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.

<table>
<thead>
<tr>
<th>TEST USED</th>
<th>n</th>
<th>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/I/z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGURE</td>
<td>WHICH TEST?</td>
<td>SECTION &amp; PARAGRAPH #</td>
<td>EXACT VALUE</td>
<td>DEFINED?</td>
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<tr>
<td>1a</td>
<td>one-way ANOVA</td>
<td>Fig. legend</td>
<td>9, 9, 10, 15</td>
<td>mice from at least 3 litters/group</td>
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<tr>
<td>results para 6</td>
<td>unpaired t-test</td>
<td>Results para 6</td>
<td>15</td>
<td>slices from 10 mice</td>
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</table>

Nature Neuroscience: doi:10.1038/nn.4373
<table>
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<tr>
<th>FIGURE NUMBER</th>
<th>WHICH TEST?</th>
<th>TEST USED</th>
<th>n</th>
<th>DESCRIPTIVE STATS (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
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<tbody>
<tr>
<td>1b</td>
<td></td>
<td>Linear mixed-effects regression</td>
<td>47 /48</td>
<td>47 samples from 28 ASD cases/48 samples from 28 controls</td>
<td>RESULTS, para 2; Figure 1 legend</td>
<td>EFFECT SIZE (BETA VALUE), STANDARD ERROR</td>
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<tr>
<td></td>
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<td></td>
<td>Supplementary Table 2</td>
<td>df = 52; t-values for each miRNA can be computed as beta value/standard error</td>
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<tr>
<td>1c</td>
<td></td>
<td>Pearson correlation</td>
<td>58</td>
<td>miRNAs</td>
<td>RESULTS, para 2; Figure 1 legend</td>
<td>PEARSON CORRELATION COEFFICIENT</td>
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<tr>
<td>2b-2C</td>
<td></td>
<td>Pearson correlation</td>
<td>109, 47, 42</td>
<td>Brain tissue samples</td>
<td>Figure 2 legend</td>
<td>PEARSON CORRELATION COEFFICIENT</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Figure 2b</td>
<td>df = 107, 45, 40; R values indicated in Figure 2b</td>
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<tr>
<td>3a, 3d</td>
<td></td>
<td>Fisher’s exact test</td>
<td>See Supplementary Table 6 for the n for each test</td>
<td>Genes</td>
<td>Supplementary Table 6</td>
<td>ODDS RATIO</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Figure 3a, 3d; Supplementary Table 6</td>
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<tr>
<td>3b</td>
<td></td>
<td>Logistic regression</td>
<td>12291 (for the strongest targets), 12243 (for the most conserve d targets)</td>
<td>Genes</td>
<td>Supplementary Table 6</td>
<td>ODDS RATIO</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Figure 3b; Supplementary Table 6</td>
<td>df = 12288, 12240</td>
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<tr>
<td>3c</td>
<td></td>
<td>The INRICH algorithm</td>
<td>see Supplementary Table 6 for genomic interval and gene numbers</td>
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<td>Supplementary Table 6</td>
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<td></td>
<td>Supplementary Table 6</td>
<td>NA</td>
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<tr>
<td>4a-4c</td>
<td></td>
<td>Pearson correlation</td>
<td>101</td>
<td>Brain tissue samples</td>
<td>RESULTS, para 2; Figure 4 legend</td>
<td>PEARSON CORRELATION COEFFICIENT</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Figure 4a-4c</td>
<td>df = 99; R values indicated in Figure 4a-4c</td>
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<tr>
<td>Step</td>
<td>Method</td>
<td>Results</td>
<td>p-values</td>
<td>Fishers exact test</td>
<td>Logistic regression</td>
<td>One-sided t-test</td>
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<td>4d</td>
<td>Pearson correlation</td>
<td>Brain tissue samples</td>
<td>RESULTS para 21; Figure 4 legend</td>
<td>Figure 4d</td>
<td>df = 99, 43, 43; R values indicated in Figure 4d</td>
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<td>5a</td>
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<td>See Supplementary Table 6 for the n for each test</td>
<td>Genes</td>
<td>Supplementary Table 6</td>
<td>See Figure 5a and Supplementary Table 6 for the P value for each test</td>
<td>Figure 5a; Supplementary Table 6</td>
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<td>See Supplementary Table 6 for the n for each test</td>
<td>Genes</td>
<td>Supplementary Table 6</td>
<td>See Figure 5a and Supplementary Table 6 for the P value for each test</td>
<td>Figure 5c-5d; Supplementary Table 6</td>
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<td>6a</td>
<td>One-sided t-test</td>
<td>See Figure 6a</td>
<td>Genes</td>
<td>Figure 6a</td>
<td>Indicated in Figure 6a</td>
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<tr>
<td>6b</td>
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<td>See Supplementary Table 6 for the n for each test</td>
<td>Genes</td>
<td>Supplementary Table 6</td>
<td>Figure 6b, 6d, 6e; Supplementary Table 6</td>
<td>Figure 6b, 6d, 6e; Supplementary Table 6</td>
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<td>Figure 6c; Supplementary Table 6</td>
<td>Figure 6c; Supplementary Table 6</td>
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<td>One-sided t-test</td>
<td>See Figure 7a</td>
<td>Genes</td>
<td>Figure 7a</td>
<td>Indicated in Figure 7a</td>
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<td>8a-8c</td>
<td>One-sided t-test</td>
<td>See Figure 8a-8c</td>
<td>Genes</td>
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<td>8d-8e</td>
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<td>See Supplementary Table 6 for the n for each test</td>
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<td>Supplementary Table 6</td>
<td>Figure 8d-8e; Supplementary Table 6</td>
<td>Figure 8d-8e; Supplementary Table 6</td>
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<td>s</td>
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<td>Supplemenary Figure 2 legend</td>
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<td>s3f-3g</td>
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<td>Supplementary Figure 3 legend</td>
<td>5 - 8 (indicate d in Supplementary Figure 3)</td>
<td>Brain tissue samples</td>
<td>Supplementary Figure 3</td>
<td>medians and quartiles</td>
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<td>s8a-8h</td>
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<td>s9a-9c</td>
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<td>Supplementary Figure 9 legend</td>
<td>101, 54, 47 (for combine d, ASD, and CTL samples, respectiv ely)</td>
<td>Brain tissue samples</td>
<td>Supplementary Figure 9 legend</td>
<td>Pearson correlation coefficient</td>
</tr>
</tbody>
</table>
Representative figures

