Innate immune memory in the brain shapes neurological disease hallmarks

Ann-Christin Wendeln1,2,3,12, Karoline Degenhardt1,2,3,12, Lalit Kaurani4,5, Michael Gertig4,5, Thomas Ulas6, Gaurav Jain5,7, Jessica Wagner1,2,3, Lisa M. Häser1,2, Katleen Wild1,2, Angelos Skodras1,2, Thomas Blank8, Ori Staszewski8, Mounita Datta8, Tatiana Pena Centeno9, Vincenzo Capece9, Md. Rezaul Islam4, Clemil Kerimoglu4, Matthias Staufenbiel1,4, Joachim L. Schultzze6,9, Marc Beyer10, Marco Prinz8,11, Mathias Jucker1,2, André Fischer4,5 & Jonas J. Neher1,2

Innate immune memory is a vital mechanism of myeloid cell plasticity that occurs in response to environmental stimuli and alters subsequent immune responses. Two types of immunological imprinting can be distinguished—training and tolerance. These are epigenetically mediated and enhance or suppress subsequent inflammation, respectively. Whether immune memory occurs in tissue–resident macrophages in vivo and how it may affect pathology remains largely unknown. Here we demonstrate that peripherally applied inflammatory stimuli induce acute immune training and tolerance in the brain and lead to differential epigenetic reprogramming of brain–resident macrophages (microglia) that persists for at least six months. Strikingly, in a mouse model of Alzheimer’s pathology, immune training exacerbates cerebral β-amyloidosis and immune tolerance alleviates it; similarly, peripheral immune stimulation modifies pathological features after stroke. Our results identify immune memory in the brain as an important modifier of neuropathology.

Contrary to the long-held assumption that immunological memory exists only in cells of the adaptive immune system, recent evidence has indicated that myeloid cells also display memory effects1–9. For example, certain immune stimuli train blood monocytes to generate enhanced immune responses to subsequent immune insults1,2. By contrast, other stimuli induce immune tolerance—suppression of inflammatory responses to subsequent stimuli3–5. Innate immune memory lasts for several days in vitro and for up to three months in circulating monocytes in vivo and is mediated by epigenetic reprogramming in cultured cells, with chromatin changes also apparent in vivo6,7,8. However, it is unclear whether immune memory occurs in long-lived tissue-resident macrophages and whether it alters tissue-specific pathology. Microglia (brain-resident macrophages) are very long-lived cells8,9. This makes them particularly interesting for studying immune memory, as virtually permanent modification of their molecular profile appears possible. As microglia are also involved in many neurological diseases10–12, we investigated whether immune memory occurs in microglia in vivo and how it affects neuropathology.

Acute immune memory in the brain

It is well-established that inflammation in the periphery can prompt immune responses in the brain13. To evaluate whether immune memory can be induced in the brain by peripheral stimulation, we gave mice daily intraperitoneal injections of low-dose lipopolysaccharides (LPS) on four consecutive days, leading to mild sickness behaviour and temporary weight loss (Fig. 1a and Extended Data Fig. 1a). Three hours after the first LPS injection (1 × LPS), there was a pronounced increase in blood cytokine levels, but only modest increases in brain cytokines. Upon the second injection (2 × LPS), the blood levels of the pro-inflammatory cytokines IL-1β, TNF, IL-6, IL-12 and IFN-α were diminished compared to their levels after 1 × LPS, whereas IL-10 release was not reduced, indicating peripheral immune tolerance. In sharp contrast, brain cytokines were markedly increased by 2 × LPS injections, indicating a brain-specific training effect induced by the first LPS stimulus (Fig. 1b, c and Extended Data Fig. 2). Accordingly, a conspicuous morphological change in microglia occurred after 2 × LPS, whereas the number of activated (GFAP+) astrocytes increased only after 3 × LPS (Extended Data Fig. 1b–d). Notably, 4 × LPS virtually abolished TNF, IL-13 and IL-6 release in the brain whereas IL-10 remained elevated, indicating immune tolerance.

Next, we examined the contribution of microglia to immune memory in the brain using inducible CX3CR1-CreER (Cre) mice crossed with mouse lines carrying loxpPlanked genes, in which tamoxifen-induced Cre expression results in persistent recombination in long-lived microglia but not in short-lived myeloid cells, including blood monocytes14. We induced microglial knockout of either transforming growth factor-β-activated kinase 1 (Tak1, also known as Map3k7), which results in inhibition of the NF-κB, JNK and ERK1/2 pathways14, or histone deacetylases 1 and 2 (Hdac1/2), two major regulators of epigenetic reprogramming and macrophage inflammatory responses15. As expected, tamoxifen-induced knockout of either Tak1 or Hdad1/2 did not alter the peripheral inflammatory response. Furthermore, brain cytokine levels were indistinguishable after 1 × LPS, but the training effect following 2 × LPS was virtually abolished in Cre+ mice. Notably, the cytokines showing the most pronounced training and tolerance effects (IL-1β, TNF, IL-6) were also the most affected by microglial gene knockout (Fig. 1b, c and Extended Data Fig. 2), indicating that immune memory in the brain is predominantly mediated by microglia. Moreover, after 1 × LPS, Cre+ and Cre− mice showed indistinguishable weight loss (Extended Data Fig. 1a) and...
sickness behaviour (not shown); however, in animals with microglial T Ak1 knockout, sickness behaviour after 2 × LPS was noticeably alleviated (Supplementary Video 1).

After intraperitoneal injections, LPS was found in the blood but not in the brain, indicating that at this dose neither significant entry of LPS into the brain nor opening of the blood–brain barrier occurred, as previously reported36. The latter was confirmed by the absence of blood iron in the brain parenchyma. Also, using type 2 CC chemokine receptor (CCR2) reporter mice37, we found no extravasation of circulating monocytes (Extended Data Fig. 1c–g), confirming that immune memory is mediated by brain-resident macrophages alone.

**Immune memory shapes neuropathology**

Next, we analysed whether the training- and tolerance-inducing stimuli (1 × LPS and 4 × LPS, respectively) could lead to long-term alterations in brain immune responses and thereby modify disease pathogenesis. APP23 mice are a model of Alzheimer’s disease pathology in which plaques of insoluble amyloid-β (Aβ) develop from 6 months of age. Amyloid plaques lead to activation of microglia18, thereby providing a stimulus that should reveal microglial immune memory. We injected 3-month-old APP23 mice with either 1 × LPS or 4 × LPS, then analysed pathology 6 months later (Fig. 2a). Strikingly, 1 × LPS increased both plaque load and total Aβ levels compared to control animals, whereas 4 × LPS decreased both plaque load and Aβ levels (Fig. 2b), with plaque-associated neuritic damage correlating directly with plaque size in all treatment groups (Extended Data Fig. 3a–c). In addition, the protein levels of Aβ precursor protein (APP) and its cleavage products were indistinguishable among the groups, indicating equivalent Aβ generation (Extended Data Fig. 3d). Furthermore, neither the total number of microglia nor the number of microglia clustering around plaques was altered by LPS treatments (Fig. 2c), whereas the training: tolerance occurs after 3 × LPS or 4 × LPS. Cytokines return to baseline within 24 h (1 × LPS, 1 × PBS and 4 × LPS + 1 day). Microglia-specific knockout of Tak1 or Hdac1/2 selectively prevents immune training in the brain. In b and c, n = 16, 11, 12, 9, 7, 5, 13, 4, 6, 9, 4, 5 from left to right. *P < 0.05, **P < 0.01, ***P < 0.001 for independent-samples median test with correction for multiple comparisons. Data are means ± s.e.m.

