

NADPH attenuates tau pathology and disease-associated microglial phenotype in a truncated tau-induced mouse model

Jiayi Da

Southeast University

Ran Zhang

Southeast University

Lian Xu

Southeast University

Ruozhen Wu

Nantong University

Shuqiang Zhang

Nantong University

Han Wang

Southeast University

Narjes Baazaoui

King Khalid University

Jianhua Shi

Southeast University

Fei Liu

New York State Institute for Basic Research in Developmental Disabilities

Nana Jin

yongna0321@ntu.edu.cn

Southeast University

Research Article

Keywords: Tau pathology, Disease-associated microglia, NADPH, Alzheimer's disease

Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11151367/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Truncated tau is increasingly recognized as a pivotal pathogenic event in Alzheimer's disease (AD). Although the 151–391 fragment (τ_{Tr}) exhibits strong pathological capacity, whether τ_{Tr} is sufficient to induce a redox-inflammatory brain response *in vivo* and whether this response is therapeutically modifiable remain unclear. Here, we show that τ_{Tr} drives robust aggregation and hyperphosphorylation *in vitro*, and induces pronounced tau pathology accompanied by microglial activation *in vivo*. Transcriptomic profiling further revealed that τ_{Tr} elicits a ROS-associated and pro-inflammatory program, characterized by activation of NF- κ B signaling and closely resembling transcriptional signatures observed in tau transgenic models. Notably, NADPH treatment markedly attenuated τ_{Tr} aggregation and hyperphosphorylation *in vitro*, and significantly reduced tau pathology and microglial activation *in vivo*. At the molecular level, NADPH treatment was associated with reduced expression of genes involved in ROS production, neuroinflammation, and microglial activation, including markers related to disease-associated microglia and led to the normalization of microglial morphology and the decreased proportion of Dectin-1 and TREM2-positive microglia. Together, these findings demonstrate that truncated tau is sufficient to drive a ROS-NF- κ B-dependent neuroinflammatory microglial program *in vivo*, and that NADPH can partially reverse this pathogenic cascade, highlighting redox modulation as a potential therapeutic strategy in tau-related neuroinflammation.

Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder worldwide, classically characterized by extracellular amyloid-beta ($A\beta$) plaques and intracellular neurofibrillary tangles (NFTs) composed of hyperphosphorylated and truncated tau^{1,2}. While $A\beta$ clearance therapies have shown limited clinical efficacy in restoring cognition, accumulating evidence suggests that tau pathology correlate more closely with cognitive decline^{3,4}.

Tau protein is encoded by *MAPT* and physiologically functions to stabilize axonal microtubules, support neuronal structure, and axonal transport. Under pathological conditions, tau undergoes abnormal hyperphosphorylation and proteolytic cleavage, generating aggregation-prone truncated fragments that exhibit enhanced seeding activity, synaptic dysfunction, mitochondrial impairment, and increased oxidative stress^{4,5}. We previously identified a tau fragment spanning residues 151–391 (τ_{Tr}) as a particularly pathogenic species, which displays strong self-aggregation, site-specific hyperphosphorylation, and aggregation seeded by AD brain-derived pathological tau (AD P-tau), suggesting its utility as a robust model for studying tau-driven neurotoxicity^{6–8}. However, whether τ_{Tr} is sufficient to induce a redox-inflammatory microglial program *in vivo* and whether this program is therapeutically reversible remain unknown.

Neuroinflammation plays a critical role in the pathogenesis and progression of AD, with microglia the principal mediators of neuroinflammation that become markedly activated and drive disease-associated

inflammatory responses⁹. With advances in single-cell transcriptomic technologies, microglia have been recognized as a highly heterogeneous population in AD^{10–13}. Distinct microglial subpopulations have been identified, such as disease-associated microglia (DAM), interferon-responsive microglia, and MHC class II-expressing microglia^{10,12}. Among these, DAM represent a prominent activation state characterized by enhanced phagocytic and lipid metabolic programs¹³, but sustained or excessive DAM-like activation may contribute to chronic neuroinflammation, synaptic dysfunction, and neurodegeneration. Although initially considered protective due to their debris-clearing capacity, sustained DAM activation during disease progression has been increasingly linked to chronic neuroinflammation, synaptic loss, and neurodegeneration, highlighting microglial state modulation as a potential therapeutic avenue¹³.

