformation (linear vs. nonlinear) used and image types being transformed clearly described? Where (section, paragraph #)?

13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?

14. Were any additional regressors (behavioral covariates, motion etc) used?
15. Is the contrast construction clearly defined?
16. Is a mixed/random effects or fixed inference used?
- a. If fixed effects inference used, is this justified?
17. Were repeated measures used (multiple measurements per subject)?
- a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?
19. Are statistical inferences corrected for multiple comparisons?
- a. If not, is this labeled as uncorrected?
20. Are the results based on an ROI (region of interest) analysis?
- a. If so, is the rationale clearly described?
- b. How were the ROI's defined (functional vs anatomical localization)?
21. Is there correction for multiple comparisons within each voxel?
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?

## ► Additional comments

Additional Comments