nature research

Corresponding author(s): Jonas Neher

☐ 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.

Stated in Materials and Methods under "Statistical Analysis": 'Minimum sample sizes were determined a priori using power analyses or as dictated by the methodology (e.g. ChIP-Seq)'.

2. Data exclusions

Describe any data exclusions.

Stated in Materials and Methods under "Statistical Analysis": Statistical outliers were identified using the "Explore" function of IBM SPSS Statistics 22 with default parameters. Significant outliers were removed from the data set.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Stated in Materials and Methods under "Statistical Analysis": 'All experiments were performed at least twice and in independent batches of animals for key findings (figures show the pooled data)'. No unsuccessful replication attempts occurred.

4. Randomization

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

Stated in Materials and Methods under "Peripheral immune stimulation": '3 month-old mice were randomly assigned to treatment groups ...'.

5. Blinding

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

Wherever possible (i.e. with the exception of Western Blotting analyses, where samples were grouped by treatment groups), analyses were performed by blinded observers and/or software-automated analyses.

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.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Only commercial or freely available software was used for this study, which is stated throughout the manuscript's Methods section. These are IBM SPSS 22, Prism 5.0, HOMER v4.8 software (http://homer.salk.edu/homer/), R v.3.2.2 scripts and Bioconductor v.3.2, DESeq2 package (v.1.10.1), Aida v.4.27, IMARIS 8.3.1, Fiji, Illumina scripts (bcl2fastq v.2.18.0), FASTQC v.0.11.5 and STAR aligner v.2.5.2b.

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.	No unique materials were generated for this study - all next generation data sets have been publicly deposited with GEO (see below).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Individual antibodies with manufacturer's details are described in Materials and Methods, 'Western Blotting analysis', 'immunostaining' , 'Isolation of microglia and fluorescence-activated (FACS) analysis' subsections. References: Iba1 (Wako, catalogue no. 019-19741): Varvel et al., J.Exp.Med, 2015, 212(11):1803-1809 CN3 (custom made): Eisele et al., Science, 2010, 330(6006):980-982 Pu.1 (New England Biolabs, catalogue no. 2258S. Clone 9G7): Ueki et al., Oncogene, 2008, 27, 300–307 HIF1a: (IHC): Novus Biologicals, catalogue no. NB100-105. Clone H1alpha67 or (IF): Thermo Fischer, catalogue no. MA1-516. Clone mgc3 CD11b (Millipore, catalogue no. MAB1387Z. Clone M1/70): Sato et al., J. Neuroinflammation, 2012, 9:65, Miron et al., Nat. Neurosci., 2013, 16:1211-1218. GFAP: Biozol, catalogue no. Z0334 FACS antibodies: CD11b-APC: BioLegend, catalogue no. 101212. Clone M1/70 CD45-FITC: eBioscience, catalogue no. 11-0451-82. Clone 30-F11: Goldmann et al. Nat. Neurosci., 2013, 16:1618-1626.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	no eukaryotic cell lines were used.
b. Describe the method of cell line authentication used.	no eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	no eukaryotic cell lines were used.
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.	no eukaryotic cell lines were used.

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.

Stated in Materials and Methods under "Animals": For all experiments, 3 month-old hemizygous APP23 transgenic (C57BL/6J-Tg(Thy1-APPK670N;M671L)23), APP23 transgene-negative littermates or C57BL/6J (wildtype) mice (Jackson Laboratory) were used.
For experiments analysing immune responses after acute LPS stimulation (see below), both male and female mice were used. For microglia-specific gene knockouts, CX3CR1-CreER animals were crossed with Tak1 fl/fl animals and Cre recombinase expression was induced by subcutaneous tamoxifen injections as previously described15. Similarly, microglial-specific knockout of HDAC1/2 was achieved after crossing CX3CR1-CreER animals with a Hdac1/2 fl/fl line16. Male and female Tak1 fl/fl and Hdac1/2 fl/fl were injected at 2-3 months of age and were incubated for four weeks without further treatment. Tamoxifen-injected CX3CR1-Cre negative littermates were used as controls (because responses in CX3CR1-Cre negative animals were indistinguishable in Hdac1/2 fl/fl and Tak1 fl/fl lines, pooled data are shown in Fig. 1).
As there is a significant gender effect on the pathology of both brain ischemia and cerebral β-amyloidosis50,51, only female mice were used for the analyses of brain pathology. APP23 mice express a transgene consisting of human amyloid-β precursor protein (APP) with the KM670/671NL mutation under the Thy-1 promoter, and have been backcrossed with C57BL/6J mice for >20 generations. Female mice develop cerebral β-amyloid lesions in the neocortex around 6 months of age.
Animals were maintained under specific pathogen-free conditions. All experiments were performed in accordance with the veterinary office regulations of Baden-Württemberg (Germany) and were approved by the Ethical Commission for animal experimentation of Tübingen and Freiburg, Germany.