Oxidative stress is both a trigger and a consequence of tau pathology, creating a feedback loop that accelerates neurodegeneration¹⁴. Nicotinamide adenine dinucleotide phosphate (NADPH) plays a central role in maintaining redox homeostasis by regenerating reduced glutathione (GSH) and neutralizing reactive oxygen species (ROS)¹⁵. Modulating NADPH homeostasis has demonstrated significant neuroprotective potential across various neurological disorders¹⁶. Further, direct administration of exogenous NADPH has also been shown to confer robust neuroprotection in experimental models of cerebral ischemia^{16,17}.

Despite these insights, it remains unclear whether exogenous NADPH administration can modulate tau pathology and neuroinflammatory responses in tauopathy. In this study, we established a pathologically relevant AAV-tau_{Tr} mouse model and performed transcriptomic profiling to characterize the underlying molecular alterations induced by truncated tau. Using this model, we further investigated the effects of NADPH treatment on tau pathology and microglial responses. Our findings demonstrate that NADPH not only attenuates tau aggregation and hyperphosphorylation, but also alleviates the disease-associated microglial phenotype and suppresses neuroinflammatory responses, suggesting that its protective effects extend beyond a classical antioxidant function. Collectively, these results provide mechanistic and functional evidence supporting redox modulation as a potential therapeutic strategy targeting tau truncation-driven pathology in AD.

Results

Truncated tau is more prone to aggregation and exhibits site-specific hyperphosphorylation

Our previous study has shown that, the truncated tau fragment (residues 151–391, designed as tau_{Tr}) is more readily captured by AD-derived pathological tau (designed as AD P-tau) and exhibits a higher propensity to aggregation seeded by AD P-tau compared with full-length tau (designed as tau_{FL}) and other truncated tau isoforms⁸. To directly examine the aggregation propensity of truncated tau, we

overexpressed HA-tau_{FL} or HA-tau_{Tr} in HEK-293T cells. Six hours later, cells were treated without/with AD P-tau for 42 hours, lysed with RIPA buffer, and fractionated into RIPA-soluble and -insoluble components for Western blots analysis. In the absence of AD P-tau, tau_{FL} and tau_{Tr} exhibited comparable levels in the soluble fraction, whereas RIPA-insoluble tau_{FL} was virtually undetectable. In contrast, tau_{Tr} readily formed RIPA-insoluble tau (Fig. 1A). Upon AD P-tau treatment, a small amount of RIPA-insoluble tau_{FL} was detected, whereas tau_{Tr} showed robust accumulation in the insoluble fraction, including SDS- and β -mercaptoethanol-resistant high-molecular-weight aggregates (~ 60 kDa, as dimer according to the molecular weight) (Fig. 1A). These results indicate that tau_{Tr} is intrinsically more prone to aggregation and highly responsive to AD P-tau seeding in cultured cells.

To determine whether these tau aggregates adopt β -sheet-rich structures, we utilized Thioflavin-T (ThT), a dye commonly used to detect amyloid fibrils enriched in cross- β sheet structures. HA-tau_{FL} or HA-tau_{Tr} was overexpressed in HeLa cells, followed by AD P-tau treatment. Cells were incubated with 10 μ M ThT for 24 hours prior to collection, and tau aggregates were visualized by HA immunofluorescence. Due to the limited aggregation of tau_{FL}, cells were treated with AD P-tau for 5 days, no ThT-positive puncta were observed (Fig. 1B). In contrast, tau_{Tr}-expressing cells displayed distinct intracellular punctate aggregates 48 hours post AD P-tau treatment, which were strongly ThT-positive (Fig. 1B). These results indicate that tau_{Tr} is more readily induced to aggregate by AD P-tau, forming β -sheet-rich structures recognizable by ThT staining.