1. Are any representative images shown (including Western blots and immunohistochemistry/staining) in the paper?
   If so, what figure(s)?
   Yes. Figure 7b.

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 #)?
   Yes. Figure 7 legend.

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 pre-determine sample sizes as effect sizes were not known a priori, but our sample sizes are larger than those reported in previous publications that detected miRNA changes (ref. 41, 43, 44).

2. Are statistical tests justified as appropriate for every figure?
   Where (section, paragraph #)?
   Yes. METHODS para 28-29.

   a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?
      Yes. Summarized in METHODS para 25-29. Statistical tests clearly defined in RESULTS, FIGURE LEGENDS, and/or METHODS.

   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 #)?
      For DGE analyses using linear mixed-effects and linear models (Fig. 1b-1c, Supplementary Fig. 3a-3e, Supplementary Table 2), normality was not formally tested for each miRNA. For the main DGE analysis of 95 cortex samples, we also computed permutation-based P values and found that the P value rankings of miRNAs were highly concordant with those observed in the original sample set (Pearson’s R = 0.99, P < 2.2e-16). For calculation of Pearson correlations (Fig. 1c, 2b-2d, 4a-4d, Supplementary Fig. 3a-3e, 9a-9c), normality was not formally tested. One-sided t-tests were used for Fig. 6a, 7a, 8a-8c, Supplementary Fig. 5 because the distributions are approximately normal and sample sizes were reasonably large (n = 88-11695). For Supplementary Fig. 3f-3g, normality was tested by the Shapiro-Wilk test. All groups except for the CTL group for hsa-miR-10a-5p (P = 0.005) and the ASD group for hsa_can_1155-m (P = 0.03) meet the normality assumption (P > 0.05). Two-sided t-tests were used for all groups. For differential gene expression analysis after miRNA over-expression in hNPCs (Supplementary Table 6), one-sided t-tests were used but normality was not formally tested for each gene. One-tailed Wilcoxon rank sum tests were performed in Supplementary Fig. 8a-8h because the distributions do not appear to be normal. Described in METHODS para 28-29.
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 #)?

For all t-tests, equal variances were not formally tested, and so all tests were performed assuming unequal variance. For DGE analyses using linear mixed-effects and linear models, equal variances were not formally tested for each miRNA. Described in METHODS para 28.

Yes. Described in RESULTS, FIGURE LEGENDS, and/or METHODS.

Yes. The Benjamini–Hochberg procedure was used unless otherwise indicated. Described in FIGURE LEGENDS and/or METHODS.

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

e. Are there adjustments for multiple comparisons?

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.

No bar graphs were used to report statistics.

4. Are criteria for excluding data points reported? Was this criterion established prior to data collection? Where is this described (section, paragraph #)?

Inclusion criteria for cases were a clinical documentation of autism. Controls had to be free of known neuropsychiatric disorders and other conditions. Sample selection was also based on sequencing data quality control, outlier detection, and matching of covariates. The methodology for sample selection was described in METHODS para 1, 8-12.

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 #)?

Tissue dissection, library preparation, and sequencing of brain samples were randomized for balance of diagnostic status, age, sex, and brain region as described in METHODS para 1-2.