Fig. 1 | Peripheral immune stimulation evokes immune memory in microglia. a. Experimental approach. s.c., subcutaneous injection. b. Peripheral cytokine levels in wild-type or APP23 mice (white bars) and in mice with microglia-specific knockout of Tak1 or Hdac1/2 (coloured bars) following lipopolysaccharide (LPS) injections. Note that tolerance is induced with repeated injections. c. Brain cytokine levels, as in b. 2 × LPS amplifies IL-1β and TNF release in control mice, demonstrating immune

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
**Microglial molecular profiles**

In vitro, immune memory in macrophages results from epigenetically mediated alterations in the enhancer repertoire, leading to transcriptional changes. As our data indicate that acute immune memory in the brain is mediated predominantly by microglia, we isolated microglia by cell sorting (Extended Data Fig. 6) from 9-month-old mice injected with 1 × LPS versus 4 × LPS showed enrichment for the thyroid hormone signalling pathway, including a putative enhancer for hypoxia inducible factor-1α (HIF-1α). Similarly, enhancers with higher H3K4me1 levels in APP mice injected with 1 × LPS versus 4 × LPS were enriched for the HIF-1 signalling pathway. On the other hand, APP mice treated with 4 × LPS showed increased H3K4me1 levels in putative enhancers related to phagocytic function (Table 1a). Notably, we found no pathway enrichment when comparing H3K4me1 levels in microglia from APP and wild-type control mice (Table 1a), indicating that H3K4me1 levels were altered predominantly in response to LPS stimulation.

Next, we analysed enhancer activation by testing for differential regulation of H3K27ac levels. In line with the requirement of an acute stimulus for H3K27ac deposition, differential enhancer activation was more pronounced in APP mice (where amyloid plaques activate microglia) than in wild-type mice (190 ± 18 in APP, 69 ± 5 in wild-type; Extended Data Fig. 7c; Supplementary Table 2). For example, differentially regulated H3K27ac levels in microglia from APP mice treated with 1 × LPS versus control APP mice were enriched for the

**Microglial activation (mm³)**

In wild-type and APP23 mice (n = 8, 7, 7 and n = 14, 10, 10 mice), i.p., intraperitoneal. Scale bar, 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001 for one-way (b) and two-way ANOVA (c, d) with Tukey correction. Data are means ± s.e.m.

---

**Fig. 2** Cerebral β-amyloidosis is altered after peripheral immune stimulation. **a**, Experimental design. **b**, Analysis of cortical Aβ plaque load (n = 22, 10, 10 mice from left to right) and protein levels (n = 14, 10, 10 animals). **c, d**, Analysis of total cortical and plaque-associated microglia (c, n = 7, 7, 14, 10, 10 mice) and cytokine levels of IL-10 and IL-1β (d) in wild-type and APP23 mice (n = 8, 7, 7 and n = 14, 10, 10 mice), i.p., intraperitoneal. Scale bar, 50 μm. *P < 0.05, **P < 0.01, ***P < 0.001 for one-way (b) and two-way ANOVA (c, d) with Tukey correction. Data are means ± s.e.m.

**Fig. 3** Stroke pathology is altered after peripheral immune stimulation. Pathological features of brain ischaemia induced one month after intraperitoneal (i.p.) injection with 1 × LPS or 4 × LPS. **a**, Neuronal damage (cresyl violet, n = 6, 6, 7, 6 mice from left to right) and number of microglia (Iba1-positive, n = 6, 6, 6, 6 mice). **b**, Cytokine profiles 1 day post-ischaemia (n = 5, 7, 5, 5 mice). **c, d**, Overview of microglial activation in the infarct (c) and quantification of neuronal damage and microglial activation (d) 7 days post-ischaemia (n = 3, 13, 8, 9 mice). Scale bar, 500 μm. *P < 0.05, **P < 0.01, ***P < 0.001 for one-way ANOVA with Tukey correction. Data are means ± s.e.m.
**Table 1 | The microglial enhancer repertoire 6 months after immune stimulation**

**a KEGG pathway enrichment of differentially regulated H3K4me1 regions (threshold ≥ 1.5-fold)**

| Condition 1 | Condition 2 | Increased in condition 1 | log\(P\) | Increased in condition 2 | log\(P\) |
|-------------|-------------|--------------------------|--------|--------------------------|--------|
| Wild-type   |             |                          |        |                          |        |
| APP PBS     | Wild-type PBS| Renal cell carcinoma     | −10    | Focal adhesion            | −6     |
|             |             | MAPK signalling pathway  | −5     | Chemokine signalling pathway | −5    |
|             | 1 × LPS     | Endocytosis               | −7     | Proteoglycans in cancer   | −5     |
|             | PBS         | MAPK signalling pathway   | −5     | Colorectal cancer         | −5     |
|             | 4 × LPS     | Proteoglycans in cancer   | −4     | Thyroid hormone signalling pathway | −4    |
|             | PBS         | Transcriptional misregulation in cancer | −8    |
|             |             | Leucocyte transendothelial migration | −5    |
|             | 4 × LPS     | Leucocyte transendothelial migration | −1    |
|             | PBS         | Cytokine–cytokine receptor interaction | −6    |
|             |             | Adherens junction         | −6     |
|             |             | FcR-mediated phagocytosis | −6     |
|             | 1 × LPS     | Rap1 signalling pathway   | −5     |
|             | 4 × LPS     | MAPK signalling pathway   | −5     |
|             |             | Endocytosis               | −5     |
|             |             | TGF-β3-signalling pathway | −4     |
|             | 1 × LPS     | Transcriptional misregulation in cancer | −4    |
|             | 4 × LPS     | Chemokine signalling pathway | −4    |
|             |             | Salmonella infection      | −6     |
|             | 1 × LPS     | Chagas disease            | −5     |
| APP         | PBS         | HIF-1 signalling pathway  | −5     |
|             | 4 × LPS     | Toxoplasmosis             | −5     |
|             |             | MAPK signalling pathway   | −5     |
|             |             | Endocytosis               | −5     |
|             |             | MAPK signalling pathway   | −5     |
|             |             | Transcriptional misregulation in cancer | −4    |

**b KEGG pathway enrichment of differentially regulated H3K27ac regions (threshold ≥ 1.5-fold)**

| Condition 1 | Condition 2 | Increased in condition 1 | log\(P\) | Increased in condition 2 | log\(P\) |
|-------------|-------------|--------------------------|--------|--------------------------|--------|
| Wild-type   |             |                          |        |                          |        |
| APP PBS     | Wild-type PBS| Thyroid hormone signalling pathway | −6    |
|             |             | mTOR signalling pathway  | −5     |
|             | 1 × LPS     | Transcriptional misregulation in cancer | −5    |
|             | PBS         | HIF-1 signalling pathway  | −8     |
|             | 4 × LPS     | Thyroid hormone signalling pathway | −7    |
|             |             | Carbohydrate digestion and absorption | −6    |
|             |             | Osteoclast differentiation | −5     |
|             |             | AMPK signalling pathway   | −5     |
|             | 4 × LPS     | Chronic myeloid leukaemia | −5     |
|             | PBS         | Rap1 signalling pathway   | −5     |
|             |             | MAPK signalling pathway   | −5     |
|             |             | mTOR signalling pathway   | −5     |
|             |             | Endocytosis               | −5     |
|             |             | MAPK signalling pathway   | −5     |
|             |             | Transcriptional misregulation in cancer | −6    |
|             |             | Osteoclast differentiation | −12    |
|             |             | Bacterial invasion of epithelial cells | −11    |
|             |             | Toll-like receptor signalling pathway | −10    |
|             |             | Ras signalling pathway    | −9     |
|             |             | Thyroid hormone signalling pathway | −9    |
|             |             | FcR-mediated phagocytosis | −9     |
|             |             | MAPK signalling pathway   | −8     |
|             |             | Rap1 signalling pathway   | −8     |
|             |             | MAPK signalling pathway   | −8     |
|             |             | Phospholipid signalling system | −7    |
|             |             | Focal adhesion            | −7     |
|             |             | Transcriptional misregulation in cancer | −6    |
|             |             | Oestrogen signalling pathway | −6    |
|             |             | TNF signalling pathway    | −6     |
|             |             | HIF-1 signalling pathway  | −6     |
|             |             | PI3K-Akt signalling pathway | −6    |
|             |             | Chronic myeloid leukaemia | −6     |
|             |             | Acute myeloid leukaemia   | −5     |