Given that neurofibrillary tangles in AD brains consist of hyperphosphorylated tau, we next assessed whether tau_{Tr}- and AD P-tau-induced aggregation is associated with site-specific tau phosphorylation. Cells overexpressed HA-tau_{FL} or HA-tau_{Tr} treated with AD P-tau were lysed in Laemmli buffer, and phosphorylation at multiple epitopes was examined by Western blots. AD P-tau treatment did not substantially alter the phosphorylation of tau_{FL} (Fig. 1C and D). In contrast, tau_{Tr} exhibited pronounced hyperphosphorylation at multiple sites, including Ser202, Thr205, AT8, Ser214, Thr217, and Ser262 (Fig. 1C and D). These findings demonstrate that truncated tau is more prone to hyperphosphorylation upon AD P-tau treatment.

Tau is a microtubule-associated protein that plays a critical role in maintaining neuronal processes. To further assess the impact of tau_{Tr} on neurites, we infected primary neurons with AAV2/8-mCherry-P2A-HA-tau_{FL} and AAV2/8-mCherry-P2A-HA-tau_{Tr}. HA and AT8 immunofluorescence staining showed that tau_{FL} was uniformly distributed throughout the neuronal soma and processes, consistent with its normal physiological role (Fig. 1E and F). In contrast, tau_{Tr} exhibited a relatively homogeneous distribution within the soma, but formed discontinuous, bead-like aggregates along neuronal processes (Fig. 1E and F). These findings suggest that tau_{Tr} tends to aggregate within neurites, which may compromise neuronal function.

Neuronal overexpression of truncated tau induces tau pathology and microglia activation in vivo

To examine its pathogenic potential of truncated tau *in vivo*, we stereotactically injected AAV2/8-mCherry-P2A or AAV2/8-mCherry-P2A-HA-tau_{Tr} into the hippocampus of 6-month-old wild-type mice (Fig. 2A). Six-month post-injection, no HA immunoreactivity was detected in the hippocampus of mice receiving AAV2/8-mCherry-P2A (Con) (Fig. 2B). In contrast, in the AAV2/8-mCherry-P2A-HA-Tau_{Tr} group, robust HA immunofluorescence was observed in neurons within the CA1, CA3, and dentate gyrus (DG) regions of the hippocampus (Fig. 2B), whereas no HA signal was detected in Iba1-positive microglia (Fig. 2C), indicating neuron-specific expression of tau_{Tr}. The hippocampal tau pathology was evaluated by immunofluorescence using the AT8 antibody, which specifically recognizes tau phosphorylated at Ser202/Thr205. Notably, tau_{Tr} formed aggregates that were AT8-positive (Fig. 2D and E). Notably, tau pathology labeled by a phospho-Thr217 (pT217) tau antibody colocalized with the HA immunofluorescence signal in tau_{Tr} overexpressing mice (Fig. 2F), confirming that the observed phospho-tau aggregates arose from virally expressed tau_{Tr}. To determine whether tau pathology is associated with microglial activation, we performed Iba1 immunofluorescence on the same brain sections. Mice injected with tau_{Tr} displayed a marked increase in Iba1-positive signal intensity in the hippocampus compared with controls (Fig. 2G and H). Three-dimensional reconstruction was next performed to assess the morphological alterations in microglia induced by tau_{Tr}. Tau_{Tr} overexpression markedly activated microglia, as indicated by reduced process-process interactions and the decreased number of microglia branches and terminals (Fig. 2I-K), suggesting a shift toward an activated microglial phenotype. Collectively, these results demonstrate that neuronal overexpression of truncated tau *in vivo* leads to the formation of AT8- and pT217-positive pathological tau aggregates and is sufficient to induce microglial activation in the hippocampus.

Truncated tau elicits a robust inflammatory transcriptional response in the hippocampus

To elucidate the molecular alterations associated with tau_{Tr}-induced pathology, we performed bulk RNA sequencing (RNA-Seq) of hippocampal tissues from mice with over-expression of truncated tau and the controls. Differential expression analysis revealed 1,131 upregulated and 345 downregulated genes in the truncated tau group compared with controls (Fig. 3A). Functional enrichment analysis showed that the upregulated genes were predominantly associated with inflammatory and immune response pathways, whereas the downregulated genes were enriched in processes related to axonal transport, synaptic function, learning and memory, and nervous system development (Fig. 3B and C). To further identify potential upstream regulatory mechanisms, we applied TRRUST analysis to predict transcription factors regulating the upregulated gene set. The top-ranked regulators included key inflammatory and immune-related transcription factors such as *Nfkb1*, *Spi1*, *Rela*, *Irf1*, and *Stat1* (Fig. 3D). Consistently, qPCR validation of selected genes confirmed the RNA-seq results, including the transcription factor *Nfkb1* and multiple pro-inflammatory mediators (*Il6*, *Il1b*, *Tnf* etc) (Fig. 3E). Also, we validated the protein expression of *Nfkb1* and observed a significant upregulation in the truncated tau group (Fig. 3F and G). Gene set enrichment analysis (GSEA) further corroborated these findings, revealing significant

