Activation of microglia is a prominent pathological feature in tauopathies, including Alzheimer’s disease. How microglia activation contributes to tau toxicity remains largely unknown. Here we show that nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, activated by tau, drives microglial-mediated tau propagation and toxicity. Constitutive activation of microglial NF-κB exacerbated, while inactivation diminished, tau seeding and spreading in young PS19 mice. Inhibition of NF-κB activation enhanced the retention while reduced the release of internalized pathogenic tau fibrils from primary microglia and rescued microglial autophagy deficits. Inhibition of microglial NF-κB in aged PS19 mice rescued tau-mediated learning and memory deficits, restored overall transcriptomic changes while increasing neuronal tau inclusions. Single cell RNA-seq revealed that tau-associated disease states in microglia were diminished by NF-κB inactivation and further transformed by constitutive NF-κB activation. Our study establishes a role for microglial NF-κB signaling in mediating tau spreading and toxicity in tauopathy.
Abnormal aggregation and spreading of the microtubule-associated protein tau is the key defining feature of a group of heterogeneous neurodegenerative diseases known as tauopathies. Alzheimer’s disease (AD) is the most common tauopathy, with a hallmark of neurofibrillary tangles (NFTs) composed of insoluble tau fibrils. Tau pathology correlates more closely with synaptic loss, neurodegeneration, and cognitive decline than does amyloid pathology. Understanding the pathogenic mechanism induced by pathological tau is critical for developing effective therapeutics for AD and other tauopathies.

Neuroinflammation, the immune response in the central nervous system (CNS) characterized by reactive gliosis and increased inflammatory molecules, is one of the early and sustained pathological features of tauopathies. Microglia, the resident innate immune cells in the CNS, are key players in neuroinflammation. Recent genetic and gene network analysis of late-onset AD (LOAD) identified several risk variants predominantly expressed in microglia, implicating a pivotal role of microglia in AD pathogenesis. Reactive microglia have been observed to associate with NFTs in AD, primary tauopathies, and tau transgenic models. In vitro studies confirm that tau directly activates microglia and triggers a proinflammatory profile. Accumulated evidence also suggests that microglia are involved in tau-mediated pathobiology, including tau phagocytosis, post-translational modification and aggregation, cell proliferation, and apoptosis in the central nervous system. Itk kinase (IKK) activates NF-κB via phosphorylation and subsequent degradation of Itkα, an inhibitor of NF-κB. Genetic manipulations of Itk and IKK using the Cre-lox system enable conditional activation or inactivation of NF-κB and the dissection of functions of NF-κB in specific cell types. Such studies have shown that neuronal NF-κB plays an essential role in synaptic plasticity and regulating learning and memory behaviors in basal conditions, whereas in pathological conditions, the anti-apoptotic role of neuronal NF-κB is associated with neuroprotective effects. Microglial NF-κB also regulates synaptic plasticity.

Dysregulation of NF-κB has been implicated in AD pathogenesis. Indeed, in a meta-analysis, NF-κB signaling was found to be among the most perturbed pathways in LOAD brains. NF-κB is known to be activated by amyloid β (Aβ) and to contribute to Aβ production. We previously showed that specific inhibition of microglial NF-κB activation via deacetylation of p65 protected against Aβ toxicity in glial-neuron cocultures. However, very little is known about the role of microglial NF-κB activation in tauopathy.

Our current study establishes NF-κB signaling as a central transcription factor driving tau responses in vitro and in vivo models of tauopathy. By genetically deleting or activating IKKβ kinase selectively in microglia, we investigated how microglial NF-κB signaling contributes to tau processing, seeding, and spreading, as well as tau toxicity using behavioral studies. Using bulk and single-nuclei RNA-sequencing (RNA-seq), we dissected how microglial NF-κB activation and inactivation modify overall transcriptomic changes, tau-associated microglial states, and underlying pathways in microglia. Our findings uncover mechanisms by which microglial NF-κB activation drives disease progression in tauopathy.

**Results**

**Tau activates NF-κB pathway in microglia.** To determine tau-induced transcriptome changes in microglia, we treated primary mouse microglia with synthetic 0N4R full-length wild-type (FL_WT) tau fibrils for 24 h and analyzed them by RNA-seq. Synthetic recombinant tau monomers and fibrils contained negligible endotoxin (Supplementary Data 1). Out of 2975 differentially expressed genes (DEGs, False Discovery Rate (FDR) < 0.05) (Fig. 1a and Supplementary Data 2), Ingenuity Pathway Analysis (IPA) revealed that the top affected canonical pathways were associated with cellular immune responses, morphological changes, cell movement, survival, and proliferation (Fig. 1b, Supplementary Data 3). NF-κB signaling, which is involved in the upstream or downstream regulation of many other canonical pathways, such as Tumor Necrosis Factor Receptor 2 (TNFR2), toll-like receptor (TLR), interferon, and interleukin 12 (IL-12) signaling, was among the top altered cellular immune response pathways. Many NF-κB target genes were upregulated, including chemokines and receptors, such as C-C Motif Chemokine Ligand (CCL5), C-X-C Motif Chemokine Ligand 9 (CXCL9), complement component C3, and proinflammatory cytokines interleukin 1 beta (IL1B), Interleukin 12b (IL12b), and Tumor Necrosis Factor (TNF). Fc fragment of IgG receptors involved in phagocytosis such as Fc receptor, IgG, high affinity I (Fcg1), and the NF-κB pathway component NF-κB Inhibitor Alpha (Nfkbia) (Fig. 1a).

NF-κB transactivation was also measured with a reporter assay. Primary microglia were infected with lentivirus expressing EGFP under the control of the 5× κB enhancer element (Lenti-κBDEGF). Both FL_WT and K18/PL tau fibrils, a truncated form of human tau fibrils containing only microtubule-binding domains with the P301L MAPT mutation, significantly induced EGFP expression, indicating the activation of NF-κB promoter by tau fibrils (Fig. 1c, d). Wild-type or P301L mutant tau monomers also induced EGFP expression in microglia infected with Lenti-κBDEGF, confirming that both tau fibrils and monomers can activate the microglial NF-κB pathway (Supplementary Fig. 1a, b). The extent of NF-κB activation induced by 2–2.5 μg/ml tau fibrils was comparable to that of 50 ng/ml lipopolysaccharide, which contains 250–2000 fold more endotoxin (Supplementary Data 1).

