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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

No statistical methods were used to pre-determine sample sizes; however, our sample sizes are similar to those reported in recently published similar studies (Ref 15, 16) and even more animals were used in our long-term study

2. Data exclusions

Describe any data exclusions.

No data excluded except one oulier in Supplemental Fig. 4D, this is mentioned in the figure legend

3. Replication

Describe whether the experimental findings were reliably reproduced.

Either N/A or mentioned in the figure legend

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No randomization since all triple or double trangenic mice of both sexes were used and are reported. Same for the analysis of the labeled cells, all labeled cells were included in the analysis.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Histological and stereological quantification was done blinded as stated in the method section

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☒	☐	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

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Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	This is decribed in the method section under "Statistics" All tests were conducted using GraphPad Prism Version 6

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	N/A

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Company and exact description is given for all antibodies

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	NA
b. Describe the method of cell line authentication used.	NA
c. Report whether the cell lines were tested for mycoplasma contamination.	NA
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	NA

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	Trangenic mice, 3 lines all on a B6 background were used male and female details are given in the method

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	NA

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Date: 24.07.2017

Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	The brain was dissected, the cerebellum and brain stem were removed and discarded. The forebrain was finely minced in ice-cold HBSS (Invitrogen) containing 15 mM HEPES, 0.54% D-Glucose and 0.1% DNase (w/v) (Sigma). Minced tissue was sequentially processed in glass Dounce and Potter homogenisers and resulting homogenates were filtered through a 70 µm cell strainer and centrifuged at 300g for 10 min, 4 °C. The resulting pellet was resuspended in 70% isotonic Percoll solution, overlayed with 37% and 30% isotonic Percoll layers and centrifuged for 30 min, 800 g, 4 °C. Cells were recovered from the 70/37% interphase and washed in FACS buffer (PBS, 2% fetal calf serum, 10 mM EDTA). Cells were resuspended and incubated with Fc block (BD Bioscience) for 10 minutes on ice, followed by staining for 15 minutes at 4°C with CD11b-brilliant violet 785 (1:200, Biolegend) and CD45-Alexa Fluor 700 (1:100, Biozol).
6. Identify the instrument used for data collection.	Sony SH800Z cell sorter.
7. Describe the software used to collect and analyze the flow cytometry data.	Manufacturer's software was used for analysis of flow cytometry data.
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Cell purity was assessed in representative samples by re-sorting the CD45low/CD11bhigh microglia population. Cell purity was >99% microglia.
9. Describe the gating strategy used.	Cells were identified as a distinct population in the FSC/BSC blot for each individual experiment (see Supplementary Figure 5 for an example). Single cells were then identified based on FSC-W and FSC-H. Tdtomato-positive and negative cell populations were gated and assessed for their expression of microglial markers (CD11bhigh/CD45low).

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒

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