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>DESCRIBITIVE STATS (AVERAGE, VARIANCE)</th>
<th>P VALUE</th>
<th>DEGREES OF FREEDOM &amp; F/T/Z/R/ETC VALUE</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHICH TEST</td>
<td>SECTION &amp; PARAGRAPH #</td>
<td>EXACT VALUE</td>
<td>DEFINED?</td>
<td>REPORTED?</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.4160
<table>
<thead>
<tr>
<th>FIGURE NUMBER</th>
<th>TEST USED</th>
<th>WHICH TEST?</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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</thead>
<tbody>
<tr>
<td>2e</td>
<td>One way ANOVA</td>
<td>Methods</td>
<td>3,3,3,3,3, 3,3,3,3</td>
<td>Biological triplicates / cell type preparation</td>
<td>Results</td>
<td>LFQ intensity per replicate shown in heatmap</td>
</tr>
<tr>
<td>2e</td>
<td>Fisher’s exact test</td>
<td>Fig. legend</td>
<td>exact 'n' reported in Table 58</td>
<td>Total proteins and cluster specific proteins per annotation term</td>
<td>Fig. legend and Table 58 Enrichment, P value</td>
<td>P-value &lt; 0.02 ; exact P values reported in Table 58</td>
</tr>
<tr>
<td>3d</td>
<td>Fisher’s exact test</td>
<td>Fig. legend</td>
<td>exact 'n' is variable for each annotation term</td>
<td>Total proteins and &gt; 10 fold enriched proteins per annotation term</td>
<td>Fig. legend Enrichment, P value</td>
<td>P-value &lt; 0.02</td>
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<tr>
<td>5d</td>
<td>Fisher’s exact test</td>
<td>Fig. legend</td>
<td>exact 'n' is variable for each annotation term</td>
<td>Total proteins and &gt; 4 fold enriched proteins per annotation term</td>
<td>Fig. legend Enrichment, P value</td>
<td>P-value &lt; 0.02</td>
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<tr>
<td>6a</td>
<td>Wilcoxon-Mann-Whitney test</td>
<td>Methods</td>
<td>8147 quantified proteins</td>
<td>Methods position score per replicate per KEGG pathway annotation term</td>
<td>Fig 6a color coded heat map</td>
<td>FDR threshold of 0.02</td>
</tr>
<tr>
<td>6a</td>
<td>One way ANOVA</td>
<td>Methods</td>
<td>61</td>
<td>position scores for significant annotation terms for all cell type preparations</td>
<td>Methods position score per replicate per annotation term, P value</td>
<td>Fig 6a color coded heat map and table S10 FDR threshold of 0.001; exact P values reported in Table S10</td>
</tr>
<tr>
<td>6c</td>
<td>unpaired two sample t-tests</td>
<td>Methods</td>
<td>3,3,3,3,3, 3,3,3,3</td>
<td>Biological triplicates / cell type preparation</td>
<td>Results error bars are mean +/- SD</td>
<td>Fig 6a color coded heat map</td>
</tr>
<tr>
<td>8e</td>
<td>unpaired two-tailed t-test</td>
<td>Fig. legend</td>
<td>5,5,5,5,3, 3</td>
<td>mice/genotype</td>
<td>Fig. legend error bars are mean +/- SD</td>
<td>Fig. legend p=0.0055; P=0.0006; P=0.46</td>
</tr>
<tr>
<td>8f</td>
<td>One way ANOVA and Dunnett post hoc test for multiple comparison against control (pcDNA)</td>
<td>Fig. legend</td>
<td>3,3,3,3,3, 3</td>
<td>Experimental replicates, in which 10 fields were analyzed per condition per experiment</td>
<td>Fig. legend Error bars are mean +/- SEM</td>
<td>Fig. legend Left panel: ANOVA p=0.0087 Right panel: ANOVA p=0.0025 For pairwise comparison, *&lt;0.05, **&lt;0.01</td>
</tr>
<tr>
<td>8d</td>
<td>unpaired two tailed t-test</td>
<td>Fig. legend</td>
<td>3,3,3,3,3, 3</td>
<td>mice/genotype</td>
<td>Fig. legend error bars are mean +/- SD</td>
<td>Fig. legend P=0.252; P=0.0105; P=0.99</td>
</tr>
</tbody>
</table>
**Chi square test**

1. **Total fibers**
   - (P20) KO: 346; total fibers WT: 251.
   - 0.4-0.7 μm KO: 32 WT: 4
   - 0.7-1.0 μm KO: 252, WT: 146
   - 1.0-1.3 μm KO: 62, WT: 101
   - WT versus KO: 0.5-1.0 μm versus 0.7-1.0 μm versus 1.0-1.3 μm
   - Chi(2) = 43.041

