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Main Figures: 8

Supplementary Figures: 7

Supplementary Tables: 13

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
+ -												

FIGURE NUMBER	TEST USED		n			DESCRIPTIVE STATS (AVERAGE, VARIANCE)		P VALUE		DEGREES OF FREEDOM & F/t/z/R/ETC VALUE	
	WHICH TEST?	SECTION & PARAGRAPH #	EXACT VALUE	DEFINED?	SECTION & PARAGRAPH #	REPORTED?	SECTION & PARAGRAPH #	EXACT VALUE	SECTION & PARAGRAPH #	VALUE	SECTION & PARAGRAPH #
+ - 1b	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ - 1d	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ - 1e	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ - 1f	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ - 4c	one-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, methods qPCR section, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ - 4d	one-way ANOVA + Bonferroni correction	fig legend	3	microglia from 3 independent cell preparations	Methods flow cytometry section, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ - 4e	one-way ANOVA + Bonferroni correction	fig legend	3	microglia from 3 independent cell preparations	Methods flow cytometry section, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ - 4h	one-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13

+ -	5a	one-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values for all comparisons presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001 NB Only difference with greatest statistical significance displayed to avoid overcrowding on graphs	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	5g	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ -	5h	two-way ANOVA + Bonferroni correction	fig legend	3	Triplicate repeats pooled from 8 mice representative of two independent cell preparations	methods bacterial phagocytosis and replication assay section, fig legend	data are mean +/- SEM	fig legend	specific p values presented in Supp Table 13 *p<0.05	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	8c	two-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001 vs 4 months; #p<0.05, ##p<0.01, ###p<0.001 vs 12 months	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	8d	two-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001 vs 4 months; #p<0.05, ##p<0.01, ###p<0.001 vs 12 months	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	8f	two-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001 vs 4 months; #p<0.05, ##p<0.01, ###p<0.001 vs 12 months	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13

+ -	8h	two-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001 vs 4 months; #p<0.05, ##p<0.01, ###p<0.001 vs 12 months	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	Supp fig 1d	n/a	n/a	2	2 mice	Fig legend	data are mean +/- SEM	Fig legend	n/a	n/a	n/a	n/a
+ -	Supp fig 5c	one-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	Supp fig 6a	one-way ANOVA + Dunnett post-test (Cbm as "control" group)	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SEM	fig legend	specific p values presented in Supp Table 13 *p<0.05, **p<0.01, ***p<0.001	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13
+ -	Supp fig 6b	two-way ANOVA + Bonferroni correction	fig legend	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	*p<0.05, **p<0.01, ***p<0.001	fig legend	F values presented in Supp Table 13	Supp Table 13
+ -	Supp fig 6c	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ -	Supp fig 6d	n/a	n/a	4	microglia from 4 independent samples, each sample pooled from 8 mice	Methods para 3, fig legend	data are mean +/- SD	fig legend	n/a	n/a	n/a	n/a
+ -	Supp fig 7	one-way ANOVA + Bonferroni correction	fig legend	2	2 mice	Fig legend	data are mean +/- SEM	fig legend	specific p values presented in Supp Table 13 *p<0.05	fig legend, Supp Table 13	F values presented in Supp Table 13	Supp Table 13

► Representative figures

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

If so, what figure(s)?

Fig 1a
Fig 1g
Supp fig 1b
Supp fig 1c

- 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 #)?

Fig 1a - representative of four independent cell preparations, reported in fig legend
Fig 1g - representative of two independent cultures, reported in fig legend
Supp fig 1b - representative of staining on three independent cell preparations, reported in fig legend
Supp fig 1c - representative of four independent samples, reported in fig 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 formal a priori statistical methods were used to pre-determine sample sizes due to insufficient previous data to enable this. Sample sizes were chosen based on estimates of anticipated variability through previous general experience of microarray analysis and accounting for pooling of tissues reducing inter-replicate variance.

Statement included in Statistical analysis section of Methods

2. Are statistical tests justified as appropriate for every figure?
 Where (section, paragraph #)?
 - a. If there is a section summarizing the statistical methods in the methods, is the statistical test for each experiment clearly defined?

Yes, Statistical analysis section of 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 #)?

Assumptions for each statistical test were considered and in almost all cases these were met. Stated in Statistical analysis section of Methods.

 - 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, SDs or SEMs are presented in figures. In general, variance was similar across compared groups. Stated in Statistical analysis section of Methods.

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

All tests were two-sided. Stated in Statistical analysis section of Methods.

 - e. Are there adjustments for multiple comparisons?

Yes. FDR was used to filter microarray data and is stated throughout the manuscript at appropriated places where this was applied. Bonferroni correction was used for ANOVA tests and is stated in methods and figure legends. Default correction adjustments were applied for GO enrichment analysis.

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

No data were excluded.

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 #)?

Randomisation was performed using random number generator software (<https://www.randomizer.org/>) to assign to treatment group (age) and to distribute mice from different cages equally among replicate pools. Stated in Statistical analysis section of Methods.

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 #)?
- Data collection and analysis were performed with the assessor unaware of allocation to treatment group. Stated in Statistical analysis section of Methods.
6. For experiments in live vertebrates, is a statement of compliance with ethical guidelines/regulations included?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
7. Is the species of the animals used reported?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
8. Is the strain of the animals (including background strains of KO/transgenic animals used) reported?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
9. Is the sex of the animals/subjects used reported?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
10. Is the age of the animals/subjects reported?
Where (section, paragraph #)?
- Yes. Methods paragraph 3, throughout text and in fig legends.
11. For animals housed in a vivarium, is the light/dark cycle reported?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
12. For animals housed in a vivarium, is the housing group (i.e. number of animals per cage) reported?
Where (section, paragraph #)?
- Yes. Statement included in Methods paragraph 2 (Mice).
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 #)?
- 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 #)?
- None 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 #)?

Stated in Methods

b. Where were the validation data reported (citation, supplementary information, Antibodypedia)?
Where does this appear (section, paragraph #)?

Not reported

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 #)?

n/a

▶ Data deposition

Data deposition in a public repository is mandatory for:

- Protein, DNA and RNA sequences
- Macromolecular structures
- Crystallographic data for small molecules
- 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.

- Are accession codes for deposit dates provided?

Where (section, paragraph #)?

Yes, GSE62420. Sated in Methods paragraph 1 (Accession codes).

▶ 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.

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

All software publicly or commercially available. URLs provided throughout text.

- 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.

n/a

▶ Human subjects

- Which IRB approved the protocol?

Where is this stated (section, paragraph #)?

n/a

- Is demographic information on all subjects provided?

Where (section, paragraph #)?

n/a

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

Where (section, paragraph #)?

n/a

- 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
- a. How was this region determined?
- n/a

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?

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