Pathway enrichment of putative enhancers (with Benjamini-Hochberg correction) with differentially regulated H3K4me1 and H3K27ac levels (based on nearest gene; cumulative \(P\) < 0.0001). 

\(n = 2\) replicates (8–10 mice per replicate).
HIF-1 signalling pathway, with enhancer regions also being enriched for HIF-1α binding motifs (Table 1b and Extended Data Fig. 8), in line with changes in H3K4me1 levels (Table 1a) and the reported key role of HIF-1α in trained immunity and macrophage inflammatory responses.21,22

Active enhancers in microglia from 4 × LPS-treated APP mice versus control APP mice showed enrichment only for the Rap1 signalling pathway, which has been implicated in phagocytosis of opsonized targets,22,23, again matching changes in H3K4me1 levels (Table 1). Strikingly, comparison of microglia from APP mice that received the training- (1 × LPS) or tolerance-inducing (4 × LPS) stimuli showed no pathway enrichment for active enhancers in mice injected with 4 × LPS, whereas enhancers in 1 × LPS-treated mice were enriched for a large number of inflammation-related pathways, highlighting the differential effects of the two immune memory states. Finally, comparison of microglia from vehicle-treated wild-type and APP mice demonstrated a small number of differentially activated enhancers with enrichment for the thyroid hormone signalling pathway (including a putative active enhancer for Hif1α) and the mTOR signalling pathway (Table 1b), indicating that microglia are also epigenetically reprogrammed in response to brain pathology alone.

We next examined microglial mRNA levels under the same conditions to determine whether epigenetic alterations were reflected in gene expression (Supplementary Table 3). First, we determined the concordance between 772 enhancers with significantly increased or decreased H3K27ac levels (Supplementary Table 2) and the direction of change in the expression of their nearest gene. Indeed, there was a significant (albeit modest) concordance between alterations in H3K27ac levels and gene expression (median concordance of pairwise comparisons, 58%; P = 0.05). This suggested that gene expression is directly affected by the microglial active enhancer repertoire. Accordingly, weighted gene correlation network analysis (WGCNA)24 revealed striking parallels to epigenetic changes (Fig. 4a–c and Supplementary Table 4). For example, the red module (MEred; Fig. 4a) contained the HIF-1 signalling pathway and correlated strongly with the 1 × LPS-injected APP group. Furthermore, gene expression in MERed was upregulated in APP versus wild-type control mice and further increased by 1 × LPS, but downregulated by 4 × LPS.

HIF-1α activation in inflammatory-stimulated macrophages can occur downstream of mitochondrial hyperpolarization; enhanced HIF-1α signalling in turn promotes glycolysis, measurable as lactate release.25 Accordingly, the green module (MEgreen; Fig. 4a), which correlated positively with control and 1 × LPS-treated APP groups but negatively with control and 4 × LPS-treated wild-type groups, was found to be enriched in genes of the glycolysis pathway. Microglial gene expression in MEGreen was upregulated in APP versus wild-type control mice and further increased in APP mice by 1 × LPS but decreased in mice that received 4 × LPS. Therefore, we analysed mitochondrial membrane potential and lactate release in microglia. Strikingly, microglia from 1 × LPS-treated APP mice showed strongly increased...
mitochondrial membrane potential, which correlated positively with the release of lactate (Fig. 4d), functionally corroborating the epigenetic and transcriptional alterations in trained microglia. Additionally, immunostaining confirmed an increase in protein levels of HIF-1α in plaque-associated microglia; these levels were further increased in 1 × LPS-treated APP mice (Fig. 4e, f). Thus, HIF-1α signalling and a metabolic switch to glycolysis are activated in response to cerebral β-amyloid deposition, and are enhanced by immune training but reduced by immune tolerance in microglia.

In contrast to MErEd and MEGreen, the MEGrey module correlated positively with the control wild type but negatively with the control APP and 1 × LPS-treated APP groups. Compared to wild-type controls, microglial gene expression in MEGrey was downregulated in APP control animals and further decreased by 1 × LPS, but showed unchanged levels in APP mice injected with 4 × LPS compared with wild-type controls (Fig. 4a–c). Notably, MEGrey was enriched for phagocytosis-related pathways, including the Rap1 signalling pathway (Fig. 4a–c), again reflecting epigenetic changes (Table 1). We therefore tested whether phagocytosis of Aβ was enhanced in 4 × LPS-treated APP mice. Indeed, microglial Aβ content was increased around 1.75-fold in these mice compared to APP control mice (Fig. 4g), providing further functional validation of the microglial enhancer repertoire and gene expression profiles.

Recent data have indicated that context-specific microglial phenotypes exist, for example, disease-associated microglia (DAM26) and the microglial neurodegenerative phenotype (MGN32). Notably, the MEBrown module, which was upregulated by both LPS treatments in wild-type mice as well as in all APP groups, contained a number of homeostatic microglial genes (for example, Hebx, Cx3cr1 and Csf1r) but also all of the stage 1 DAM core genes except Apoe, as well as more than twice stage 2 core genes26 (Fig. 4c). Interestingly, the gene encoding Apoe, which may be crucial for promoting a detrimental microglial phenotype27,28, was found in the same module (MEred) as Hif1α. MEREd also contained other genes genetically linked to risk for Alzheimer’s disease, namely Csl3 and Inpp5d3, suggesting that HIF-1α may also be a detrimental modulator of Alzheimer’s disease pathology.

The epigenetic landscape of microglia has been described only under homeostatic conditions30–33. Our data now demonstrate epigenetic modifications in microglia in response to peripheral immune stimulation but also as a result of cerebral β-amyloidosis, including activation of the HIF-1α and mTOR pathways, and leading to transcriptional and functional alterations. Although the global epigenetic and transcriptional changes were relatively modest, they are likely to have been driven by a small number of microglia that received the required secondary immune stimulation, as evidenced for example by increased levels of HIF-1α in plaque-associated microglia (Fig. 4). mTOR activation is a well-known event in early Alzheimer’s disease33 and was recently shown in microglia, where it activated HIF-1α and glycolysis to sustain microglial energy demand in models of Alzheimer’s disease pathology.34 Our data now indicate that mTOR activation may be mediated by epigenetic microglial reprogramming in response to cerebral β-amyloidosis and that HIF-1α signalling downstream of mTOR could be a detrimental event, because augmentation or suppression of HIF-1α signalling occurred concomitantly with aggravated or alleviated Aβ deposition, respectively.