2. **Total fibers**
   - (P30) KO: 317; total fibers WT: 261.
   - 0.4-0.7 μm KO: 125 WT: 7
   - 0.7-1.0 μm KO: 144, WT: 147
   - 1.0-1.3 μm KO: 48, WT: 57
   - WT versus KO: 0.5-1.0 μm versus 0.7-1.0 μm versus 1.0-1.3 μm
   - P = 2.8 x 10^-5
   - Chi(2) = 20.98

3. **Total fibers**
   - (P60) KO: 323; total fibers WT: 365.
   - <0.4 μm KO: 29, WT: 29
   - 0.4-0.7 μm KO: 185, WT: 222
   - 0.7-1.0 μm KO: 96, WT: 92
   - 1.0-1.3 μm KO: 13, WT: 22
   - WT versus KO: <0.4 versus 0.5-1.0 μm versus 0.7-1.0 μm versus 1.0-1.3 μm
   - P = 0.36
   - Chi(3) = 3.21

**S12d**

- Unpaired two tailed t-test
  - Mice/genotype
  - Fig. legend
  - t(4) = 2.675
  - P = 0.468

**Table S6**

- Wilcoxon-Mann-Whitney test
  - Quantified proteins
  - Methods
  - Page 33
  - Table S10
  - FDR threshold of 0.02
# Table S10

**One way ANOVA**

| Methods | 993 position scores for significant annotation terms for all cell type preparations | Methods | position score per replicate per annotation term, P value | Table S10 | FDR threshold of 0.001; exact P values reported in Table S10 | Methods | - | - |

# Table S15

**unpaired two tailed t-test**

| Methods | 4,4 mice | Results | Average expression and expression per replicate | Table S15 | P-value < 0.0001 | Methods | - | - |

# Table S16

**Wilcoxon-Mann-Whitney test**

| Methods | 10,783 ratio of quantified proteins between liver and brain | Results | position score per annotation term, P Value | Table S16 | FDR threshold of 0.02; exact P values reported in Table S16 | Methods and Table S16 | - | - |

# Table S13b

**Wilcoxon-Mann-Whitney test**

| Methods | 2,737 position scores for significant annotation terms for all cell type preparations | Methods | position score per cell type per annotation term | Fig S13b color coded heat map | P-value < 0.005 | Fig. legend | - | - |

# Table S4

**Wilcoxon-Mann-Whitney test**

| Methods | 119 position scores for significant annotation terms for P5, P14 and P24 stages if mouse cerebellum | Methods | position score per replicate per annotation term | Fig S4 color coded heat map | P-value < 0.005 | Fig. legend | - | - |

# Table S6b

**Wilcoxon-Mann-Whitney test**

| Methods | 10,008 quantified proteins | Methods | position score per annotation term per cell type | Show n as scatter plot in Fig S6b | Benjamini-Hochberg FDR threshold of 0.02 | Methods | - | - |

# Table S6c

**Wilcoxon-Mann-Whitney test**

| Methods | 10,008 quantified proteins | Methods | position score per annotation term per cell type | Show n as scatter plot in Fig S6c | Benjamini-Hochberg FDR threshold of 0.02 | Methods | - | - |

# Table S4

**Fisher’s exact test**

| Methods | exact ‘n’ reported in Table S3 | Total transcripts and transcripts not seen as proteins per annotation term | Table S3 | Enrichment, P value | Table S3 | P-value < 0.02; exact P values reported in Table S3 | Table S3 | - | - |

# Table S11

**Fisher’s exact test**

| Methods | exact ‘n’ reported in Table S11 | Total proteins and >10 fold enriched proteins per annotation term | Table S11 | Enrichment, P value | Table S11 | P-value < 0.02; exact P values reported in Table S11 | Table S11 | - | - |

# Table S12

**Fisher’s exact test**

| Methods | exact ‘n’ reported in Table S12 | Total transcripts and >10 fold enriched transcripts per annotation term | Table S12 | Enrichment, P value | Table S12 | P-value < 0.02; exact P values reported in Table S12 | Table S12 | - | - |

# Table S14

**Wilcoxon-Mann-Whitney test**

| Methods | 10,008 quantified proteins | Methods | position score per replicate per annotation term | Table S14 | FDR threshold of 0.02 | Methods and Table S14 | - | - |

# Table S18

**unpaired two sample t-tests**

| Methods | 4,4,4,4,4, 4,4,4,3,3 Biological quadruplicates / brain region And triplicates for optic nerve and corpus callosum | Legend to Figure 1 | Log2 Fold Expression (=t-test difference), Standard deviation p-value | Table S18 | pval < 0.05 | Table S18 | - | - |
### Representative figures

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

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?
   - The number of experiments are indicated in the figure legends

### Statistics and general methods

1. Is there a justification of the sample size?
   - We chose the sample size based on previous literature in the field.

2. Are statistical tests justified as appropriate for every figure?
   - The statistics were used based on the properties on the data points. Details are included in the figure legend.

