nature neuroscience

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Main Figures: 5

Supplementary Figures: 9

Supplementary Tables: 1

Supplementary Videos: N/A

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
+ -	1B	Two-tailed unpaired T-test	Fig. legend	19	cells from 2 batches of culture	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.02	Results para 2	F(1,17) = 2.567	Fig. legend

Nature Neuroscience: doi:10.1038/nn.3752

		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 #
+ -	1C	ANOVA followed by Dunnett's post hoc using wildtype Halo57 as reference	Fig. legend	46	cells from 2 batches of culture	Fig legend.	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(3,42) = 5.473	Fig. legend
+ -	1F	ANOVA followed by Newman-Keuls post hoc	Fig. legend	98	cells from 2 batches of culture	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.001	Fig. legend	F(2,95) = 14.02	Fig. legend
+ -	2C	ANOVA	Fig. legend	105	units from 6 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(4,105) = 22.1345	Fig. legend
+ -	2D	Kolmogorov -Smirnov test	Fig. legend	105	units from 6 mice	Fig. legend	N/A	Fig. legend	< 0.05	Fig. legend	----	----
+ -	2E	ANOVA	Fig. legend	103	units from 6 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(4,105) = 37.08	Fig. legend
+ -		ANOVA	Fig. legend	103	units from 6 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig legend	F(4,105) = 32.53	Fig. legend
+ -		ANOVA	Fig. legend	103	units from 6 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(4,105) = 10.43	Fig. legend
+ -	3C	ANOVA followed by Newman-Keuls post hoc	Fig. legend	106	units from 16 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(4,101) = 7.379	Fig. legend
+ -	3E	ANOVA followed by Newman-Keuls post hoc	Fig. legend	58	units from 9 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.05	Fig. legend	F(2,116) = 2.826	Fig. legend
+ -	3G	Two-tailed paired T-test	Fig. legend	16	units from 3 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.01	Fig. legend	F(1,14) = 4.485	Fig. legend
+ -	3H	Two-tailed paired T-test	Fig. legend	16	units from 3 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.001	Fig. legend	F(1,14) = 9.078	Fig. legend
+ -	4E	Two-tailed unpaired T-test (1.6 mW/mm2)	Fig. legend	10	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.7791	Fig. legend	F(1,8) = 0.2902	Fig. legend
+ -		Two-tailed unpaired T-test (6.3 mW/mm2)	Fig. legend	11	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.031	Fig. legend	F(1,4) = 3.264	Fig. legend
+ -		Two-tailed unpaired T-test (12.7 mW/mm2)	Fig. legend	11	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.0068	Fig. legend	F(1,9) = 3.494	Fig. legend
+ -		Two-tailed unpaired T-test (25.4 mW/mm2)	Fig. legend	8	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.0452	Fig. legend	F(1,6) = 2.422	Fig. legend

Nature Neuroscience: doi:10.1038/nn.3752

+ -		Two-tailed unpaired T-test (41.4 mW/mm2)	Fig. legend	8	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.0016	Fig. legend	F(1,6) = 5.422	Fig. legend
+ -		Two-tailed unpaired T-test (61.6 mW/mm2)	Fig. legend	12	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.0001	Fig. legend	F(1,10) = 11.4	Fig. legend
+ -		Two-tailed unpaired T-test (130 mW/mm2)	Fig. legend	11	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	< 0.0001	Fig. legend	F(1,9) = 9.275	Fig. legend
+ -		Two-tailed unpaired T-test (450 mW/mm2)	Fig. legend	10	units from 4 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.0025	Fig. legend	F(1,8) = 4.338	Fig. legend
+ -	4F	Two-tailed paired T-test (Jaws)	Fig. legend	5	units from 2 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.5685	Fig. legend	F(1,4) = 0.6205	Fig. legend
+ -		Two-tailed paired T-test (eNpHR3.0)	Fig. legend	6	units from 2 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.0121	Fig. legend	F(1,5) = 3.843	Fig. legend
+ -		Two-tailed paired T-test (Jaws-ER2)	Fig. legend	6	units from 2 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.1952	Fig. legend	F(1,5) = 1.496	Fig. legend
+ -	5J	Two-tailed paired T-test	Fig. legend	8	units from 6 mice	Fig. legend	Error bars are +/- SEM.	Fig. legend	0.5894	Fig. legend	F(1,7) = 0.5655	Fig. legend

Representative figures

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

   If so, what figure(s)?

   Fig. 2, 4, 5 and Supplementary Fig. 5.

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 #)?

   Page 19.

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.

   Yes, page 20. Sample sizes in this manuscript were similar to previous papers from our group and were chosen to reflect sample sizes that might be reflected by end users.

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

   Where (section, paragraph #)?

   Yes, page 20.

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

   Yes.

