nature neuroscience

Corresponding Author:	Mann, Simons	# Main Figures:	8
Manuscript Number:	NN-RS50056C	# Supplementary Figures:	14
Manuscript Type:	Resource	# Supplementary Tables:	18
		# Supplementary Videos:	0

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 ST (AVERAGE, VARIA		P VALU	JE	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 #
+	2e	One way ANOVA	Metho ds	3,3,3,3,3, 3,3,3,3	Biological triplicates /cell type preparation	Results	LFQ intensity per replicate shown in heatmap	Fig. legend	FDR threshold of 0.05	Methods	-	-
+	2e	Fisher's exact test	Fig. legend	exact 'n' reported in Table S8	Total proteins and cluster specific proteins per annotation term	Fig. legend and Table S8	Enrichment, P value	Fig. legen d and Table S8	P-value < 0.02 ; exact P values reported in Table S8	Fig. legend and Table S8	-	-
+	3d	Fisher's exact test	Fig. legend	exact 'n' is variable for each annotati on term	Total proteins and > 10 fold enriched proteins per annotation term	Fig. legend	Enrichment, P value	Show n in figure 3d	P-value < 0.02	exact P values shown in fig 3d	-	-
+	5d	Fisher's exact test	Fig. legend	exact 'n' is variable for each annotati on term	Total proteins and > 4 fold enriched proteins per annotation term	Fig. legend	Enrichment, P value	Show n in figure 3d	P-value < 0.02	exact P values shown in fig 3d	-	-
+	6a	Wilcoxon- Mann- Whitney test	Metho ds	8147	quantified proteins	Methods	position score per replicate per KEGG pathway annotation term	Fig 6a color coded heat map	FDR threshold of 0.02	Methods	-	-
+ -	ба	One way ANOVA	Metho ds	61	position scores for significant annotation terms for all cell type preparations	Methods	position score per replicate per annotation term, P value	Fig 6a color coded heat map and table \$10	FDR threshold of 0.001; exact P values reported in Table S10	Methods	-	-
+	6c	unpaired two sample t-tests	Metho ds	3,3,3,3,3, 3,3,3,3	Biological triplicates /cell type preparation	Results	error bars are mean +/- SD	Fig. legend	FDR threshold of 0.02; exact P values reported in Table S5	Methods	-	-
+	8e	unpaired two-tailed t- test	Fig. legend	5,5,5,5,3,	mice/genotype	Fig. legend	error bars are mean +/- SD	Fig. legend	p=0055; P=0006; P=0.46	Fig. legend	t(8)=3.768; t(8)=5.464 t(4)=0.8099	-
+ -	8f	One way ANOVA and Dunnett post hoc test for multiple comparison against control (pcDNA)	Fig. legend	3,3,3,3,3,	Experimental replicates, in which 10 fields were analyzed per condition per experiment	Fig. legend	Error bars are mean +/- SEM	Fig. legend	Left panel: ANOVA p=0.0087 Right panel: ANOVA p=0.0025 For pairwise comparison, *=<0.05, **<0.01	Fig. legend	Left panel: F (5, 12) = 5,254 Right panel: F (6, 14) = 6,114	-
+	8d	unpaired two tailed t- est	Fig. legend	3,3,3,3,3,	mice/genotype	Fig. legend	error bars are mean +/- SD	Fig. legend	P=0.252; P=0.0105; P=0.99	Fig. legend	t(4)=1.338; t(4)=4.538; t(4)=0.0019	-