We next profiled microglial transcriptional changes in a tauopathy mouse model of isolated microglia from 11-month-old PS19 mice. Disease-associated microglia (DAM) are identified as a subset of microglia associated with AD and other neurodegenerative diseases with a unique transcriptional signature. Some DAM signature genes, such as Cst7, Axl, Lpl, Itxag, Clec7a, Cox6a2, Ank, Csfr1, and NF-κB target genes, such as Tnf, Il1rn, Trh2, and Traf3, were among the upregulated DEGs (FDR < 0.05, Supplementary Data 4) (Fig. 1e). Fifty-nine of 187 upregulated DEGs in PS19 microglia, including DAM genes (e.g., Clec7a, Cst7, Lpl) and NF-κB target genes (e.g., Tnf, Il1rn, Trh2), were also upregulated in tau fibrils stimulated microglia, indicating a similarity between tau-induced transcriptomic changes in microglia in vitro and in vivo. (Fig. 1a, e and Supplementary Data 5). NF-κB signaling was also identified as one of the top differentially regulated pathways in PS19 microglia (Fig. 1f). Other pathways and DEGs included cell growth and death, mitochondrial dysfunction, and autophagy pathways, together with cytochrome-c oxidases, the terminal enzymes of the mitochondrial respiratory chain, such as Cox6a2, Cox8a, and endo-lysosome associated genes, such as Ctsb, Lamp1, Gnr, Rab34, all of which were upregulated in PS19 microglia (Fig. 1e, f and Supplementary Data 6). Consistent with the canonical pathways associated with NF-κB activation, cell movement, and migration, inflammatory responses and phagocytosis were the top activated biological functions in tau-stimulated microglia and...
PS19 microglia (Supplementary Fig. 1c, d). Specifically, IκB kinase complex subunit IKKβ (ikbb) was identified as one of the top upstream regulators responsible for tau-mediated transcriptomic changes in primary microglia (Fig. 1g) and PS19 microglia (Fig. 1h) using IPA upstream regulatory analysis. ikbb gene itself was also upregulated in tau-stimulated microglia, and predicted to be activated in PS19 microglia (Fig. 1i, j). Indeed, a large repertoire of DEGs was predicted to be regulated by IKKβ, supporting IKKβ activation as a master regulator of tau-mediated microglial NF-κB activation.

NF-κB transforms transcriptomes in cultured microglia. To directly investigate the cell-autonomous effects of NF-κB in
microglia, we activated IKKβ in microglia by crossing Cx3cr1CreERT2 mice with R26-StopIkk2a mice, in which a constitutively active form of IKKβ was inserted into the Flxedox-Rosa locus with a stop codon (hereafter referred to as "IkkkbCA/Fm" mouse) 38. We treated primary microglia from Cx3cr1CreERT2/+, IkkkbCA/Fm mice with 4-hydroxy tamoxifen 39 to induce Cre expression (Fig. 2a). Elevation of Ikkkb was confirmed by RT-qPCR (Fig. 2b). We compared the transcriptomes induced by IkkkbCA with those induced by tau stimulation, and observed 693 shared DEGs (556 upregulated and 137 downregulated) (Fig. 2c and Supplementary Data 7), suggesting that tau-induced alterations may be partially mediated by NF-κB activation. IPA analyses of shared DEGs showed that in tau-stimulated microglia, enhanced cell proliferation, movement, phagocytosis, cytotoxicity, and reduced cell death (Fig. 2d), as well as elevation of immune response pathways such as interferon, TLR signaling, and inhibition of apoptosis signaling (Fig. 2c and Supplementary Data 8), may be mediated through NF-κB activation.

In complementary experiments, we selectively inactivated NF-κB in microglia by crossing Cx3cr1CreERT2 mice with IkkkbKO mice 40, and treated primary microglia with 4-hydroxy tamoxifen 39 to induce Cre expression (Fig. 2f). Deletion of Ikkkb in microglia was confirmed by RT-qPCR (Fig. 2g). To further examine NF-κB-dependent microglial transcriptomes, we next compared the DEGs between IKKβ null and IkkkbCA microglia. Inactivation of NF-κB in microglia resulted in 208 unique DEGs (78 upregulated and 130 downregulated), while activation resulted in 1381 unique DEGs (775 upregulated and 606 downregulated) (Fig. 2h, Supplementary Data 9). IPA analysis revealed that inactivation of microglial NF-κB led to pathways associated with elevated cell death, and decreased cell movement and proliferation. In direct contrast, activation of microglial NF-κB altered pathways associated with decreased cell death, but elevated cell movement, proliferation, and phagocytosis (Fig. 2i, j). Analyses of the top affected canonical pathways revealed that those unique for IKKβ null microglia were associated with cell cycle regulation, nucleotide biosynthesis, and DNA damage repair, whereas those unique for IkkkbCA were enriched for integrin, TNFR2, and PI3K/Akt signaling (Supplementary Fig. 2a, b and Supplementary Data 10). Surprisingly, ~400 DEGs were shared by activating and inactivating microglial NF-κB (Fig. 2h and Supplementary Data 11). Among the shared DEGs, a great fraction was involved in interferon signaling (Supplementary Fig. 2c and Supplementary Data 10). Specifically, interferon regulatory factor 3 and 7 (IRF3, IRF7), interferon-α/β receptor (IFnar), and interferon-γ (IFng) were the top upstream regulators predicted to be activated for these transcriptomic changes (Supplementary Fig. 2d). These results support the extensive crosstalk between interferons and the NF-κB pathway in microglia 41.

NF-κB promotes microglial processing and release of tau species with seeding activity. In cultured conditions, we found that tau fibrils can be readily taken up by microglia, but not neurons, in a time-dependent manner, and once inside the cell, they colocalize with the late endosome/lysosome labeled by Dextran 42 (Supplementary Fig. 3a, b), where they can be proteolytically processed 43,44. To determine the effects of NF-κB signaling on this process, we examined tau fibril accumulation in IKKβ null and IkkkbCA microglia by comparing them to their respective littermate wild-type controls (Ikkkb WT). Inactivation of NF-κB enhanced, while activation diminished, the number of tau fibrils remaining in microglia (Fig. 3a, b). To dissociate uptake from clearance, we performed a pulse-chase assay by preloading microglia with tau fibrils and assessing the time-dependent clearance in the next 24 h (Fig. 3c). Compared with corresponding littermate wild-type controls, inhibition of NF-κB slowed down, while activation accelerated, tau clearance in microglia (Fig. 3d–g).

Next, we isolated sarkosyl-insoluble tau (AD-tau) from postmortem human AD frontal cortical tissue 44 (Fig. 3h). After incubation with microglia, AD-tau was internalized and detected by phosphorylated tau (pTau) antibody AT8 as intracellular puncta (Fig. 3i, j). After chasing in tau-free medium for 24 h, ~23% (~7.3%) of total AT8-positive-pTau taken up by microglia was released in the conditional medium (CM) and ~22.5% (~5.2%) remained in the cells (Fig. 3k), suggesting that ~55% internalized pTau were degraded or processed by microglia.

We next examined the seeding activity of microglia-released tau using HEK human tau RPD301S biosensor cells 45. The AD-tau microglial conditional medium (AD-tau CM) induced intracellular tau aggregation in HEK biosensor cells while CM of microglia without tau loading did not (Control CM) (Fig. 3l, m), demonstrating a potent seeding activity of AD-tau processed and secreted by microglia. Moreover, NF-κB inhibitor TPCA-146 significantly reduced AT8-positive pTau released from microglia (Fig. 3n) with a modest increase of intracellular pTau (Fig. 3o). Thus, inhibition of NF-κB reduces the microglial processing and release of pathologic tau species.

Microglial NF-κB activation promotes tau seeding and spreading in PS19 mice. Tau pathology spreads from the entorhinal cortex to the hippocampal region in the early stage of AD 47. To model the seeding and spread of tau inclusions in vivo, we inoculated young PS19 mice with exogenous tau seeds as described previously 43 (Supplementary Fig. 4a). To investigate the role of microglia, we depleted microglia by feeding young PS19 mice a diet containing the colony-stimulating factor 1 receptor...
inhibitor PLX5622 (PLX)\(^{48}\), followed by inoculating with either brain extract from progressive supranuclear palsy (PSP) patients (Supplementary Fig. 4b) or synthetic K18/PL tau fibrils (Fig. 4a) unilaterally into the hippocampus. K18/PL tau fibrils-injected mice were continuously fed with PLX for one month to prevent microglial repopulation, which reduced the number of microglia by over 80% (Supplementary Fig. 4c, d). Depletion of microglia significantly reduced the number of tau inclusions in AT8+ neurons in both contralateral and ipsilateral cortex of PS19 mice inoculated with PSP brain extract, consistent with the notion that microglia promote tau seeding and spreading in mouse tauopathy models\(^{17}\) (Supplementary Fig. 4e, f). Similarly, microglial depletion significantly reduced the seeding and spreading of tau in PS19 mice inoculated with synthetic K18/PL tau fibrils, which
induced tau spreading within 1 month (Fig. 4a–c). Importantly, no AT8+ or MC1+ neurons were detected in PS19 mice inoculated with PBS, or in non-transgenic control mice inoculated with tau seeds (Supplementary Fig. 4g).