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.

This study did not involve human subjects.

Corresponding Author: Jonas Neher

Date: Feb 9, 2018

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.	All microglia were obtained from 9 month-old, female mice. 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-APC (1:200, Biolegend) and CD45-FITC (1:200, Biolegend).
6. Identify the instrument used for data collection.	FACS Aria for ChipSeq sample collection. Sony SH800 for RNAseq and DiOC experiments.
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.	The gating strategy was performed as previously described (Fueger et al., Nat. Neurosci., 2017). In brief, cells were identified as a distinct population in the FSC/SSC blot for each individual experiment (see Extended Data Figure 6 for an example). Single cells were then identified based on SSC-W and SSC-H (not shown). Finally, microglia were gated based on CD11bhigh/CD45low signals (see Extended Data Figure 6). For this microglia population, DIOC6(3) intensity was determined.

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

Corresponding Author: Jonas Neher

Date: Feb 9, 2018

ChIP-seq Reporting Summary

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

Data deposition

1. For all ChIP-seq data:

☒ a. Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☒ b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all necessary reviewer access links. (The entry may remain private before publication.)

http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=kjudwqcojpwrtsn&acc=GSE82170

3. Provide a list of all files available in the database submission.

Fastq files:
input_WT_rep1.fastq.gz
input_WT_rep2.fastq.gz
input_APP_rep1.fastq.gz
input_APP_rep2.fastq.gz
H3K4me1_WT_PBS_rep1.fastq.gz
H3K4me1_WT_PBS_rep2.fastq.gz
H3K4me1_WT_1xLPS_rep1.fastq.gz
H3K4me1_WT_1xLPS_rep2.fastq.gz
H3K4me1_WT_4xLPS_rep1.fastq.gz
H3K4me1_WT_4xLPS_rep2.fastq.gz
H3K4me1_APP_PBS_rep1.fastq.gz
H3K4me1_APP_PBS_rep2.fastq.gz
H3K4me1_APP_1xLPS_rep1.fastq.gz
H3K4me1_APP_1xLPS_rep2.fastq.gz
H3K4me1_APP_4xLPS_rep1.fastq.gz
H3K4me1_APP_4xLPS_rep2.fastq.gz
H3K27ac_WT_PBS_rep1.fastq.gz
H3K27ac_WT_PBS_rep2.fastq.gz
H3K27ac_WT_1xLPS_rep1.fastq.gz
H3K27ac_WT_1xLPS_rep2.fastq.gz
H3K27ac_WT_4xLPS_rep1.fastq.gz
H3K27ac_WT_4xLPS_rep2.fastq.gz
H3K27ac_APP_PBS_rep1.fastq.gz
H3K27ac_APP_PBS_rep2.fastq.gz
H3K27ac_APP_1xLPS_rep1.fastq.gz
H3K27ac_APP_1xLPS_rep2.fastq.gz
H3K27ac_APP_4xLPS_rep1.fastq.gz
H3K27ac_APP_4xLPS_rep2.fastq.gz

UCSC browser files:
H3K4me1_WT_PBS.gz
H3K4me1_WT_1xLPS.gz
H3K4me1_WT_4xLPS.gz
H3K4me1_APP_PBS.gz
H3K4me1_APP_1xLPS.gz
H3K4me1_APP_4xLPS.gz
H3K27ac_WT_PBS.gz

H3K27ac_WT_1xLPS.gz
H3K27ac_WT_4xLPS.gz
H3K27ac_APP_PBS.gz
H3K27ac_APP_1xLPS.gz
H3K27ac_APP_4xLPS.gz

4. If available, provide a link to an anonymized genome browser session (e.g. UCSC).

Methodological details

5. Describe the experimental replicates.

Two independent replicates (each containing microglia pooled from 8-10 animals) were analysed for each condition (2 genotypes X 3 treatments). Average Pearson correlation coefficient for replicates: r=0.850 for H3K4me1 and r=0.897 for H3K27ac.

6. Describe the sequencing depth for each experiment.

After purifying the precipitated chromatin and isolating the DNA, DNA libraries were generated using the NEB Next Ultra DNA Library Prep Kit for Illumina and the NEB Q5 polymerase (both from New England Biolabs). Multiplexing of samples was done using 6 different index-primers from the Library Prep Kit. One sample from each condition (genotype and treatment) was pooled for that purpose to rule out amplification and sequencing biases within the final data. Input samples were pooled and processed accordingly. The ideal number of amplification cycles was estimated via RealTime PCR to avoid over-amplification. Accordingly, samples were amplified for 13-15 cycles and the DNA was isolated afterwards. Individual libraries were pooled whereby each pool represented one whole batch of samples for each condition and targeted histone modification and was set to a final DNA concentration of 2 nM before sequencing (50 bp) on a HiSeq 2000 (Illumina) according to the manufacturer's instructions.