+ -	8c	Chi square test	Fig. legend	Total fibers (P20) KO: 346; total fibers WT: 251. 0.4-0.7µ m KO: 32 WT: 4 0,7-1,0µ m KO: 252, WT: 146 1,0-1,3µ m KO: 62, WT:	WT versus KO: 0,5-1,0μm versus 0,7-1,0μm versus 1,0-1,3μm	Fig. legend	-	-	P=4,5x10-10	Fig. legend	Chi(2)=43.041	-
+-	8c	Chi square test	Fig. legend	Total fibers (P30) KO: 317; total fibers WT: 261. 0.4-0.7µ m KO: 125 WT: 57 0,7-1,0µ m KO: 144, WT: 147 1,0-1,3µ m KO: 48, WT: 57	WT versus KO: 0,5-1,0μm versus 0,7-1,0μm versus 1,0-1,3μm	Fig. legend	-	-	P=2.8 x 10-5	Fig. legend	Chi(2)=20.98	-
+ -	8c	Chi square test	Fig. legend	Total fibers (P60) KO: 323; total fibers WT: 365. <0,4µm KO: 29,WT: 29 0.4-0.7µ m KO: 185, WT: 222 0,7-1,0µ m KO: 96, WT: 92 1,0-1,3µ m KO: 13, WT: 22	WT versus KO: <0,4 versus 0,5-1,0μm versus 0,7-1,0μm versus 1,0-1,3μm	Fig. legend	-	-	P= 0,36	Figure	Chi(3)=3,21	-
+	S12d	unpaired two tailed t- est	Fig. legend	3,3,4,4	mice/genotype	Fig. legend	error bars are mean +/- SD	Fig. legend	P=0.055; P=0.468	Fig. legend	t(4)=2.675 t(4)=0.8006	-
+	Tabl e S6	unpaired two sample t-tests	Metho ds	3,3,3,3,3, 3,3,3,3	Biological triplicates /cell type preparation	Results	Log2 Fold Expression, t-test difference, p-value	Table S6	FDR threshold of 0.02 ; exact P values reported in Table S5	Methods	-	-
+	Tabl e S10	Wilcoxon- Mann- Whitney test	Metho ds	8147	quantified proteins	Methods	position score per replicate per annotation term	Table S10	FDR threshold of 0.02	Methods Page 33 and Table S10	-	-

+	Tabl e S10	One way ANOVA	Metho ds	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	-	-
+	Tabl e S15	unpaired two tailed t- est	Metho ds	4,4	mice	Results	Average expression and expression per replicate	Table S15	P-value < 0.0001	Methods	-	-
+	Tabl e S16	Wilcoxon- Mann- Whitney test	Metho ds	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	-	-
+	S13b	Wilcoxon- Mann- Whitney test	Metho ds	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	-	-
+	S4	Wilcoxon- Mann- Whitney tes	Metho ds	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	-	-
+	S6b	Wilcoxon- Mann- Whitney test	Metho ds	10,008	quantified proteins	Methods	position score per annotation term per cell type	Show n as scatte r plot in Fig S6b	Benjamini- Hochberg FDR threshold of 0.02	Methods	-	-
+	S6c	Wilcoxon- Mann- Whitney tes	Metho ds	10,008	quantified proteins	Methods	position score per annotation term per cell type	Show n as scatte r plot in Fig S6c	Benjamini- Hochberg FDR threshold of 0.02	Methods	-	-
+	Tabl e S4	Fisher's exact test	Metho ds	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	-	-
+	Tabl e S11	Fisher's exact test	Metho ds	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	-	-
+	Tabl e S12	Fisher's exact test	Metho ds	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	-	
+	Tabl e S14	Wilcoxon- Mann- Whitney test	Metho ds	10,008	quantified proteins	Methods	position score per replicate per annotation term	Table S14	FDR threshold of 0.02	Methods Page 27 and Table S14	-	-
+	Tabl e S18	unpaired two sample t-tests	Metho ds	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	-	-

+ - S5	Wilcoxon- Mann- Whitney test	Metho ds	10,008	quantified proteins	Methods	position score per annotation term per cell type	Show n as scatte r plot in Fig S5	Benjamini- Hochberg FDR threshold of 0.02	Methods	-	-
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▶ Representative figures

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

If so, what figure(s)?

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 #)?

All images are shown as representatives

The number of experiments are indicated in the figure legends

▶ 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.

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

Where (section, paragraph #)?

We chose the sample size based on previous literature in the field.

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)?

Where is this described (section, paragraph #)?

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

Is the variance similar between groups that are being statistically compared?

Where is this described (section, paragraph #)?

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

e. Are there adjustments for multiple comparisons?