upregulation of reactive oxygen species biosynthetic process, and immune- and inflammation-related gene sets, including positive regulation of NF- κ B transcription factor activity and interleukin-6 signaling, alongside marked downregulation of gene sets involved in synaptic function, axoneme assembly and axon transport (Fig. 3H). The PS19 mouse model is a well-established transgenic model for studying tauopathies related neurodegeneration. Previous studies have reported prominent alterations in lysosomal and inflammatory pathways in the hippocampus of 9-month-old PS19 mice¹⁸. To determine whether truncated tau-induced molecular changes resemble those observed in the PS19 model, we analyzed hippocampal transcriptomic data from PS19 mice using the same analytical pipeline and statistical thresholds. Gene overlap analysis using the GeneOverlap R package revealed that 55% of upregulated genes and 30.9% of downregulated genes were shared between the two datasets, with a highly significant overlap ($p < 0.001$) (Fig. 3I). Functional enrichment analysis showed that shared upregulated genes were significantly enriched in NF- κ B signaling, microglial activation, and neuroinflammatory responses, whereas shared downregulated genes were significantly enriched in neuron projection extension, regulation of synaptic plasticity, and cell growth during development (Fig. 3I). These results indicate that overexpression of truncated tau induces neuronal dysfunction and neuroinflammation, recapitulating key molecular signatures of the PS19 tauopathy model, particularly those associated with neuroinflammation and immune activation.

NADPH attenuates tau aggregation and site-specific hyperphosphorylation via ROS reduction

GSEA identified a significant enrichment of the ROS biosynthetic process in the tau_{Tr} group, characterized by increased expression of NADPH oxidase 2 (NOX2) components (*Cybb*, *Cyba*, *Ncf1/2*, Fig. 3H and Fig. 4A), thereby suggesting enhanced ROS production and oxidative stress under truncated tau overexpression. NADPH is essential for GSH regeneration and thereby protect cells against the ROS-mediated toxicity^{19,20}. To determine whether NADPH protects cells from ROS toxicity induced by truncated tau expression and aggregation, HEK-293T cells expressing HA-tau_{Tr} were treated with 2 μ M NADPH in the absence or presence of AD P-tau. In HEK-293T cells, tau_{Tr} overexpression (Fig. 4B and C) and tau_{Tr} overexpression combined with AD P-tau treatment (Fig. 4D and E) could markedly increase intracellular ROS (Fig. 4B and D) and LDH release (Fig. 4C and E). Notably, NADPH treatment significantly attenuated both the ROS elevation and LDH release induced by tau_{Tr} alone and by tau_{Tr} plus AD P-tau (Fig. 4B-E), a redox-modulatory and cytoprotective effect of NADPH in tau_{Tr}-expressing cells.

Truncated tau exhibited substantially higher aggregation and phosphorylation than full-length tau. To assess the effect of NADPH on tau aggregation, HA-tagged tau_{Tr} was overexpressed in HeLa cells, which were then co-treated with NADPH and AD P-tau as described above (Fig. 4F). Upon AD P-tau exposure, ~22% of tau-expressing cells contained intracellular tau aggregates. NADPH treatment markedly reduced the proportion of cells harboring tau aggregates to ~12%, without altering total tau expression, as indicated by the unchanged proportion of HA-positive cells. (Fig. 4F and G). We next examined the effect