We here provide evidence of both immune training and tolerance in microglia and demonstrate their impact on neuropathology for the first time. While we cannot completely exclude the possibility that other cell-types contribute to immune memory and modulation of pathology in the brain, microglial-specific gene knockout of Takt1 or Hdac1/2 virtually abolished immune training (Fig. 1), indicating that microglia are likely to be the major effectors of immune memory. Notably, in our experiments, the effects of immune memory mostly became apparent following a secondary inflammatory stimulus, corroborating the concept of innate immune memory1,3. However, while in the periphery training may be beneficial owing to enhanced pathogen elimination7,35,36, and tolerance may be detrimental owing to higher rates of infection resulting from immune suppression3, we found that training promotes, while tolerance alleviates, neuropathology. This is consistent with the beneficial effects of preventing microglial pro-inflammatory responses in models of Alzheimer’s disease pathology and stroke12,37 and the worsening of cerebral β-amyloidosis in response to pro-inflammatory peripheral stimuli in animal models.38 Similarly, immune training has recently been described in epithelial stem cells, where it promotes wound healing but may also underlie autoimmune disorders39. Thus, immune memory in the brain could conceivably affect the severity of any neurological disease that presents with an inflammatory component, but this will need to be studied for each individual condition.

Our data provide proof-of-principle for innate immune memory in microglia, and while our different LPS injection paradigms may not necessarily model physiological stimuli, we found that individual cytokines applied peripherally may also elicit immune memory effects in the brain (Extended Data Fig. 9). These results suggest that a wide variety of immune challenges may induce microglial immune memory and provide a possible mechanism for LPS-induced immune memory in the brain. It will be crucial to determine which other stimuli may lead to long-term modulation of microglial responses and thereby contribute to the severity of many neurological diseases.

Online content
Any Methods, including any statements of data availability and Nature Research reporting summaries, along with any additional references and Source Data files, are available in the online version of the paper at https://doi.org/10.1038/s41586-018-0023-4.

Received: 21 August 2016; Accepted: 23 February 2018; Published online 11 April 2018.

1. Netea, M. G., Latz, E., Mills, K. H. G. & O’Neill, L. A. J. Innate immune memory: a paradigm shift in understanding host defense. Nat. Immunol. 16, 675–679 (2015).
2. Netea, M. G. et al. Trained immunity: A program of innate immune memory in health and disease. Science 352, eaat1098 (2016).
3. Saeed, S. et al. Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. Science 345, 1251086 (2014).
4. Cheng, S. C. et al. mTOR- and HIF-1α-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 345, 1250684 (2014).
5. Bliswaj, S. K. & Lopez-Collazo, E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. Trends Immunol. 30, 475–487 (2009).
6. Novakovic, B. et al. β-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. Cell 167, 1354–1368 (2016).
7. Kleinijenhuis, J., et al. Baciile Calmette-Guérin induces NOD2-dependent non-specific protection from reinfection via epigenetic reprogramming of monocytes. Proc. Natl Acad. Sci. USA 109, 17537–17542 (2012).
8. Tay, T. L. et al. A new fate mapping system reveals context-dependent random or clonal expansion of microglia. Nat. Neurosci. 20, 793–803 (2017).
9. Füger, P. et al. Microglia turnover with aging and in an Alzheimer’s model via long-term in vivo single-cell imaging. Nat. Neurosci. 20, 1371–1376 (2017).
10. Prinz, M. & Priller, J. Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nat. Rev. Neurosci. 15, 300–312 (2014).
11. Heneka, M. T., Kummer, M. P. & Latz, E. Innate immune activation in neurodegenerative disease. Nat. Rev. Immunol. 14, 463–477 (2014).
12. d’Alessio, C. & Anrather, J. The immunology of stroke: from mechanisms to translation. Nat. Med 17, 796–808 (2011).
13. Perry, V. H., Cunningham, C. & Holmes, C. Systemic infections and inflammation affect chronic neurodegeneration. Nat. Rev. Immunol. 7, 161–167 (2007).
14. Goldmann, T. et al. A new type of microglia gene targeting shows TAK1 to be pivotal in CNS autoimmune inflammation. Nat. Neurosci. 16, 1618–1626 (2013).
15. Datta, M. et al. Histone deacetylases 1 and 2 regulate microglial function during development, homeostasis, and neurodegeneration in a context-dependent manner. Immunity http://www.cell.com/immunity/fulltext/S1074-7613(18)30075-X (2018).
16. Banks, W. A. & Robinson, S. M. Minimal penetration of lipopolysaccharide across the murine blood-brain barrier. Brain Behav. Immun. 24, 102–109 (2010).
17. Saenderup, N. et al. Selective chemokine receptor usage by central nervous system myeloid cells in CCR2-red fluorescent protein knock-in mice. PLoS One 5, e16393 (2010).
18. Sturchler-Pierrat, C. et al. Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. Proc. Natl Acad. Sci. USA 94, 13287–13292 (1997).
19. Ostuni, R. et al. Latent enhancers activated by stimulation in differentiated cells. Cell 152, 157–171 (2013).
20. Kaikkonen, M. U. et al. Remodeling of the enhancer landscape during macrophage activation is coupled to enhancer transcription. Mol. Cell 51, 310–325 (2013).
21. Cramer, T. et al. HIF-1α is essential for myeloid cell-mediated inflammation. Cell 112, 645–657 (2003).
22. Cramer, T. et al. HIF-1α protects against peripheral inflammatory macrophages. Cell 167, 457–470 (2016).
23. Keren-Shaul, H. et al. A unique microglia type associated with restricting neuroinflammation in Alzheimer’s disease. Cell 169, 1276–1290 (2017).
24. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559 (2008).
25. Mills, E. L. et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167, 457–470 (2016).
26. Krasemann, S. et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity 47, 566–581 (2017).
27. Shi, Y. et al. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature 549, 523–527 (2017).
28. Lambert, J. C. et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease. Nat. Genet. 45, 1452–1458 (2013).
29. Gosselin, D. et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 159, 1327–1340 (2014).
30. Lavin, Y. et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159, 1312–1326 (2014).
31. Gosselin, D. et al. An environment-dependent transcriptional network specifies human microglia identity. Science 367, eaai2222 (2017).
32. Wang, C. et al. Targeting the mTOR signaling network for Alzheimer’s disease therapy. Mol. Neurobiol. 49, 120–135 (2014).
33. Ulland, T. K. et al. TREM2 maintains microglial metabolic fitness in Alzheimer’s disease. Cell 170, 649–663 (2017).
34. Kaufmann, E. et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell 172, 176–190 (2018).
35. Naik, S. et al. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature 550, 475–480 (2017).
36. Arts, R. J. W. et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe 23, 89–100 (2018).
37. Heneka, M. T., Golenbock, D. T. & Latz, E. Innate immunity in Alzheimer’s disease. Nat. Immunol. 16, 229–236 (2015).
38. O’Banion, M. K. Does peripheral inflammation contribute to Alzheimer disease? Evidence from animal models. Neurology 83, 480–481 (2014).
39. Li, Y. et al. Rap1a null mice have altered myeloid cell functions suggesting distinct roles for the closely related Rap1a and 1b proteins. J. Immunol. 179, 8322–8331 (2007).
40. Langfelder, P. & Horvath, S. WGCNA: an R package for weighted correlation network analysis. BMC Bioinformatics 9, 559 (2008).
41. Klumpp, K. et al. Targeting the mTOR signaling network for Alzheimer’s disease. Cell 167, 457–470 (2016).
42. Krasemann, S. et al. The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. Immunity 47, 566–581 (2017).
43. Shi, Y. et al. ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. Nature 549, 523–527 (2017).
44. Lambert, J. C. et al. Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer’s disease. Nat. Genet. 45, 1452–1458 (2013).
45. Gosselin, D. et al. Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. Cell 159, 1327–1340 (2014).
46. Lavin, Y. et al. Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159, 1312–1326 (2014).
47. Gosselin, D. et al. An environment-dependent transcriptional network specifies human microglia identity. Science 367, eaai2222 (2017).
48. Wang, C. et al. Targeting the mTOR signaling network for Alzheimer’s disease therapy. Mol. Neurobiol. 49, 120–135 (2014).
49. Ulland, T. K. et al. TREM2 maintains microglial metabolic fitness in Alzheimer’s disease. Cell 170, 649–663 (2017).
50. Kaufmann, E. et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell 172, 176–190 (2018).
51. Arts, R. J. W. et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe 23, 89–100 (2018).
52. Heneka, M. T., Golenbock, D. T. & Latz, E. Innate immunity in Alzheimer’s disease. Nat. Immunol. 16, 229–236 (2015).
53. O’Banion, M. K. Does peripheral inflammation contribute to Alzheimer disease? Evidence from animal models. Neurology 83, 480–481 (2014).
54. Naik, S. et al. Inflammatory memory sensitizes skin epithelial stem cells to tissue damage. Nature 550, 475–480 (2017).