110

the tests are two-sides

yes, in Fig.8f

3.	Are criteria for excluding data points reported?	no data were excluded
	Was this criterion established prior to data collection?	
	Where is this described (section, paragraph #)?	
		\(\frac{1}{2}\)
4.	Define the method of randomization used to assign subjects (or samples) to the experimental groups and to collect and process data.	N/A
	If no randomization was used, state so.	
	Where does this appear (section, paragraph #)?	
5.	Is a statement of the extent to which investigator knew the group allocation during the experiment and in assessing outcome included?	data analyzed in Fig8 and FigS12 was performed blinded
	If no blinding was done, state so.	
	Where (section, paragraph #)?	
C	For experiments in live vertebrates, is a statement of compliance with	
Ь.	ethical guidelines/regulations included?	no
	Where (section, paragraph #)?	
7.	Is the species of the animals used reported?	yes
	Where (section, paragraph #)?	
8.	Is the strain of the animals (including background strains of KO/ transgenic animals used) reported?	yes
	Where (section, paragraph #)?	
9.	Is the sex of the animals/subjects used reported?	yes
	Where (section, paragraph #)?	
10	Is the age of the animals/subjects reported?	yes, legends
20.	Where (section, paragraph #)?	, 100, 100, 100
11.	For animals housed in a vivarium, is the light/dark cycle reported?	no
	Where (section, paragraph #)?	
12	For animals housed in a vivarium, is the housing group (i.e. number of	no
12.	animals per cage) reported?	110
	Where (section, paragraph #)?	
13.	For behavioral experiments, is the time of day reported (e.g. light or dark cycle)?	N/A
	Where (section, paragraph #)?	

14.		evious history of the animals/subjects (e.g. prior drug ration, surgery, behavioral testing) reported?	N/A
	Where (s	ection, paragraph #)?	
	a.	If multiple behavioral tests were conducted in the same group of animals, is this reported? Where (section, paragraph #)?	N/A
		where (section, paragraph #)?	
15.	If any ani	imals/subjects were excluded from analysis, is this reported?	no animals were excluded
	Where (s	ection, paragraph #)?	
	a.	How were the criteria for exclusion defined?	N/A
		Where is this described (section, paragraph #)?	
	b.	Specify reasons for any discrepancy between the number of animals at the beginning and end of the study.	N/A
		Where is this described (section, paragraph #)?	
		ibodies been validated for use in the system under study d species)?	Yes
	a.	Is antibody catalog number given?	yes, methods
		Where does this appear (section, paragraph #)?	
	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 i	dentity	N/A
	a.	Are any cell lines used in this paper listed in the database of commonly misidentified cell lines maintained by <u>ICLAC</u> and <u>NCBI Biosample</u> ?	
		Where (section, paragraph #)?	
	b.	If yes, include in the Methods section a scientific justification of their useindicate 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://proteomecentral.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. Identify all custom software or scripts that were required to conduct

the study and where in the procedures each was used.

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.

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 #)?

2.	Is demographic information on all subjects provided
	Where (section, paragraph #)?

N/A			
N/A			

3.	Is the number of human subjects, their age and sex clearly defined?	N/A		
	Where (section, paragraph #)?			
4.	Are the inclusion and exclusion criteria (if any) clearly specified?	N/A		
	Where (section, paragraph #)?			
_				
5.	How well were the groups matched?	N/A		
	Where is this information described (section, paragraph #)?			
6.	Is a statement included confirming that informed consent was obtained from all subjects?	N/A		
	Where (section, paragraph #)?			
7.	For publication of patient photos, is a statement included confirming that consent to publish was obtained?	N/A		
	Where (section, paragraph #)?			
	CAADL L. I			
1	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		
	If yes, is the number rejected and reasons for rejection described?	N/A		
	Where (section, paragraph #)?			
2.	Is the number of blocks, trials or experimental units per session and/ or subjects specified?	N/A		
	Where (section, paragraph #)?			
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?	N/A		
	Where (section, paragraph #)?			
	/222121, 62120.261.11).			
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?	N/A
If not, state area of acquisition.	
a. How was this region determined?	N/A
O to the Cold stormath (in Texts) of the MRI content state (2)	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
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	N/A
localization)?	
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