Sample	total reads	unique reads
Input DNA WT rep1	43,653,649	22,521,424
Input DNA APP rep1	57,360,101	20,063,703
Input DNA WT rep2	18,705,038	10,131,874
Input DNA APP rep2	62,054,242	25,550,040
H3K4me1 APP PBS rep1	32,181,918	18,238,592
H3K4me1 APP 1xLPS rep1	32,191,666	19,840,661
H3K4me1 APP 4xLPS rep1	20,857,099	15,165,311
H3K4me1 WT PBS rep1	49,012,493	26,828,670
H3K4me1 WT 1xLPS rep1	39,838,235	25,828,048
H3K4me1 WT 4xLPS rep1	32,950,540	20,860,044
H3K4me1 APP PBS rep2	29,131,210	19,138,719
H3K4me1 APP 1xLPS rep2	56,587,216	33,723,235
H3K4me1 APP 4xLPS rep 2	40,409,585	28,324,774
H3K4me1 WT PBS rep2	47,078,338	29,001,036
H3K4me1 WT 1xLPS rep2	34,536,801	22,105,718
H3K4me1 WT 4xLPS rep2	33,256,659	19,634,814
H3K27ac APP PBS rep1	37,808,377	27,605,016
H3K27ac APP 1xLPS rep1	37,051,423	26,952,487
H3K27ac APP 4xLPS rep1	34,648,071	25,771,385
H3K27ac WT PBS rep1	34,381,399	25,113,140
H3K27ac WT 1xLPS rep1	36,872,457	24,181,969
H3K27ac WT 4xLPS rep1	47,424,833	32,688,700

H3K27ac APP PBS rep2 37,453,302 26,605,454
H3K27ac APP 1xLPS rep2 43,997,870 31,847,085
H3K27ac APP 4xLPS rep2 19,690,027 15,281,216
H3K27ac WT PBS rep2 49,760,486 31,691,677
H3K27ac WT 1xLPS rep2 40,300,910 29,665,604
H3K27ac WT 4xLPS rep2 35,786,320 24,396,825

7. Describe the antibodies used for the ChIP-seq experiments.

H3K4me1: Abcam ab8895, ChIP grade: Manufacturer's statement: Specific for mono-methylated Lysine 4 of histone H3. Does not recognise di- or tri-methyl Lysine 4 nor methylation at Lysine 9.

H3K27ac: Abcam ab4729, ChIP grade: Manufacter's statement: All batches of ab4729 are tested using peptide arrays and show less than 30% cross reactivity with both Histone H3 acetyl K9 and unmodified Histone H3 peptides in this application.

Both antibodies have been validated for ChIP by independent investigators (see e.g. the antibody validation database at www.compbio.med.harvard.edu/antibodies/targets; or the Amit lab website: http://www.weizmann.ac.il/immunology/AmitLab/data-and-method/co_chip/verified-antibodies).

8. Describe the peak calling parameters.

Data were processed using HOMER software (http://homer.salk.edu/homer/). Tag directories were created from bam files using 'makeTagDirectory' for individiual samples and inputs, and peak calling was performed using 'findpeaks -style histone' with 4-fold enrichment over background and input, a Poisson p-value of 0.0001, and a peak width of 500 bp for H3K4me1 and 250 bp for H3K27ac. Peaks common to both replicates were determined using 'mergepeaks' (-prefix) function. To focus analysis on enhancers, peaks within ±2.5 kb of known TSS were filtered out. Union peak files for H3K4me1 and H3K27ac marks were then created for group-wise comparisons using 'mergepeaks' function (-d given). Active enhancers, i.e. genomic regions containing both H3K4me1 and H3K27ac peaks, were identified using the 'window' function of bedtools, requiring peaks of both marks to be located within a genomic region of 4 kb. Union peak files of active enhancers were then used for comparisons between groups using the 'getDifferentialPeaks' function (using a fold-change cut-off of 1.5 and a cumulative Poisson p-value of 0.0001). Finally, differential peaks were annotated using the 'annotatepeaks.pl' function, including gene ontology analysis.

9. Describe the methods used to ensure data quality.

For peak calling, we used a threshold of 4-fold enrichment over background and a conservative cumulative Poisson p-value of 0.0001 and limited the analysis to peaks that were independently replicated in the two batches of samples. In this way, we identified we identified 20,241 putative active enhancers across all conditions. Due to the long-term experiments and the mixed in vivo microglial population (i.e. plaque-associated vs. non-plaque associated), we set the threshold for differential peak calling at 1.5-fold. This is very clearly stated throughout the manuscript.

10. Describe the software used to collect and analyze the ChIP-seq data.

Data were processed using HOMER software (http://homer.salk.edu/homer/) as described in the Materials & Methods section.