Since microglial processing of tau is regulated by NF-κB, we reasoned that microglial NF-κB activity could also affect tau seeding and spreading in vivo. We selectively deleted Ikkβ in adult microglia of PS19 mice by crossing Cx3cr1CreERT2 mice incubated with 4-hydroxytamoxifen to induce IKKc. PCR analysis of Ikkβ expression in adult microglia from Cx3cr1CreERT2/+; IkkβCA/+ and PS19 mice. Tamoxifen injection diminished IKKβ expression in adult microglia from Cx3cr1CreERT2/+; IkkβCA/+ (referred to as Ikkβ+/− mice) as confirmed with qRT-PCR analyses (Fig. 4d, e). To measure how tau seeding and spreading were affected, 3–month-old mice were inoculated with tau fibrils in one side of the hippocampus 2 weeks following tamoxifen injection (Fig. 4f). K18/PL fibrils were used to induce tau spreading since only 4 weeks post-inoculation time is needed for robust wide-spread tau propagation. Inactivation of microglial NF-κB reduced the amount of MC1+ tau inclusions significantly at the ipsilateral side of the cortex (Fig. 4g, h), similar to the effect of deleting microglia (Fig. 4c), suggesting a critical role for microglial NF-κB activity in tau spreading. In complementary experiments, tamoxifen injection enhanced IKKβ expression in adult microglia from Cx3cr1CreERT2/+; IkkβCA/+ mice (Fig. 4i, j). RNA-seq analysis of upregulated DEGs in IkkβCA/+ mouse brain (Supplementary Data 12) confirmed that NF-κB is the top transcription factor that leads the transcriptomic changes (Supplementary Fig. 5). In complementary experiments, we tested if activating microglial NF-κB could enhance tau propagation. To avoid ceiling effects, we reduced the amount of inoculated tau fibrils to that in microglial-depletion experiments. One month after the inoculation, IkkβCA/+ mice exhibited elevated tau inclusions significantly on the ipsilateral side, with a modest increase on the contralateral side of the hippocampus (Fig. 4k–m).

Our findings indicate that NF-κB activation is essential in promoting microglial-mediated tau seeding in vivo.

Inactivation of microglial NF-κB partially restores microglia homeostasis and protects against spatial learning and memory deficits in PS19 mice. Microgliosis and amoeboid morphological changes are early and sustained phenomena in PS19 mice10. Inactivation of microglial NF-κB reduced microgliosis in the hippocampus of 8–9-month-old PS19 mice (Fig. 5a, b), and a similar trend was observed in the cortex (Fig. 5c, d). Imanis analysis of microglial morphology further revealed that inhibition of microglial NF-κB resulted in more ramified morphology with longer processes and more branches, partially reversing the amoeboid morphology induced by pathogenic tau (Fig. 5e–g).

PS19 mice exhibit spatial learning and memory deficits starting at 7–8 months of age9,20. To examine the functional influence of inhibition of microglial NF-κB activity in PS19 mice, we tested 8–9-month-old Ikkβ−/−, Ikkβ−/−, IkkβCA/+; P301S+, and Ikkβ−/−; P301S+ mice in the Morris water maze (MWM) test, a hippocampus-dependent assay that evaluates spatial learning and memory deficits. Inhibition of microglial NF-κB alone did not significantly impact spatial learning and memory, measured by the learning curve and the number of times to cross platform location in a 72 h probe trial (Fig. 5h, i). Strikingly, inactivating microglial NF-κB in PS19 mice significantly improved the learning ability (Fig. 5h) and restored the spatial memory in the probe trial (Fig. 5i), without affecting swimming speed (Fig. 5j). Thus, hyperactive microglial NF-κB plays a critical role in altering microglial homeostasis and driving cognitive deficits in PS19 mice.

Inactivation of microglial NF-κB rescues tau-mediated transcriptomic changes and microglial autophagy deficits while increasing tau inclusions. We next examined if the protective effects of microglial NF-κB inactivation are mediated by reducing tau inclusions. Surprisingly, instead of reducing neuronal tau inclusions, inactivation of microglial NF-κB markedly increased the number of intraneuronal tau inclusions in both hippocampus and cortex of 9–10-month-old PS19 mice (Fig. 6a–c). This indicates that the toxicities of neuronal tau inclusions on cognition might be regulated by NF-κB-dependent maladaptive microglia responses, which are reduced by NF-κB inhibition.

To dissect the mechanisms underlying the protective effects of inhibiting microglial NF-κB activity, we performed a bulk RNA-seq of cortical tissues from 8 to 9-month-old Ikkβ+/+, Ikkβ−/−, IkkβCA/+; P301S+, and Ikkβ−/−; P301S+ mice. Remarkably, the inactivation of microglial NF-κB resulted in a reversal of more than 90% of DEGs (286 out of 311 genes) in PS19 mice (Fig. 6d, e and Supplementary Data 13), despite elevating the tau inclusions. IPA analysis of these 286 genes revealed that the majority of reversed canonical pathways were related to inflammatory responses and the endo-lysosome system, and included neuroinflammation signaling, complement system, ROS generation, TLR signaling, autophagy, and phagocytosis (Fig. 6f and Supplementary Data 14). Tau-mediated biological function alterations including inflammatory response, cell movement, phagocytosis, superoxide production, and demyelination were also reversed (Supplementary Fig. 6a). The top upstream regulators of the pathways and functions reversed by NF-κB inactivation include those regulating immune cell survival and activation (e.g., Tryopb, Spi1, Csf1r, Csf1), superoxide generation (e.g., Cybb), and the interferon pathway (e.g., Ifi7, Ifi8, Ifi16, Stat1), suggesting these pathways are likely to be associated with the toxic effects of microglia in PS19 mice (Supplementary Fig. 6b). Our previous studies showed that impairments in chaperone-mediated autophagy (CMA) induced by acetylated tau lead to aberrant tau release31, we therefore directly examined the effect of NF-κB inhibition on microglial CMA activity. We treated microglia expressing KFERQ-Dendra, a reporter for CMA52, with tau fibrils in the presence or absence of NF-κB inhibitor. We found that NF-κB inhibition by TP53A-1 fully rescued the CMA defect induced by tau fibrils, reflected by the recovery of the intracellular degradation of Dendra (Figs. 6g, h) and the amount of Dendra reporter reaching and internalized in lysosomes (Fig. 6g, i).
**Fig. 3 NF-κB promotes tau processing in primary microglia.** a, b Representative images and quantification of 24 h fluorescent tau fibrils accumulation in primary Ikbkb−/− (a) and IkbkbCA (b) microglia compared with their littermate Ikbkb+/+ and IkbkbWT control, respectively. Scale bar, 25 μm. Values are mean ± SD, relative to littermate control. Total N = 9 wells/group from three independent experiments. P-values were from multilevel mixed-effect model with experiment as hierarchical level, ***p < 0.001. c Schematic diagram of pulse-chase assay comparing Ikbkb−/− with Ikbkb+/+ microglia (d, e 25,000 cells/well) and comparing IkbkbCA with IkbkbWT microglia (f, g 20,000 cells/well). Scale bar, 25 μm. Values are mean ± SD, relative to 0 h. Total N = 16 wells (6 h, Ikbkb+/+); 20 wells (6 h, Ikbkb−/−); 14 wells/group of other conditions from three independent experiments. P-values were from multilevel mixed-effect model with experiment as hierarchical level, ***p < 0.001. h Schematic diagram illustrating the isolation procedures of human AD-tau. i Schematic diagram of quantifying release of AD-tau from microglia. j Detection of intracellular pTau by AT8 (green), cell membranes by Wheat Germ Agglutinin (WGA) (red), and nuclei by DAPI (blue). A representative image from three independent experiments. Scale bar: 10 μm. j Schematic diagram of quantifying release of AD-tau from microglia. k Quantification of secreted and the intracellular pTau by AT8 ELISA and shown as the percentage of total pTau loaded in microglia after 2 h incubation. N = 4 wells. A representative dataset from two independent experiments. Values are mean ± SEM. l Representative images of tau aggregation in HEK biosensor cells induced by CM from microglia treated with AD-tau. Insert: High magnification confocal image of tau aggregation. Scale bar: 50 μm, inset: 15 μm. m Quantification of tau aggregation positive cells. N = 4 (control) and 10 (AD-tau) fields from three independent experiments, values are mean ± SEM, two-tailed Mann–Whitney test. n, o TPCA-1-treated microglia were incubated 2 h with AD-tau followed by 24 h chase. The pTau released to medium (n) and intracellular pTau (o) were determined by AT8 ELISA. A representative dataset from two independent experiments. N = 4 wells, values are mean ± SEM, relative to DMSO, two-tailed t-test. Source data are provided as a Source data file.
Increased association of reporter to lysosome resulted from primary activation of CMA as we did not find changes in the overall abundance of endo/lysosomal compartments quantified by LAMP1 (Figs. 6j, k). These results support that inhibiting microglial NF-κB prevents tau-induced cellular toxicities including autophagy deficits.