Acknowledgements We thank P. Rizzu for experimental advice, L. Walker for manuscript comments and D. Bryce for statistical advice. This study was supported by a PhD fellowship from the Studienstiftung des Deutschen Volkes (A.-C.W.), a Roman Herzog Fellowship from the Hertie Foundation (J.J.N.), and grants from the network ‘Neuroinflammation in Neurodegeneration’ (State of Baden-Württemberg, Germany; M.J. and M.P.) and the Soberk-Stiftung (M.P.), the DFG (SFB992, Reinhart-Koselleck-Grant to M.P., SFB704 to J.L.S.), the European Research Council (A.F.) the Fortuine Program (Med. Faculty, Univ. Tubingen; 2075-1-0; J.J.N), the Fritz Thyssen Foundation (Cologne, Germany; J.J.N.) and the Paul G. Allen Family Foundation (Seattle, USA; J.J.N.). M.B. and J.L.S are members of the Excellence Cluster ImmunoSensation.

Author contributions K.D., A.-C.W., J.W., L.M.H., K.W., A.S., T.B, O.S., M.D. and J.J.N. performed microglial isolation, in vivo and ex vivo experiments and histological/biochemical analyses. M.G., L.K., G.J., T.P.C., V.C., R.I., C.K., A.F., M.B., T.U., J.L.S. and J.J.N. performed ChIP–seq and RNA-seq analyses. J.J.N conceived the study and coordinated the experiments together with M.J., A.F., M.P., M.B., J.L.S. and M.S. J.J.N. wrote the manuscript, with contributions from all authors.

Competing interests The authors declare no competing interests.

Additional information Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0023-4.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0023-4.
Reprints and permissions information is available at http://www.nature.com/reprints.
Correspondence and requests for materials should be addressed to J.J.N.
Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
METHODS

Mice. For all experiments, 3-month-old hemizygous APP23 transgenic (C57BL/6J-Tg(Thy1-APPK670N,M671L)23), APP23 transgene-negative littermate or C57BL/6J (wild-type) mice (Jackson Laboratory) or type 2 CC chemokine receptor (CCCR) reporter mice (kindly provided by R. Ransohoff, Boston) were used.

For experiments analysing immune responses after acute LPS and cytokine stimulation (see below), both male and female mice were used. For microglia-specific gene knockouts, CX3CR1-CreER animals were crossed with TacI/− mice and Cre recombinase expression was induced by subcutaneous tamoxifen injections as previously described44. Similarly, microglial-specific knockout of Hdac1/2 was achieved after crossing CX3CR1-CreER animals (kindly provided by S. Jung (Weizmann Institute, Rehovot)) with a Hdac1/2/− line (kindly provided by P. Matthias (FMI Basel)). Both TacI/− and Hdac1/2/− mice were injected with tamoxifen 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 mice were indistinguishable in Hdac1/2/− and TacI/− lines, pooled data are shown in Fig. 1).

As there is a significant gender effect on the pathology of both brain ischaemia and cerebral β-amyloidosis40,41, only female mice were used for the analyses of brain pathology. APP23 mice express a transgene consisting of human APP with the KM670/671NL mutation under the Thy-1 promoter, and have been backcrossed with C57BL/6J mice for more than 20 generations. Female mice develop cerebral Aβ lesions in the neocortex at around 6 months of age42.

Animals were maintained under specific pathogen-free conditions. All experiments were performed in accordance with the veterinary regulations of Baden-Württemberg (Germany) and were approved by the Ethical Commission for animal experimentation of Tübingen and Freiburg, Germany.

Peripheral immune stimulation. Three-month-old mice were randomly assigned to treatment groups and were injected intraperitoneally (i.p.) with bacterial lipopolysaccharides (LPS from Salmonella enterica serotype typhimurium, Sigma) at a daily dose of 500 µg per kg bodyweight. Animals received either four LPS injections on four consecutive days (4 × LPS), a single LPS injection followed by three vehicle injections on the following three days (1 × LPS or 4 × vehicle injections (PBS)). Acute stimulation showed indistinguishable cytokine responses in wild-type and APP23 transgenic animals; Fig. 1 shows the pooled data from both genotypes (see Extended Data Fig. 2 for data separated by genotype). Furthermore, as cytokine responses were indistinguishable in animals treated with one, two, three or four injections of PBS, pooled data from all time points are shown.

For peripheral cytokine treatments, recombinant mouse cytokines (TNE, IL-10, PeproTech) were aliquoted as per the manufacturer's instructions and stored at −80 °C until use. To determine whether a long-term change in the brain's immune response (training or tolerance) occurred after peripheral cytokine injection, mice were treated on four consecutive days with 0.1 µg per kg bodyweight IL-10 or once with 0.1 µg per kg bodyweight TNF-α (both from Bioxcell). Control mice received four vehicle injections (PBS). Four weeks later, cytokine- and control-treated mice were injected with LPS (1 µg per kg bodyweight) or PBS, and were killed 3 h after the injection.

At the specified time-points, animals were deeply anaesthetized using sedaxylan and ketamine (64 mg/kg and 472 mg/kg), blood was collected from the right ventricle of the heart and animals were transcardially perfused with ice-cold PBS through the left ventricle. The brain was removed and sagitally separated into the two hemispheres, which were either fixed in 4% paraformaldehyde (PFA) or fresh-frozen on dry ice. Fresh-frozen hemispheres were homogenized using a Precellys lysing kit and machine at 10 or 20% (w/v) in homogenization buffer (50 mM Tris pH 8, 150 mM NaCl, 5 mM EDTA) containing phosphatase and protease inhibitors (Pierce). Fixed hemispheres were kept in 4% PFA for 24 h, followed by cryoprotection in 30% sucrose in PBS, subsequently frozen in 2-methylbutane and coronally sectioned at 25 µm using a freezing-sliding microtome (Leica).