Tissue dissection, RNA extraction, library preparation, and sequencing were performed blind to all metadata information about the samples. Data analysis was not performed blind to metadata information about the samples. Describe in METHODS para 2, 25.

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 #)?

7. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?

NA

8. Is the species of the animals used reported? Where (section, paragraph #)?

NA

9. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? Where (section, paragraph #)?

NA

10. Is the sex of the animals/subjects used reported? Where (section, paragraph #)?

NA
11. Is the age of the animals/subjects reported?
   Where (section, paragraph #)?
   
   NA

12. For animals housed in a vivarium, is the light/dark cycle reported?
    Where (section, paragraph #)?
    
    NA

13. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
    Where (section, paragraph #)?
    
    NA

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

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

   a. If multiple behavioral tests were conducted in the same group of animals, is this reported?
      Where (section, paragraph #)?
      
      NA

16. If any animals/subjects were excluded from analysis, is this reported?
    Where (section, paragraph #)?
    
    NA

   a. How were the criteria for exclusion defined?
      Where is this described (section, paragraph #)?
      
      NA

   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 #)?
      
      NA

Reagents

1. Have antibodies been validated for use in the system under study (assay and species)?
   
   No antibody used.

   a. Is antibody catalog number given?
      Where does this appear (section, paragraph #)?
      
      NA
b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?

Where does this appear (section, paragraph #)?

NA

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.

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.

NA

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 #)?

Primary human neural progenitor cells were generated in a previous study and cultured as described (Stein JL et al. Neuron 83, 69–86 (2014)). Cells were free of mycoplasma contamination based on DAPI staining. Described in METHODS para 22.
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 #)?

METHODS para 31.

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.

   All analyses were performed using publicly available softwares, and relevant parameters are provided in METHODS.

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.

   We provide code for the DGE analysis using a linear mixed-effects model and the WGCNA analysis in Supplementary Code 1 and 2 (METHODS para 30).

Human subjects
1. Which IRB approved the protocol?  
   Where is this stated (section, paragraph #)?  
   No live human subjects were used.

2. Is demographic information on all subjects provided?  
   Where (section, paragraph #)?  
   Self-reported ethnicity was available through brain banks for a proportion of subjects and documented in Supplementary Table 1.

3. Is the number of human subjects, their age and sex clearly defined?  
   Where (section, paragraph #)?  
   Yes. Supplementary Table 1.

4. Are the inclusion and exclusion criteria (if any) clearly specified?  
   Where (section, paragraph #)?  
   Inclusion criteria for cases were a clinical documentation of autism. Controls had to be free of known neuropsychiatric disorders and other conditions. Sample selection was also based on sequencing data quality control, outlier detection, and matching of covariates. The methodology for sample selection was described in METHODS para 1, 8-12.

5. How well were the groups matched?  
   Where is this information described (section, paragraph #)?  
   Samples used for the DGE analysis were overall matched for age, sex, brain region, and other technical variables between ASD and control groups (MTHODS para 8-12; Supplementary Figure 1; Supplementary Table 1).

6. Is a statement included confirming that informed consent was obtained from all subjects?  
   Where (section, paragraph #)?  
   Tissue from subjects were made available through brain banks which had performed relevant consenting with families. Sample acquisition sources were noted in METHODS para 1.

7. For publication of patient photos, is a statement included confirming that consent to publish was obtained?  
   Where (section, paragraph #)?  
   No patient photo included.

### 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 fMRI results involved.

   a. If yes, is the number rejected and reasons for rejection described?  
      Where (section, paragraph #)?  
      NA

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

   Where (section, paragraph #)?

3. Is the length of each trial and interval between trials specified?  
   NA
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?
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>If fixed effects inference used, is this justified?</td>
<td>NA</td>
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<tr>
<td>Were repeated measures used (multiple measurements per subject)?</td>
<td>NA</td>
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<td>If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?</td>
<td>NA</td>
</tr>
<tr>
<td>If the threshold used for inference and visualization in figures varies, is this clearly stated?</td>
<td>NA</td>
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<tr>
<td>Are statistical inferences corrected for multiple comparisons?</td>
<td>NA</td>
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<tr>
<td>If not, is this labeled as uncorrected?</td>
<td>NA</td>
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<tr>
<td>Are the results based on an ROI (region of interest) analysis?</td>
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<tr>
<td>If so, is the rationale clearly described?</td>
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<td>How were the ROI’s defined (functional vs anatomical localization)?</td>
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<tr>
<td>Is there correction for multiple comparisons within each voxel?</td>
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<tr>
<td>For cluster-wise significance, is the cluster-defining threshold and the corrected significance level defined?</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Additional comments

Additional Comments

Nature Neuroscience: doi:10.1038/nn.4373