NF-κB is required for tau-associated microglial states in PS19 mice. Microglia exhibit disease-associated states in the presence of pathology\textsuperscript{35}, including tau\textsuperscript{53}, which have been characterized by single-cell RNA-seq. To characterize the effects of NF-κB on microglial transcriptomes, we performed single-nuclei RNA-seq (snRNA-seq) using cortical tissues from Ikbkb\textsuperscript{+/+}; P301S\textsuperscript{+}, Ikbkb\textsuperscript{-/-}; P301S\textsuperscript{+}, and Ikbkb\textsuperscript{CA}; P301S\textsuperscript{+}, P301S\textsuperscript{+} mice. Non-transgenic (Ikbkb\textsuperscript{+/+} and Ikbkb\textsuperscript{WT}, for simplicity, labeled as Ikbkb\textsuperscript{+/+} in all snRNA-seq analysis) mice were used as non-disease controls. Following an established snRNA-seq protocol\textsuperscript{54}, we sequenced 114,118 nuclei from all four genotypes. After removal of potential multiplets using DoubletFinder\textsuperscript{55} and filtering for low-quality nuclei determined by thresholding gene counts, UMI counts, and percentage mitochondrial genes per nuclei (Supplementary Fig. 7a–e), we used 103,681 nuclei for downstream analysis. Using reference gene markers for annotations (Supplementary Fig. 7g), we identified major cell types of the brain, which were similarly represented within each group and individual mouse (Fig. 7a, b, Supplementary Fig. 7f).
Fig. 4 Microglial NF-κB activation promotes tau seeding and spread in PS19 mice. a Schematic diagram illustrating the timeline of microglia depletion and KIB/P/PL tau fibril inoculation in PS19 mice. Pathological tau was detected by MC1 immunohistochemical staining. b, c Representative images of MC1 tau positive neurons in ipsilateral and contralateral cortex (b) and quantification of percentage of MC1-occupied area (c) in control diet feeding mice (n = 6) and PLX diet feeding mice (n = 8), 6 sections per mouse, values are mean ± SD. P-values were calculated using multilevel mixed-effect model with mouse as hierarchical level. Scale bar, 500 μm. d Breeding diagram illustrating the generation of PS19 mice with microglia conditional deletion of IKKB. e qPCR confirmed reduction of Ikbb mRNA in adult microglia isolated from Ikbb−/− mice (n = 5) compared with wild-type control Ikbb+/+ (n = 3) mice. Values are mean ± SD, two-tailed t-test. f Schematic diagram illustrating the timeline of microglial IKKβ deletion and KIB/PL tau fibrils inoculation in PS19 mice. g, h Representative images of MC1 tau positive neurons in ipsilateral and contralateral cortex (g) and quantification of the percentage of MC1-occupied area (h) in Ikbb+/+; P301S+ (n = 10) and Ikbb−/−; P301S+ (n = 15) mice. 5 sections per mouse, values are mean ± SD. P-values were calculated using multilevel mixed-effect model with mouse as hierarchical level. Scale bar, 500 μm. i Breeding diagram illustrating the generation of PS19 mice with microglia expressing IKKCa. j qPCR confirmed increase of Ikbb mRNA in adult microglia isolated from Ikbb−/− mice (n = 4) compared with wild-type control Ikbb+/+ mice (n = 3), values are mean ± SD, one-tailed Mann-Whitney test. k Schematic diagram illustrating the timeline of expressing microglial IKKCa and KIB/PL tau fibril inoculation in PS19 mice. l, m Representative images of MC1 tau positive neurons in ipsilateral and contralateral hippocampus CA1 region (l) and quantification of the percentage of MC1-occupied area (m) in Ikbb+/+; P301S−/−; P301S+/+; P301S+ (n = 9) and Ikbb+/+; P301S−/−; P301S+/+ (n = 14) mice. 8 sections per mouse, values are mean ± SD. P-values were calculated using multilevel mixed-effect model with mouse as hierarchical level. Scale bar, 200 μm. Source data are provided as a Source data file.

We then examined the trajectory of the subclusters of a total 4305 microglia (1068 from Ikbb+/+; 1275 from Ikbb−/−; 720 from Ikbb−/−; P301S+; 1242 from Ikbb−/−; P301S+; and 1242 from Ikbb+/+; P301S+) to investigate how NF-κB affects microglial states in P301S mice using Monocle56. The microglial population from the four genotypes exhibited a clearly-defined five subclusters (Fig. 7c, Supplementary Data 15). Analyses of the distribution of the five subclusters by genotypes revealed that microglia from Ikbb+/+ brains were distributed among clusters 1 and 2, while the vast majority of cluster 3 came from Ikbb+/+; P301S, likely representing tau-induced microglial states (Fig. 7d). Most microglia from clusters 4 and 5 came from Ikbb−/−; P301S, suggesting that constitutive NF-κB activation further transforms microglial states (Fig. 7d). In direct contrast, microglia from Ikbb−/−; P301S were mostly found in Cluster 1, along with wild-type Ikbb+/+ microglia, demonstrating that activation of NF-κB is required for tau-induced transcriptional changes (Fig. 7d). We next examined microglial trajectory with pseudotime from 0 to 15 (Fig. 7e). Microglia from non-transgenic control (Ikbb+/+) were enriched at the starting point of the trajectory (pink, Fig. 7e, f). Microglia from PS19 mice with wild-type IKKB (Ikbb+/+; P301S+) exhibit trajectory away from those of non-transgenic control (green, Fig. 7f), while those lacking IKKB (Ikbb−/−; P301S+) partially overlaps with that of Ikbb+/+ control, illustrating their failure to advance in the direction of that of PS19 microglia (purple, Fig. 7e). The other end of the trajectory (yellow, Fig. 7e) was populated with microglia from Ikbb−/−; P301S+ mice, extending beyond the tau-associated disease states (cyan, Fig. 7f). Thus, tau-associated disease states in microglia require NF-κB activation.