Focal brain ischaemia. For the induction of a focal cortical stroke, we modified existing models of endothelin-1 (ET-1)-induced brain ischaemia43 to avoid traumatic injury to the brain. Under anaesthesia and analgesia (fentanyl, midazolam, centred on Bregma as described43), the dura mater was carefully removed. Under anaesthesia and analgesia (fentanyl, midazolam, centred on Bregma as described43), the dura mater was carefully removed. Under anaesthesia and analgesia (fentanyl, midazolam, centred on Bregma as described43), the dura mater was carefully removed. The brain homogenates were quantified with a microplate bicinchoninic acid (BCA) assay (Pierce) and adjusted accordingly. Samples were then analysed on NuPage Bis-Tris gels (Invitrogen) using standard procedures. Proteins were transferred to nitrocellulose membranes, blocking was performed with 5% milk in PBS containing 0.05% Tween (PBST) for 1 h and blots were incubated with mouse anti-Aβ (6E10; 1:1,000, Covance) in PBST overnight at 4°C. Membranes were then probed with secondary HRP-labelled antibodies (1:20,000, Jackson ImmunoLaboratories). Protein bands were detected using chemiluminescent peroxidase substrate (BCL prime, GE Healthcare). Densitometric values of the protein band intensities were analysed with the software package Aida v.4.27 and normalized to GAPDH intensities.

Immunostaining. Immunohistochemical staining was performed on free-floating sections using either Vectastain Elite ABC kits (VECTOR laboratories) or fluorescent secondary antibodies (Jackson Immunolaboratories). Unless otherwise noted, brain sections were blocked for 1 h with 5% normal serum of the secondary antibody species, followed by primary antibody incubation overnight at 4°C. Primary antibodies used were: rabbit anti-Pu.1 (1:1,000, Cell Signalling), rabbit anti-IXba1 (1:1,000; Wako; catalogue no. 019-19741), rabbit anti-GFAP (1:500, Biozol; catalogue no. Z03334), rabbit anti-Aβ (CN3; 1:2,000)44, mouse anti-HIF-1α (1:500; Novus Biologicals, catalogue no. NB100-105, clone H1alpha67), rat anti-CD11b (1:2,000; Millipore, catalogue no. MAB1387Z), rabbit anti-APP (antibody 5313 to the ectodomain of APP, 1:750; kindly provided by C. Haass, DZNE Munich). Sections were then washed and incubated with secondary antibodies. Cresyl violet and Congo red staining was conducted according to standard procedures. Fluorescent plaque staining was achieved using Methoxy-X04 (4% vol of 10 mg/ml methoxy-X04 in methanol, 2% vol of 7.7% vol Cremophor EL in 88.3% vol PBS) for 20 min at room temperature.

Images were acquired on an Axiosplan 2 microscope with Axiosplan MRm and AxioVision 4.7 software (Carl Zeiss). Fluorescent images were acquired using a LSM 510 META (Axiovert 200 M) confocal microscope with an oil immersion 63 × 1.4N.A objective and LSM software 4.2 (Carl Zeiss), using sequential excitation of fluorophores. Maximum-intensity projections were generated using IMARIS 8.3.1 software (Bitmap).

For quantitative comparisons, sections from all groups were stained in parallel and analysed with the same microscope settings by an observer blinded to the treatment groups. To quantify the intensity of total microglial HIF-1α staining, high-resolution bright-field images were acquired using fixed camera exposure time and lamp intensity and subsequently analysed with Fiji software. Colour channels were split and a fixed intensity threshold was applied to the red channel. On each image, the thresholded area over the total image area was calculated. Area fractions were measured on images of at least 9 plaques and 15 plaque-free regions per animal. To exclude an influence of plaque size on microglial HIF-1α levels, plaques of similar size were selected for analysis of HIF-1α levels in the control and cytokine treatment groups (average plaque size: PBS p.i.: 1.73 ± 0.15 × 1.84 ± 0.19, 4 × LPS p.i.: 2.27 ± 0.39% Congo red area fraction).

For nuclear HIF-1α staining, a modified staining protocol was used. In brief, sections were blocked with mouse immunoglobulin blocking reagent (Vector laboratories) for 1 h at room temperature, followed by blocking with normal donkey serum for 1 h at room temperature. Sections were then incubated overnight with mouse anti-HIF1α (clone mgc3, 1:50; Thermo Fisher Scientific, catalogue no. MA1-516) and rabbit anti-Pu.1 (1:250; New England Biolabs, catalogue no. 2258S. Clone 9G7) at 4 °C. To quantify the intensity of nuclear HIF-1α staining, z-stacks from three plaques and plaque-free regions per animal were acquired with the same microscope settings and subsequently analysed with IMARIS 8.3.1 software. Using the surfaces tool, a mask based on microglial nuclei was created using staining for Pu.1. A filter for area was applied to exclude background staining. The created surface was used to mask the HIF-1α channel. The mean masked HIF-1α intensity was then determined.

To quantify neuronal dystrophy, fluorescent images from 5–10 plaques per animal were acquired with the same microscope settings and subsequently analysed with Fiji software. Maximum intensity projections were generated to choose the region of interest consisting of APP staining and the plaque. Fluorescence channels were split, and intensity thresholds were applied to each channel. For every plaque, the thresholded area within the region of interest was calculated as a measure of plaque size and dystrophic area.

Stereological and morphological quantification. Stereological quantification was performed by a blinded observer on random sets of every 12th systematically sampled 25-µm thick sections throughout the neocortex. Analysis was conducted using the Stereo Investigator software (Stereo Investigator 6; MBF Bioscience) and a motorized x–y–z stage coupled to a video microscopy system (Optronics). For quantification of plaque-associated cells, the optical fractionator technique was used with 3D dissector as previously described45. For the quantification of plaque-associated cells, plaques were identified by Congo red staining and cells in their immediate vicinity were counted. Plaque load was determined by analysing the

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
cortical area covered by Congo Red and/or anti-Aβ staining using the area fraction fractionator technique. The volume of neuronal damage and microglial activation after brain ischaemia was determined using the Cavalieri estimator technique.

To analyse microglial morphology, we acquired three images from three non-consecutive lba-1 immunostained brain sections per animal using identical camera acquisition settings, at 20 × 0.50NA magnification. In order to perform the filament tracing in IMARIS (v8.3.1), images were pre-processed in Fiji to optimize the contrast for reconstruction. The image background was subtracted using the inbuilt Fiji plugin to obtain an evenly distributed intensity and enhance contrast to the cells; subsequently the images were sharpened and their intensity was adjusted to the respective minimum and maximum histogram values. Filaments were then traced in IMARIS using the in-built Autopath algorithm. Reconstruction parameters were kept constant among all images; each cell was reconstructed as a ‘filament’ element in IMARIS, associated with a total length and volume.

**Enzyme-linked immunosorbent assay (ELISA).** For quantification of Aβ3 by ELISA (Meso Scale Discovery) in brain homogenates or by single molecule array (SOMO, Quanterix) in isolated microglial cells, samples were pre-treated with formic acid (Sigma-Aldrich, final concentration: 70% vol/vol), sonicated for 35 s on ice, and centrifuged at 25,000g for 1 h at 4°C. Neutralization buffer (1 M Tris base, 0.5 M Na2HPO4, 0.05%NaN3 (wt/vol)) was then added at a 1:20 ratio. Aβ3 was measured by an observer blinded to the treatment groups using human (6E10) Aβ3 triplex assay (Meso Scale Discovery, MSD) in brain homogenates or Simoa Human Abeta 42 2.0 Kit (Quanterix) in isolated microglia according to the manufacturer’s instructions.