Nature Neuroscience: doi:10.1038/nn.3752

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, page 20.
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, see attached checklist.
d. Are tests specified as one- or two-sided?	Yes.
e. Are there adjustments for multiple comparisons?	Yes, this was done by applying a Newman-Keuls post-hoc test.
3. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?	Yes, pages 15 and 20. Electrophysiology data criterion was established prior to collection; light propagation data criterion were not (but are reported, p. 20).
4. 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 carried out. This is on p. 20.
5. 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 was carried out. This is on p. 20.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?	Yes, see page 14.
7. Is the species of the animals used reported? Where (section, paragraph #)?	Yes, see page 14, 15, 16, 17 and Supplementary Table 1.
8. Is the strain of the animals (including background strains of KO/ transgenic animals used) reported? Where (section, paragraph #)?	Yes, see page 14, 15, 16, 17 and Supplementary Table 1.
9. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?	Yes, see page 14, 15, 16, 17 and Supplementary Table 1.
10. Is the age of the animals/subjects reported? Where (section, paragraph #)?	Yes, see page 14, 15, 16, 17 and Supplementary Table 1.

Nature Neuroscience: doi:10.1038/nn.3752

11. For animals housed in a vivarium, is the light/dark cycle reported? Where (section, paragraph #)?	Yes, see page 14.
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?	Yes, see page 14.
13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?	N/A
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported? Where (section, paragraph #)?	Yes, see page 14.
a. If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	N/A
15. If any animals/subjects were excluded from analysis, is this reported? Where (section, paragraph #)?	Yes, see page 20.
a. How were the criteria for exclusion defined? Where is this described (section, paragraph #)?	Some light propagation data was not saved, accidentally, and thus was not possible to analyze. See page 20.
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.
a. Is antibody catalog number given? Where does this appear (section, paragraph #)?	Yes, see page 19.
b. Where were the validation data reported (citation, supplementary information, Antibodypedia)? Where does this appear (section, paragraph #)?	The staining was done as carried out in a previous manuscript, see page 19.

Nature Neuroscience: doi:10.1038/nn.3752

2. If cell lines were used to reflect the properties of a particular tissue or disease state, is their source identified?

   Where (section, paragraph #)?

   N/A

   a. Were they recently authenticated?

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

   N/A

Data deposition

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.

1. Are accession codes for deposit dates provided?

   Where (section, paragraph #)?

   These will be provided on page 14 by the final paper publication date.

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.

   N/A

2. Is computer source code/software provided with the paper or deposited in a public repository? Indicate in what form this is provided or how it can be obtained.

   N/A

Human subjects

1. Which IRB approved the protocol?

   Where is this stated (section, paragraph #)?

   N/A

2. Is demographic information on all subjects provided?

   Where (section, paragraph #)?

   N/A

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

   Where (section, paragraph #)?

   N/A

Nature Neuroscience: doi:10.1038/nn.3752

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

   Where (section, paragraph #)?

   N/A

5. How well were the groups matched?

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

   N/A

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

   Where (section, paragraph #)?

   N/A

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

   Where (section, paragraph #)?

   N/A

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?

   N/A

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

      Where (section, paragraph #)?

      N/A

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

   Where (section, paragraph #)?

   N/A

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

   N/A

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.

   N/A

5. Is the task design clearly described?

   Where (section, paragraph #)?

   N/A

6. How was behavioral performance measured?

   N/A

7. Is an ANOVA or factorial design being used?

   N/A

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

   If not, state area of acquisition.

   N/A

Nature Neuroscience: doi:10.1038/nn.3752

a. How was this region determined?	N/A
9. Is the field strength (in Tesla) of the MRI system stated?	N/A
a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?	N/A
b. Are the field-of-view, matrix size, slice thickness, and TE/TR/ flip angle clearly stated?	N/A
10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?	N/A
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 #)?	N/A
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 #)?	N/A
13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?	N/A
14. Were any additional regressors (behavioral covariates, motion etc) used?	N/A
15. Is the contrast construction clearly defined?	N/A
16. Is a mixed/random effects or fixed inference used?	N/A
a. If fixed effects inference used, is this justified?	N/A
17. Were repeated measures used (multiple measurements per subject)?	N/A
a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?	N/A
18. If the threshold used for inference and visualization in figures varies, is this clearly stated?	N/A
19. Are statistical inferences corrected for multiple comparisons?	N/A
a. If not, is this labeled as uncorrected?	N/A

Nature Neuroscience: doi:10.1038/nn.3752

20. Are the results based on an ROI (region of interest) analysis?	N/A
a. If so, is the rationale clearly described?	N/A
b. How were the ROI's defined (functional vs anatomical localization)?	N/A
21. Is there correction for multiple comparisons within each voxel?	N/A
22. For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?	N/A

Additional comments

Additional Comments	

Nature Neuroscience: doi:10.1038/nn.3752