We further dissected the pathways modified by microglial NF-κB in PS19 mice. We identified 961 DEGs in Ikbb−/−; P301S+ microglia and 691 DEGs in Ikbb+/+; P301S+ (vs. Ikbb−/−; P301S+) (FDR < 0.05, |log2FC| > 0.1, Supplementary Fig. 8a, b, Supplementary Data 16). We found that genes induced by tau positively correlated with DAM genes (Supplementary Fig. 8c), while those induced by Ikbb+/+; P301S+ and Ikbb+/+ (vs. Ikbb−/−; P301S+ and Ikbb−/−; P301S+) partially overlapped with those upregulated by constitutive NF-κB activation, with 439 DEGs uniquely upregulated in Ikbb+/+; P301S+ microglia (vs. Ikbb−/−; P301S+) (FDR < 0.05, |log2FC| > 0.1, Fig. 7g, Supplementary Data 17). Given the protective effects of NF-κB inactivation against tau-mediated toxicity, we were particularly interested in the 200 DEGs downregulated by NF-κB inactivation by comparing gene expression in cluster 1 vs. cluster 3 (Fig. 7h), which could underlie its protective effects against functional deficits and tau seeding/spread. Investigation of the overlapping pathways downregulated by NF-κB inactivation using gene set enrichment analysis (GSEA) identified complement, the IL2/STAT5, and lipid binding pathways (Fig. 7h, Supplementary Fig. 8a). Consistent with our finding that microglial NF-κB promotes tau processing, release, seeding, and spread, both proteinysis and exocytosis functions of microglia were down-regulated in Ikbb−/−; P301S+ microglia and upregulated in Ikbb+/+; P301S+ microglia by comparing gene expression in clusters 4 and 5 vs. cluster 1 (Fig. 7i). These pathways include secretory granule complex, vesicular/protein transport, and exocytosis, trafficking (Fig. 7h, i). The reprogramming of microglial disease states by NF-κB inactivation provides molecular underpinnings for microglial-mediated tau seeding and tau-mediated cognitive deficits.

Discussion

Here, we show that microglial NF-κB activation is required for microglial-mediated tau spreading and tau-mediated spatial learning and memory deficits in tauopathy mice (Fig. 8). NF-κB signaling was among the top altered cellular immune response pathways in response to tau in microglia isolated from PS19 tauopathy mice. By genetically activating or inactivating microglial NF-κB, we found that NF-κB accelerates microglial processing of tau in cultured microglia, and promotes tau seeding and spreading in vivo. Moreover, inactivation of NF-κB in tauopathy mice partially restored microglial homeostasis, reversed tau-mediated changes in the transcriptome and the CMA function, and rescued spatial learning and memory deficits. By identifying tau-associated microglial states that were diminished by NF-κB inactivation, our snRNA-seq analyses further reveal potential molecular mechanisms underlying microglial-mediated tau seeding/spreading and toxicity.

In both tau-stimulated primary microglia and isolated microglia from aged PS19 mice, we found that NF-κB is among the top upstream regulators, consistent with a previous study of microglia from rTg4510 mice57. We also analyzed microglial DEGs from another study of Tau-P301S model (GSE9318058, Supplementary Data 18) and confirmed the activation of NF-κB pathway (Supplementary Fig. 9). In contrast, NF-κB target genes were not enriched in a microglial transcriptome study from APPswe/PS1dE9 mice, an Aβ driven AD model59, suggesting distinct disease-associated transcriptional programs induced by tau vs. Aβ. Microglial NF-κB could be activated by soluble tau monomers, in agreement with a previous study showing that microglial NF-κB is activated as early as 2 months of age in the rTg4510 model57. We observed that some of the pathways and functions...
Fig. 5 Inactivation of microglial NF-κB partially rescues microgliosis, morphological alterations and protects against spatial learning and memory deficits in PS19 mice.  

a–d Representative immunohistochemical staining (a, c) and quantification (b, d) of Iba1+ microglia in the hippocampus (a, b) and cortex (c, d) of Ikbkb+/+ (n = 3), Ikbkb−/− (n = 3), Ikbkb+/+; P301S+ (n = 9), and Ikbkb−/−; P301S+ (n = 12) mice. Scale bar, hippocampus 250 μm, cortex 100 μm, values are mean ± SD, two-way ANOVA with Sidak’s multiple comparisons post-test. 

e Representative immunohistochemical confocal images showing morphological features of microglia from the hippocampus of Ikbkb+/+, Ikbkb−/−, Ikbkb+/+; P301S+, and Ikbkb−/−; P301S+ mice and the corresponding 3D reconstructions using Imaris. Scale bar, 10 μm. 

f, g Quantification of the total length of microglial processes (f) and the number of microglia process branch points (g). N = 4 mice per genotype (four sections per mouse) were imaged and a total of 893 (Ikbkb+/+), 902 (Ikbkb−/−), 3455 (Ikbkb+/+; P301S+), and 2143 (Ikbkb−/−; P301S+) microglia were analyzed. Values are shown as boxplot. The box extends from the 25th to 75th percentiles with the median is shown in the middle of box. Whiskers show from the smallest value to the largest. P-values were calculated using multilevel mixed-effect model with mouse and section as hierarchical levels, ***P < 0.001. 

h–j Morris Water Maze test was performed using Ikbkb+/+ (n = 13), Ikbkb−/− (n = 13), Ikbkb+/+; P301S+ (n = 8), and Ikbkb−/−; P301S+ (n = 12) mice. h Escape latency was plotted against the training days; i Times of crossing the platform location in the 72 h probe trial; j swimming speed. Values are mean ± SD, two-way ANOVA with Tukey’s multiple comparisons post-test. Source data are provided as a Source data file.
induced by tau overlapped with those induced by NF-κB activation, including proliferation and cell movement/migration, and that these could be reversed by inactivating microglial NF-κB in vitro and in PS19 mice. Moreover, inhibition of microglial NF-κB activity in PS19 mice reduced microgliosis, and resulted in longer and more branchy processes, partially reverting cells to a more homeostatic microglial state. Our snRNA-seq analyses further revealed that NF-κB is required for tau-associated microglial states in PS19 mice. NF-κB inactivation diminished the tau-associated disease states. In contrast, constitutive NF-κB activation in PS19 mice further extended microglial states beyond the tau-associated disease states, supporting a feed-forward
mechanism between tau toxicity and microglial NF-κB activation (Fig. 8).