Soluble AβP3 containing the Swedish mutations (as present in the APP23 transgenic model) was measured using the sw soluble APP kit (Mesoscale Discovery) following the manufacturer’s instructions after extraction with 1% Triton X-100 and ultracentrifugation for 1 h (135,000g at 4°C). For cytokine measurements, brain homogenates were centrifuged at 25,000g for 30 min at 4°C. Supernatants were analysed using the mouse pro-inflammatory panel 1 V-plex plate (Mesoscale Discovery) according to the manufacturer’s instructions. To determine blood cytokines, serum was obtained by coagulation of whole blood in Vacuette (Greiner Bio-One) for 10 min at room temperature and centrifugation for 10 min at 2,000g. Serum samples were diluted 1:2 before measurements. The investigator was blinded to the treatment groups.

Measurements were performed on a Mesoscale Sector Imager 6000 or a Simoa HD-1 Analyzer. For analyses of brain homogenates, protein levels were normalized against total protein amount as measured by BCA protein assay (Pierce).

To determine levels of LPS in blood and brain homogenates, the Limulus Amebocyte Lysate assay was used according to the manufacturer’s instructions (Pierce LAL Chromogenic Endotoxin Quantitation Kit). Standards were prepared either in serum or brain homogenate from non-injected control animals. Serum samples were diluted 1:100 and brain homogenates 1:5 to eliminate matrix effects. Isolation of Aβ from brain homogenate was performed by 1% Triton X-100 extraction and ultracentrifugation for 1 h at 4°C. The pellet was resuspended in 100 µl Nelson buffer (50 mM Tris, 150 mM NaCl, 20 mM EDTA, 1% vol/vol Triton X-100, 0.5% vol/vol NP-40) and frozen on dry ice.

**Chromatin immunoprecipitation, library preparation and analysis.** To isolate microglia for chromatin purification, 1 ml sodium butyrate, an inhibitor of histone deacetylases, was added to the dissection medium and FACS buffers. After 2 h, the suspensions were fixed in 1% PFA for 10 min at room temperature, followed by addition of glycerine (final concentration: 125 mM) for 5 min and washing in HBSS. Microglia were then sorted into homogenization buffer (0.32 M sucrose, 5 mM CaCl2, 5 mM magnesium acetate, 50 mM HEPES, 0.1 mM EDTA, 1 mM DTT, 0.1% vol/vol Triton X-100) and centrifuged at 950g for 5 min at 4°C. The pellet was resuspended in 100 µl Nelson buffer (50 mM Tris, 150 mM NaCl, 20 mM EDTA, 1% vol/vol Triton X-100, 0.5% vol/vol NP-40) and frozen on dry ice.

**Chromatin immunoprecipitation with sequencing (ChIP–seq)** was performed as previously described. Briefly, chromatin samples were digested with an equal number of restriction enzymes and replications were performed for each condition and targeted histone modification. Cell lysates from 8–10 mice were pooled, giving a total cell number of approximately 0.8 million to 1 million cells per replicate. The cross-linked chromatin was sheared for 3 × 7 cycles (30 s on/off) in a Bioruptor Plus (Diagenode) to achieve an average fragment size of 350 bp. Proper shearing and chromatin concentration were validated by DNA isolation and quantification using a small amount of each sample individually. Samples were split in half and 1 µg of ChIP-grade antibody (H3K4me1: Abcam ab8895 or H3K27ac: Abcam ab4729) was added and incubated overnight at 4°C. From each sample, 1% of the total volume was taken as input control before antibody binding. Immunoprecipitation was performed by incubating samples with 30 µl BSA-blocked protein A magnetic beads (Dynabeads, Invitrogen) for 1 h at 4°C. After purifying the precipitated chromatin and isolating the DNA, DNA libraries were generated using the Next Ultra DNA Library Prep Kit for Illumina and the Q5 polymerase (New England Biolabs). Multiplexing of samples was done using six different index primers from the Library Prep Kit. For each replicate, samples from each condition (genotype and treatment) were pooled 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; each pool represented one whole batch of samples for each condition and targeted histone modification. The final mixture for the library pool was loaded onto a 2% agarose gel and the correct band size was excised for purification and quantification. The purified libraries were then sequenced using the Illumina HiSeq 2000 platform and output was used for further analyses at the NGS facility at the University of Huddersfield.

**Base calling from raw images and file conversion to fastq files** were achieved using standard Illumina pipeline scripts. Sequencing reads were then mapped to the mouse reference genome (mm10) as described in previous work using default parameters. Data were further processed using HOMER software (http://homer.salk.edu/homer/), following two recently published analyses on microglial epigenetic profiles. Tag directories were created from bam files using a Qubit 2.0 Fluorometer (Life Technologies) and Agilent 2100 Bioanalyzer (Agilent Technologies). A final library concentration of 2 nM was used for sequencing. Sequencing was performed using a 50-bp single read setup on the Illumina HiSeq 2000 platform.

**Call base from raw images and file conversion to fastq files** were achieved using standard Illumina pipeline scripts. Sequencing reads were then mapped to the mouse reference genome (mm10) as described in previous work using default parameters. Data were further processed using HOMER software (http://homer.salk.edu/homer/), following two recently published analyses on microglial epigenetic profiles.
normality criterion was met, data were analysed using a one-way ANOVA (for example, Shapiro–Wilk test) and statistical outliers using the 'explore' function. If the Tukey correction for significant main effects ($P < 0.05$). As the cytokine data for acute LPS stimulation (Fig. 1) showed inequality of variance as well as skewedness, a non-parametric, independent-samples median test was performed followed by pairwise comparison with correction for multiple comparison.

All experiments were performed at least twice and in independent batches of animals for key findings (figures show the pooled data). Owing to batch-related variation in some dependent variables, 'batch' was added as a random variable to analyses where a significant batch effect was observed. For datasets with small sample size (for example, western blotting analyses), the Kruskal–Wallis test was performed, followed by pairwise comparisons if $P < 0.05$. In the figure legends, $n$ denotes the number of animals per treatment group. Minimum sample sizes were determined prior using power analyses or as dictated by the methodology (for example, ChIP-seq).

Raw and processed data are provided in the Gene Expression Omnibus (accession number GSE82170; subseries GSE82168 for ChIP-seq and GSE104630 for RNA-seq datasets). Other data that support the findings of this study are available from the corresponding author upon reasonable request.
Extended Data Fig. 1 | Acute responses to LPS injections. a, Weight changes after injection of LPS (wild-type mice: \( n = 11, 11, 11, 11, 4 \) from left to right for PBS, \( n = 9, 9, 8, 7 \) for \( 1 \times \) LPS, \( n = 10, 10, 10, 7 \) for \( 4 \times \) LPS; APP animals: \( n = 14, 14, 14, 14 \) for PBS, \( n = 8, 8, 5, 5 \) for \( 1 \times \) LPS, \( n = 10, 10, 10, 10, 10 \) for \( 4 \times \) LPS; Cre mice: \( n = 5, 5, 4 \)). b–c, Morphological changes in microglia (\( n = 6, 6, 6, 6, 6 \) mice). Scale bar, 50 \( \mu \)m. d, Numbers of microglia and activated (GFAP \(^+\)) astrocytes (microglia: \( n = 6, 6, 6, 6, 6 \) mice, Scale bar, 50 \( \mu \)m). d, Numbers of microglia and activated (GFAP \(^+\)) astrocytes (astrocytes: \( n = 6, 8, 9, 7, 5 \) mice). e, Blood and brain levels of LPS after daily injections with 500 \( \mu \)g per kg bodyweight (\( n = 4, 3, 3, 3, 3 \) animals). f, Assessment of iron entry from the blood (detected by Prussian blue staining) shows positive staining in an aged (>25 months) APP transgenic mouse, but not after repeated intraperitoneal LPS injections (\( n = 3 \) mice analysed). g, In heterozygous mice expressing red fluorescent protein (RFP) under the type 2 CC chemokine receptor (Ccr2) promoter, no entry of CCR2-expressing blood monocytes was detected after repeated LPS injection (staining for RFP; insert shows RFP-positive monocytes in the choroid plexus; \( n = 3 \) mice analysed). Scale bar, 100 \( \mu \)m. Data are means ± s.e.m. *\( P < 0.05 \), **\( P < 0.01 \), ***\( P < 0.001 \) for one-way ANOVA with Tukey correction.
Extended Data Fig. 2 | Cytokine response after acute LPS injections. 