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

   b. Do the data meet the assumptions of the specific statistical test you chose (e.g. normality for a parametric test)?
      - Based on previous literature we assumed that the data points have a normal distribution and used t-test or ANOVA in these cases

   c. Is there any estimate of variance within each group of data?
      - no

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

   e. Are there adjustments for multiple comparisons?
      - yes, in Fig.8f
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>3. Are criteria for excluding data points reported?</td>
<td></td>
</tr>
<tr>
<td>Was this criterion established prior to data collection?</td>
<td>no data were excluded</td>
</tr>
<tr>
<td>Where is this described (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>data analyzed in Fig8 and FigS12 was performed blinded</td>
</tr>
<tr>
<td>6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included? Where (section, paragraph #)?</td>
<td>no</td>
</tr>
<tr>
<td>7. Is the species of the animals used reported?</td>
<td>yes</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported? Where (section, paragraph #)?</td>
<td>yes</td>
</tr>
<tr>
<td>9. Is the sex of the animals/subjects used reported?</td>
<td>yes</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>10. Is the age of the animals/subjects reported?</td>
<td>yes, legends</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
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</tr>
<tr>
<td>11. For animals housed in a vivarium, is the light/dark cycle reported?</td>
<td>no</td>
</tr>
<tr>
<td>Where (section, paragraph #)?</td>
<td></td>
</tr>
<tr>
<td>12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported? Where (section, paragraph #)?</td>
<td>no</td>
</tr>
<tr>
<td>13. For behavioral experiments, is the time of day reported (e.g. light or dark cycle)? Where (section, paragraph #)?</td>
<td>N/A</td>
</tr>
</tbody>
</table>
14. Is the previous history of the animals/subjects (e.g. prior drug administration, surgery, behavioral testing) reported?

Where (section, paragraph #)?

N/A

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 #)?

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

a. Is antibody catalog number given?

Where does this appear (section, paragraph #)?

yes, methods

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

Where does this appear (section, paragraph #)?

in FigS11 we report validation data other antibodies are well established and reported by the companies that provide the antibodies

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 #)?

N/A

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.

N/A
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 #)?

Yes, it has been provided in the methods section under "MS data analysis"

The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium (http://proteomcentral.proteomexchange.org) via the PRIDE partner repository with the dataset identifier PXD001250. (Please visit http://tinyurl.com/pd244vr to access the data. The dataset can also be accessed through MaxQB database (http://maxqb.biochem.mpg.de/mxdb/project/show/P009).

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.

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

1. Are accession codes for deposit dates provided?

Where (section, paragraph #)?

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.

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.

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 #)?

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?

   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?
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>8. For data acquisition, is a whole brain scan used?</td>
<td>N/A</td>
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<tr>
<td>If not, state area of acquisition.</td>
<td></td>
</tr>
<tr>
<td>a. How was this region determined?</td>
<td>N/A</td>
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<tr>
<td>9. Is the field strength (in Tesla) of the MRI system stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. Is the pulse sequence type (gradient/spin echo, EPI/spiral) stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>b. Are the field-of-view, matrix size, slice thickness, and TE/TR/flip angle clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>10. Are the software and specific parameters (model/functions, smoothing kernel size if applicable, etc.) used for data processing and pre-processing clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>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 #)?</td>
<td>N/A</td>
</tr>
<tr>
<td>13. How were anatomical locations determined, e.g., via an automated labeling algorithm (AAL), standardized coordinate database (Talairach daemon), probabilistic atlases, etc.?</td>
<td>N/A</td>
</tr>
<tr>
<td>14. Were any additional regressors (behavioral covariates, motion etc) used?</td>
<td>N/A</td>
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<tr>
<td>15. Is the contrast construction clearly defined?</td>
<td>N/A</td>
</tr>
<tr>
<td>16. Is a mixed/random effects or fixed inference used?</td>
<td>N/A</td>
</tr>
<tr>
<td>a. If fixed effects inference used, is this justified?</td>
<td>N/A</td>
</tr>
<tr>
<td>17. Were repeated measures used (multiple measurements per subject)?</td>
<td>N/A</td>
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<tr>
<td>a. If so, are the method to account for within subject correlation and the assumptions made about variance clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>18. If the threshold used for inference and visualization in figures varies, is this clearly stated?</td>
<td>N/A</td>
</tr>
<tr>
<td>19. Are statistical inferences corrected for multiple comparisons?</td>
<td>N/A</td>
</tr>
</tbody>
</table>
a. If not, is this labeled as uncorrected?

N/A

20. Are the results based on an ROI (region of interest) analysis?

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