Emerging evidence supports the hypothesis that microglia participate in tau seeding and spreading. 14,17 NLRP3–ASC inflammation–some activation was found to exacerbate exogenously seeded tau pathology as well as non-exogenously seeded intraneuronal tau aggregates, at least partially through modulating tau phosphorylation. 60,61. Our study shows that in PS19 mice, exogenous tau inoculation-induced tau seeding was accelerated by microglial NF-κB activation, but diminished by NF-κB inactivation. Indeed, inactivation of microglial NF-κB signaling alone induced a similar reduction in seeding as depleting microglia altogether, highlighting the central role of NF-κB signaling in microglia-mediated tau seeding. NF-κB activation could promote microglia to secrete more seeding-competent tau, and thus accelerate the spread of tau pathology. Indeed, in cultured microglia, we showed that NF-κB inhibition rescued deficits in CMA and reduced tau sequestration, consistent with our previous finding that impairments in CMA promote extracellular tau release in neurons. 51

How NF-κB signaling mediates the opposite effects on intraneuronal tau inclusions triggered by exogenous tau seeds vs. those induced by transgene alone is not known. These two models develop tau inclusions at vastly different timeline: 1-month post-inoculation vs. 8–9 months, and likely involve distinctive proteostasis mechanisms. Beyond modulating tau phosphorylation, 16 microglial NF-κB may also modulate the sorting, trafficking, and exocytosis of tau in neurons. Further investigation is needed to determine the extracellular soluble and seeding-competent tau released from microglia, and to identify the cellular machinery involved in sorting, transportation, degradation, and release of tau in both neurons and glia.

Microglia activation in neurodegenerative diseases can have both beneficial and detrimental effects. Similarly, in response to tauopathy, some of the microglial responses are adaptive, serving protective functions, while others could be maladaptive and promote toxicity. Inhibiting microglial NF-κB activity is sufficient to rescue the learning and memory deficits in PS19 mice, suggesting that NF-κB hyperactivation drives tau toxicity in neurons. Single-nuclei RNA-seq analyses of transcriptomes of excitatory and inhibitory neurons revealed that inactivation of microglial NF-κB caused marked changes in these neurons (Supplementary Fig. 10). Microglial NF-κB inactivation led to elevated synaptogenesis pathways in excitatory neurons, and endocannabinoid synapse and P2Y puri- genic receptor signaling pathways in inhibitory neurons, consistent with the protective effects of inactivating NF-κB. In transcriptomic analyses of tau-stimulated microglia in culture and in vivo, we identified that elevation of cytokines such as IL-1, IL-6, TNFa, and interferonα is among the major consequences of activation of microglial NF-κB. Chronic elevation of these cytokines can cause neurotoxicity. In other models of neurodegeneration, inhibition of microglial NF-κB reduced inflammatory markers, rescued motor neuron death, and extended survival of ALS mice. 62 In a kainic acid-induced neurodegenerative mouse model, deletion of IKKβ in microglia reduced expression of IL-1β and TNFα, which resulted in 30% reduction of hippocampal neuronal cell death. 63 Another potential mechanism mediating the toxic maladaptive responses could be the elevated expression of the complement system and related genes (e.g., Clqα, Clqγ), which are also positively regulated by microglial NF-κB.64,65. In AD mouse models, activated microglia were found to phagocytose synapses, a process relying on the activation of complement factors, including C3, C1q, and CR3. 66,67. In tauopathy, inactivation of C3-C3aR signaling reverses the deregulation of the immune network and rescues behavior deficits in PS19 mice. 68 The exact protective mechanism underlying inactivating microglial NF-κB remains to be determined.

Using two in vivo models, our current study revealed important roles of microglial NF-κB activity in tau spreading as well as tau-induced neuronal toxicity and cognitive deficits (Fig. 8). In young PS19 mouse, the spreading of tau inclusion is driven by exogenous tau seeds in a rather short period of time. We showed that microglial NF-κB activation accelerates while deficiency reduces the spread of tau inclusions. Combined with our finding that NF-κB inhibition reduced the release of tau from cultured primary microglia, our results suggest that microglial NF-κB activation promotes tau spreading via enhanced release of seedable tau (Fig. 8a). In contrast, in the non-seeded PS19 model, age-dependent tau aggregation is determined by tau proteostasis of neurons expressing human tau transgene. In this chronic model, we found that the protective effects of microglial NF-κB inhibition are associated with normalization of >90% of the overall transcriptome, abolishment of tau-mediated microglial states at the single-cell level, and elevation of intraneuronal tau inclusions. While the mechanisms underlying how microglial NF-κB affects tau proteostasis in neurons are unknown, it is possible that microglial NF-κB activity may stimulate tau exocytosis and/or proteolysis in these neurons, resulting in elevated intraneuronal tau inclusions in brains lacking microglial NF-κB activity (Fig. 8b).

Our surprising finding that inactivating microglial NF-κB protected against tau-mediated cognitive deficits despite elevated tau inclusions reveals that tau pathology and toxicity are multifaceted and cell-type-specific. While intraneuronal tau load can be pathological, it does not represent all pathological tau species, such as those secreted that can exert toxicity as well. Thus, the amount of intraneuronal tau inclusions may not directly correlate with the extent of neuronal injury. Importantly, microglial NF-κB
activity is necessary for executing tau-mediated neuronal toxicity and cognitive deficits. Taken together, our work shows that microglial NF-κB acts downstream of tau pathology, and directly mediates toxic effects on cognition, highlighting the potential of blocking maladaptive microglial responses instead of removing tau aggregates as a therapeutic strategy to treat tauopathy.

**Methods**

**Mice.** Tau-P301S transgenic mice (P301S+;JAX:008169) or Cx3cr1CreERT2/Cx3cr1CreERT2 mice (JAX: 021160) were crossed with IkbkbF/F mice (MGI:2445462) or R26-StopFLikk2ca (ikbkbCAF/F) mice (JAX:008242) to obtain P301S+/IkbkbF/F, P301S+/IkbkbCAF/F, Cx3cr1CreERT2/+;IkbkbF/F, and Cx3cr1CreERT2/+;ikbkbCAF/F mice. P301S+;IkbkbCAF/F mice were then crossed with Cx3cr1CreERT2/+;ikbkbCAF/F mice to obtain P301S+;Cx3cr1CreERT2/++;ikbkbCAF/F mice and littermates controls including ikbkbCAF/F, Cx3cr1CreERT2/++;ikbkbCAF/F, and P301S+;ikbkbCAF/F mice. Similarly, P301S+;ikbkbCAFFF
NF-κB is required to induce tau-associated microglia states in PS19 mice. Single-nuclei RNA-seq were performed using cortical tissues from IκkBα−/− (n = 4), IκkBβ+/−; P301S+ (n = 3), IκkBβ−/−; P301S+, and IκkBα−/−; P301S+ (n = 2) mice. a UMAP plots of all single nuclei and their annotated cell types. OPCs Oligodendrocyte progenitor cells. b Proportion of cell types for each genotype. c UMAP plot depicting different microglial cell subclusters. Each cell was color-coded based on their cluster affiliation. d Proportion of cells in each microglial subcluster for each genotype. e Pseudotime trajectory demonstrating the shift of microglial state. f Pseudotime trajectory of microglia labeled with genotypes. The trajectory illustrates the shift of microglial transcriptome from IκkBα+/−; P301S+ to IκkBα−/−; P301S− mice. g Venn diagram comparing the number and overlap of upregulated DEGs in Cluster 3 vs Cluster 1, and Cluster 4 and 5 vs Cluster 1. h i Selected GSEA hallmark and Gene Ontology pathways identified for downregulated microglia DEGs in Cluster 1 vs Cluster 3 (h) and upregulated microglia DEGs in Cluster 4 and 5 vs Cluster 1 (i). GOBP GO biological processes, GOCG GO cellular compartment, GOMF GO molecular function.