a, Additional cytokines (Fig. 1) analysed in the serum (top) and brain (bottom) 3 h after each daily intraperitoneal LPS injection on four consecutive days in 3-month-old mice (control mice received PBS injections; n = 16, 11, 12, 9, 7, 7 and 5, 13, 4, 6, 9, 4, 5 mice for groups from left to right). b, Cytokine response in the blood only in wild-type (b, n = 6, 7, 8, 5, 3, 3 mice) or APP23 mice (c, n = 10, 3, 3, 4, 3, 3 mice). d, e, Cytokine response in the brain only in wild-type (d, n = 6, 7, 8, 5, 3, 3 mice) or APP23 mice (e, n = 10, 4, 4, 4, 4, 4 mice). Data are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 for independent-samples median test with correction for multiple comparisons.
Extended Data Fig. 3 | APP levels and processing, neuritic dystrophy and astrocyte activation in 9-month-old APP23 animals. 

**a, b,** Micrograph of fluorescent staining for amyloid plaque (Methoxy-X04; green) and amyloid precursor protein (APP; red) (a) shows neuritic dystrophy surrounding the amyloid deposit, which is unchanged by LPS treatments (b; n = 5, 5, 5 animals). **c,** Overall Pearson’s correlation of plaque size with neuritic dystrophy (APP area; n = 49, 39, 42 plaques for PBS, 1 × LPS, 4 × LPS groups). **d,** Western blotting analysis (for gel source data, see Supplementary Fig. 1) of brain homogenates for APP and C-terminal fragment-β (CTFβ; n = 7, 4, 7 mice), and soluble APP β ELISA (n = 6, 6, 6 mice). **e,** Micrograph of activated astrocytes (glial fibrillar acidic protein: GFAP) surrounding an amyloid plaque (Congo red) and quantification of the number of plaque-associated GFAP-positive astrocytes (n = 6, 6, 5 mice). Scale bar, 10 μm (a), 20 μm (e). Data are means ± s.e.m. *P < 0.05 for one-way ANOVA with Tukey correction.
Extended Data Fig. 4 | Cytokine levels in 9-month-old animals.

(a) Cytokine measurements in brain homogenates of 9-month-old wild-type (n = 8, 8, 7 mice) and APP23 mice (n = 14, 10, 10 mice) treated i.p. with 1 × LPS or 4 × LPS at 3 months of age. Cytokine measurements in the serum of 9-month-old wild-type (WT; n = 14, 9, 13 mice) and APP23 mice (APP; n = 18, 12, 14 mice) after i.p. stimulation with 1 × LPS or 4 × LPS at 3 months of age and re-stimulated with an additional LPS injection (500 µg kg−1) at 9 months of age (n = 10, 7, 10 animals). Data are means ± s.e.m. *P < 0.05, **P < 0.01 for two-way ANOVA with Tukey correction. In a a significant main effect for genotype is indicated by bars spanning all conditions of the same genotype.
Extended Data Fig. 5 | Cytokine levels after brain ischaemia and in blood of 4-month-old mice. Three-month-old animals were injected i.p. with 1 × LPS or 4 × LPS and incubated for 4 weeks before receiving a stroke. a, Cytokine measurements in brain homogenates 24 h after stroke (n = 5, 7, 5 animals). b, Cytokine measurements in the serum (n = 6, 6, 6 animals). Data are means ± s.e.m. ***P < 0.001 for one-way ANOVA with Tukey correction.
Extended Data Fig. 6 | Microglial sorting strategy. Microglia were sorted as CD11b<sup>high</sup> and CD45<sup>low</sup> cells (population P4) from 9-month-old APP23 mice or wild-type littermates following i.p. injections of 1 × LPS or 4 × LPS at 3 months of age.
Extended Data Fig. 7 | Analysis of microglial enhancers. Microglial enhancers were analysed in 9-month-old wild-type and APP23 (APP) mice treated intraperitoneally with 1 × LPS or 4 × LPS at 3 months of age. a, Exemplary UCSC browser images of genomic region around the Hif1a gene (normalized to input and library dimension). b, Numbers of regions with differentially regulated H3K4me1 levels. c, Heatmaps of H3K4me1 regions (centred on H3K27ac peaks). d, Pairwise correlations between the two replicates of H3K4me1 read densities in differentially regulated regions. e–g, Analyses of H3K27ac levels analogous to b–d for H3K4me1. n = 2 replicates (8–10 mice per replicate); differential enhancers showed a cumulative Poisson P < 0.0001.
Extended Data Fig. 8 | Transcription factor motif analysis of active enhancer regions. Motif analysis was performed for selected conditions to identify transcription factors involved in the differential activation of enhancers (using putative enhancer regions present in both replicates within 500 bp around enhancer peaks). a, For all active enhancers, motif analysis was performed using the union H3K27ac peak file and standard background (random genomic sequence). b, Pairwise comparisons between conditions, using the first condition’s H3K27ac peak file as input and the second condition’s peak file as background. As motif enrichment was often relatively low, the analysis was focused on transcription factor (families), whose motifs occurred at least twice in ‘known’ (black) and ‘de novo’ motifs (blue). Motifs are identified by HOMER software using hypergeometric testing (no adjustment for multiple comparisons was made).
Extended Data Fig. 9 | Peripherally applied cytokines induce immune memory in the brain. a. Experimental design. b. Cytokine responses in the brain, four weeks after peripheral cytokine application (n = 17, 5, 21, 8, 8, 15 mice from left to right). Note that TNF dose-dependently enhances (low dose) or decreases (high dose) certain cytokines. Similar to high dose TNF, certain cytokines are also reduced by peripheral application of IL-10 four weeks earlier. c. Cytokine responses in the periphery are unaffected (n = 8, 21, 9, 5, 10 mice). Data are means ± s.e.m. *P < 0.05, **P < 0.01, ***P < 0.001 for one-way ANOVA with Tukey correction.
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.

1. **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. 22585. 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): Goldmann et al. Nat. Neurosci., 2013, 16:1618-1626.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

b. Describe the method of cell line authentication used.

c. Report whether the cell lines were tested for mycoplasma contamination.

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 described. Similarly, microglial-specific knockout of HDAC1/2 was achieved after crossing CX3CR1-CreER animals with a Hdac1/2 fl/fl line. 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-amyloidosis, 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.
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. ☒
ChIP-seq Reporting Summary

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                 |
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 rep2 | 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 | 34,648,071  | 25,771,385   |
| H3K27ac APP 1xLPS rep1 | 34,648,071  | 25,771,385   |
| 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   |
7. Describe the antibodies used for the ChIP-seq experiments.

| Antibody | Description |
|----------|-------------|
| 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: Manufacturer’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 individual 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 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.