Fig. 8 Hypothetical model of microglia NF-κB drives tau spreading/toxicity. a In an acute model of young PS19 mouse, microglia process the inoculated exogenous tau and release seedable tau to drive tau spreading in a month. Microglial NF-κB activation accelerates the spread of tau inclusions seeded by the inoculation of exogenous tau seeds, likely due to increased tau secretion and impaired autophagy, which is rescued by NF-κB inhibition. b In the non-seeded PS19 model, tau aggregation in aged neurons is determined by tau proteostasis of neurons expressing human mutant tau transgene. Microglial NF-κB activation drives while deletion rescues toxicities. NF-κB activity drives down intraneuronal inclusions via unknown mechanisms, likely via stimulating tau release and/or proteolytic cleavage in these neurons.

Tamoxifen administration. To induce efficient Cre expression and recombination in vivo, tamoxifen (T6468, Sigma-Aldrich) was dissolved in corn oil to prepare 20 mg/ml stock. Mice were given tamoxifen via intraperitoneal (IP) injection at 2 mg per day for 3 days at 2.5 months of age. To induce Cre expression and recombination in primary microglia, 4-hydroxytamoxifen (SML1666, Sigma-Aldrich) were incubated with glia mixed culture for 3 days at 37 °C to 40 °C in a 5% CO2 humidified incubator. Microglia were centrifuged to completely remove 4-hydroxytamoxifen before use. Littermate control mice or microglia that do not have Cre gene were also administered tamoxifen. Gene symbols, corresponding genotype, and IKKβ levels in microglia are summarized as follows.

| Gene symbols | Genotype | IKKβ levels in microglia |
|-------------|----------|-------------------------|
| IκkBα−/−    | Cx3cr1CreERT2/+, IκkBβ+/− | Normal |
| IκkBα+/−    | Cx3cr1CreERT2/+, IκkBβ−/− | Normal |
| IκkBβ−/−; P301S+ | Cx3cr1CreERT2/+, IκkBβ+/− | Deleted |
| IκkBβ−/−; P301S+ | Cx3cr1CreERT2/+, IκkBβ−/− | Normal |
| IκkBβ−/−; P301S+ | Cx3cr1CreERT2/+, IκkBα+/− | Normal |
| IκkBβ−/−; P301S+ | Cx3cr1CreERT2/+, IκkBα−/− | High |

Primary microglia culture. Primary microglia were prepared as described previously69. Briefly, mouse hippocampi and cortices from 2–3-day-old newborn pups were isolated in DPBS. After removing the meninges, brain tissues were cut into small pieces and digested with 0.1% trypsin at 37 °C for 20 min before neutralizing the trypsin with 30% FBS in DMEM media. Digested tissues were triturated to the cell suspension and centrifuged for 15 min at 200 × g. After resuspension in 10% FBS in DMEM, cells were plated onto poly-D-lysine (PDL)-coated T-75 flasks to generate mixed glial cultures in the medium of 10% FBS in DMEM. When confluent, on day 12, microglia were separated from the glia layer by shaking the flask at 200 rpm for 3 h. Floating microglia were collected for RNA analysis or seeded at 75,000 cells/cm² in PDL-coated plates for tau fibril stimulation and processing assay. For microglial tau release assay, 5 ng/ml GM-CSF was added for microglial culture but removed after cells were harvested. Then microglia were seeded on culture plates in the medium of 10% FBS in DMEM without GM-CSF for 24 h before treatment.

Adult microglia isolation. Adult microglia were isolated using magnetic-activated cell sorting (MACS) as described before69. Briefly, anesthetized mice were thoroughly transcardially perfused with cold PBS to remove circulating blood cells in the CNS. Dissected brains were chilled on ice and minced in digestion media containing 0.2% collagenase type 3 (LS004182, Worthington) and 3 U/mL dispase (LS02104, Worthington). After 37 °C incubation for 45 min, digestion was inactivated by 2.5 mM EDTA (15575020, Thermo Scientific) and 1% fetal bovine serum (10082165, Thermofisher). Digested brain tissues were triturated by serological pipette to the cell suspension and passed through a 70 μm cell strainer. Myelin in the cell suspension was depleted by myelin removal beads (130-096-733, Miltenyi Biotec) and magnetic LD columns (130-042-901, Miltenyi Biotec) and magnetic MS column (130-042-201, Miltenyi Biotec) for RNA isolation.
DNA was digested with Dnase I. For qPCR, RNA was reverse-transcribed to cDNA by iScript cDNA synthesis kit (1708890, Bio-Rad). The reverse transcription reactions were performed using SYBR Green PCR Master Mix (4309155, Applied Biosystems) on ABI 7900HT real-time system (Applied Bio- systems). GAPDH was used as a reference gene for normalization and the relative expression differences were calculated based on the 2^(-ΔΔCt) method. The following primers were used for RT-qPCR: Gapdh (forward) 5′-GGGAAGGCCCCATAC-CATCCT-3′, (reverse) 5′-GCGTCTCCGATGGTTGGA-3′; Ikbkβ (forward) 5′- AAGAACAGAGACGTTCG-3′ and (reverse) 5′- CAGGTCTTCGATCOCCTCTCCTG-3′.

Brain tissue collection. Mice were anesthetized with tribromoethanol and transcardially perfused with PBS. Hippocampus and cortex were dissected from one hemibrain and flash-frozen at −80 °C for biochemical analyses and snRNA-seq, whereas the other half hemibrain (or whole brain from tau spreading experiment) was fixed in 4% paraformaldehyde for 48 h, followed by 30% sucrose infiltration for 48 h at 4 °C. 30 μm-thick coronal brain sections were prepared by freezing microtome (Leica) and stored at −20 °C in cryoprotectant before staining.

Immunochemistry and image analysis. DPBS was used for immunohis-tochemistry. Six to eight pieces of brain sections per mouse that contain a series of anterior to posterior hippocampus were washed to remove cryoprotectant and then permeabilized by 0.5% Triton X-100. After blocking in 5% normal goat serum (NGS) for 1 h, brain sections were incubated with primary antibodies in the same blocking buffer overnight at 4 °C. Sections were then washed by DPBS containing 0.1% Tween-20 and incubated with Alexa-conjugated secondary antibodies (1:500) for 1 h in blocking buffer. After washing, sections were mounted on glass slides with Prolong Gold Antifade Mountant. The primary antibodies used in the immunochemistry protocol as well as the secondary antibodies were purchased from Keyence BX-Z700 microscope using 10x objective and analyzed with ImageJ (NIH)45. All images were processed with the auto local threshold Phansalkar plugin. Regions of interest including the hippocampus and cortex were hand-traced. MC1+ areas were measured by ImageJ, whereas microglia numbers were counted with the Analyze Particles function. Images for 3D reconstruction were acquired using confocal microscope LSM880 (ZEISS) at 40× magnification. Three fields per mouse of CA3 hippocampal region were randomly selected and microglia was reconstructed using the Imaris software as described before46. Experiments performing imaging and quantification were blinded.

Endotoxin detection. Endotoxin levels in tau fibrils, monomers, and LPS were detected using an endotoxin detection kit following the manufacturer’s protocol (GenScript ToxinSensor™ Chromogenic LAL Endotoxin Assay Kit). All tau samples had endotoxin levels of <1.0 EU/mL at working concentration.

Tau fibril stimulation, uptake, and clearance assay. K18/PL and full-length tau fibrils were synthesized and labeled with Alexa Fluor 647 (K18/PL) as described before28. All in vitro high-content assays were performed in 96-well PDL plates (655946, Greiner) with primary microglia at a density of 25,000 cells/well (50 μl). Microglia were infected with Lenti-CAG-mCherry (655946, Greiner) with primary microglia at a density of 25,000 cells/well (50 μl). Microglia numbers were counted with the Analyze Particles function. Images for 3D reconstruction were acquired using confocal microscope LSM880 (ZEISS) at 10× magnification. For NF-κB assay, wild-type microglia were plated for 24 h and then incubated with Alexa-647 labeled K18/PL fibrils for 24 h were analyzed with ImageJ (NIH)71. All images were processed with the auto local threshold Phansalkar plugin. Regions of interest including the hippocampus and cortex were hand-traced. MC1+ areas were measured by ImageJ, whereas microglia numbers were counted with the Analyze Particles function. Images for 3D reconstruction were acquired using confocal microscope LSM880 (ZEISS) at 40× magnification. Three fields per mouse of CA3 hippocampal region were randomly selected and microglia was reconstructed using the Imaris software as described before46. Experiments performing imaging and quantification were blinded.

High-content analysis assay. High-content analysis assay, including immunos-taining, image acquiring, and automated analysis, was used to unbiasedly detect and quantify immunofluorescence signals in cultured cells37. After treatment and trypan blue staining, cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and incubated for 1 h in a blocking solution containing DPBS, 0.01% Triton X-100, and 5% NGS. The cells were then incubated in a blocking solution containing anti-Iba1 antibody overnight at 4 °C, followed by incubation with a secondary antibody for 1 h. Nuclei were labeled with DAPI (4′,6-diamidino-2-phenylindole) and TPC1-CA for 1 h followed by 5 min incubation with 20 μM TPCA-1 and 100 mM leupeptin for 8 h to prevent degradation of the reporter translocated into lysosomes during that time. CMA activity was estimated from the degradation of...
the reporter as the increase in the intensity of KFERQ-Dendra fluorescence upon blockage of lysosomal proteolysis. The association of KFERQ-Dendra to lysosomes was confirmed by colocalization with the endo/lysosomal marker LAMP1.

**Immunostaining and imaging for CMA activity.** Microglia grown on a coverslip were fixed by 4% PFA, permeabilized with 0.3% Triton X-100, and then blocked with blocking solution (5% normal goat serum, 0.3% Triton X-100) for 1 h at RT. The coverslips were then incubated with primary antibodies in the same blocking solution at 4 °C overnight. The primary antibodies used for immunofluorescence were rat anti-CD11b (1:200, ab83940, Abcam) or rat anti-LAMP1 (1:1000, Hybridoma bank, Cat # I4b8). After brief washing with PBS (for 5 min, three times), the coverslips were incubated with secondary antibodies for 1 h at RT. The secondary antibodies used were Alexa Fluor 488- or Cy5-conjugated goat IgG against rabbit and rat (1:500). Nuclei were co-stained with Hoechst. After brief washing, the coverslips were mounted with ProLong Diamond antifade reagent (Thermo Fisher Scientific, P36965). Confocal images of sixty cells from three independent experiments were acquired with a laser scanning microscope TCS SP8 (Leica), and processed with LAS X software (Leica). ImageJ Software (v.2.1.0) (NIH) was used for image processing and analysis. DiAnA was scanned microscope TCS SP8 (Leica), and processed with LAS X software (Leica).

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Hierarchical level. All statistical difference between designated groups. The multilevel mixed-effects Fisher seeding were inoculated: (1) 3 μL of 4.3 μg/ul P53 brain extracts for microglia-depleted PS19 mice (Fig. 4a–c) and PS19 mice with microglial NF-κB inactivation (Fig. 4f–h); (2) 2 μL of 0.2 μg/ul K18/PL tau fibrils for PS19 mice with microglia-depleted PS19 mice (Fig. 4g–i); (3) 2 μL of 4.3 μg/ul K18/PL tau fibrils for microglia-depleted PS19 mice (Fig. 4a–c) and PS19 mice with microglial NF-κB activation (Fig. 4f–h). The same volumes of PBS were injected into PS19 mice or the same amount of tau seeds were injected into non-transgenic mice as control. Mice were monitored during the anesthesia until recovery. After 3 months (for P53 brain extracts) or 1 month (for K18/PL tau fibrils) of spreading, mice were perfused for whole-brain immunohistochemistry of AT8 or MCI1 tau as described.

Morris water maze. Morris water maze studies were conducted during daylight hours. The water maze consisted of a pool (122 cm in diameter) containing opaque water (20 ± 1°C) and a platform (14 cm in diameter) submerged 1.5 cm below the surface. Hidden platform training (days 1–6) consisted of 12 sessions (two per day, 2 h apart), each with two trials. The mouse was placed into the pool at alternating drop locations for each trial. A trial ended when the mouse located the platform and remained motionless on the platform for 5 s, for a maximum of 60 s per trial. Mice that failed to find the platform within the 60 s trial were led to it and placed on it for 15 s. Probe trials were conducted 72 h after the final hidden training. Mice were returned to the pool with a drop location that was 180° opposite of the original target platform location in the absence of the hidden platform. Performance was measured with EthoVision video-tracking (Noldus Information Technology). Visible platform training, where the platform was cued with a mounted black-and-white striped mast, was conducted for three sessions after the conclusion of probe trials. Pre-set criteria for exclusion from the analysis included floating and thigmotaxic behaviors, neither of which was observed in current studies.

Statistics. The sample size for each experiment was determined on the basis of previous publications. All in vitro experiments were performed with a minimum of three biological replicates. Mean values from at least three independent experiments were used for computing statistical differences. All in vivo experiments were performed with a minimum of four mice per genotype. All in vivo data were averaged to either individual mouse (microglia number counts), individual section (MCI, AT8 tau), or individual microglia (inarris morphology analysis), and mean values were used for computing statistical differences. Data visualization was done with Graphpad and R package ggplot2. Statistical analyses were performed with Graphpad prism 9.0 (t-test, one-way and two-way ANOVA (Graphpad, San Diego, California), STATA 12 (multilevel mixed-effects model) (StataCorp), R (R Foundation for Statistical Computing, Vienna, Austria). Values are reported as mean ± standard error of the mean (SEM) or standard deviation (SD). The Shapiro-Wilk test of normality and F test to compare variances were applied to normality tests for statistical significance. The sample size for each experiment was determined on the basis of

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

All data supporting this study are included in the paper and its supplementary files. All RNA-seq data were deposited in the Gene Expression Omnibus (GEO) under the following series accession numbers: bulk tissue RNA-seq GSE98013; mouse single-nuclei RNA-seq GSE198014. Source data are provided with this paper.

Code availability

All custom codes used for snrna-seq data analysis have been archived at Zenodo (https://doi.org/10.5281/zenodo.6336233) and are directly available at https://github.com/lifan36/Wang_et_al_NatC_2021.

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C.W. and L.G. designed research; C.W., L.F., R.K., L.Z., L.K., D.L., Y.Z., C.C., and W.L. performed experiments; S.M., J.G., L.T.G., W.W.S., and B.M. provided key reagents; C.W., L.F., R.K., L.Z., I.K., M.C., B.L., Y.L., D.L., C.C., A.M.C., W.L., and L.G. analyzed data; C.W., L.F., B.L., W.L., and L.G. wrote the manuscript.

Competing interests

L.G. is a founder of Aeton Therapeutics, Inc. The remaining authors declare no competing interests.

Additional information

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