of NADPH on tau hyperphosphorylation, HEK-293T cells overexpressing tau_{Tr} were treated with 2 μM NADPH in the absence (Fig. 4H and I) or presence of AD P-tau (Fig. 4J and K). In the absence of AD P-tau, NADPH treatment markedly reduced tau_{Tr} phosphorylation at AT8 (Ser202/Thr205), Thr212 and Thr231 (Fig. 4H and I). AD P-tau could further induce tau hyperphosphorylation at Ser199, Ser205, AT8, Thr217 and Ser262 (Fig. 4J and K). While 0.2 μM NADPH had little effect on AD P-tau induced tau phosphorylation, 2 μM NADPH robustly suppressed hyperphosphorylation of tau at multiple pathological epitopes, including Ser199, Ser205, AT8, Ser214, Thr217, and Ser262 (Fig. 4J and K). In primary neurons overexpressing tau_{Tr}, tau_{Tr} formed discontinuous, bead-like aggregates along neuronal processes (Fig. 4L). NADPH treatment promoted a more uniform distribution of tau_{Tr} in neurites proximal to the soma, although discontinuous aggregates were still observed in distal segments (Fig. 4L). Collectively, these results indicate that NADPH attenuates oxidative stress, tau aggregation and site-specific hyperphosphorylation driven by truncated tau, supporting a protective role of NADPH in modulating pathological tau responses.

NADPH attenuates tau_{Tr}-induced tau pathology and microglia activation in vivo

To assess the effect of NADPH on tau pathology *in vivo*, 6-month-old C57BL/6J mice received hippocampal injections of AAV2/8-mCherry-P2A-HA-tau_{Tr} and were treated intravenously with NADPH (7.5 mg/kg) every three days for 186 days (Fig. 5A). After six months of NADPH treatment, no significant changes in body weight were observed in the mice. However, NADPH treatment increased the survival rate by approximately 33% and markedly improved coat pigmentation and fur smoothness (data not shown). Six months after AAV administration, brain sections were immunostained with the AT8 and pT217 tau antibody to access tau pathology. NADPH treatment markedly reduced the number of AT8- and pT217-positive neurons in tau_{Tr}-overexpressing mice (Fig. 5B-E). In addition, HA immunofluorescence revealed that NADPH reduced total tau expression (Fig. 5D and F). Iba1 immunofluorescence revealed a significant reduction in hippocampal Iba1 signal intensity in NADPH-treated mice compared with the tau_{Tr} group, indicating reduced microglial activation (Fig. 5G and H). Together, these data demonstrate that NADPH could suppress the tau_{Tr}-driven tau pathology and microglial activation *in vivo*.

NADPH attenuates the activation of disease-associated microglial in vivo

To further elucidate molecular changes induced by NADPH, we performed bulk RNA sequencing of hippocampi from NADPH-treated and tau_{Tr}-overexpressing C57BL/6J mice and compared their transcriptomic profiles. Differential expression analysis identified 5 upregulated and 172 downregulated genes in the NADPH-treated group relative to the tau_{Tr} group (Fig. 6A). Notably, 91% of these differentially expressed genes (158 downregulated and 3 upregulated) exhibited expression changes opposite to those observed in the tau_{Tr} group compared with controls (Fig. 6B and C). We defined this gene set as reversed DEGs (rev.DEGs). Among the reversed DEGs, key NADPH oxidase components (*Cybb*, *Cyba*, and *Ncf1*) were significantly downregulated upon NADPH treatment relative to the tau_{Tr}

group, suggesting suppression of ROS-generating pathways (Fig. 6C). Functional enrichment analysis revealed that “rev.DEGs” were predominantly associated with immune and inflammatory responses, microglial activation, and phagocytic processes (Fig. 6D). Given the relatively relaxed cut-off used for defining individual DEG, we additionally performed gene set enrichment analysis (GSEA), which captures the activity of the entire gene set independent of arbitrary threshold selection. Consistently, GSEA demonstrated that gene sets related to microglial activation, positive regulation of NF- κ B transcription factor activity, and inflammatory responses were significantly downregulated following NADPH treatment (Fig. 6E). Through TRRUST enrichment analysis of the rev.DEGs, we identified a central regulatory module governed by *Spi1*, *Irf1*, and *Nfkb1* (Fig. 6F), which are key drivers of neuroinflammation and oxidative stress. Interestingly, these transcription factors are highly sensitive to the cellular redox state. Quantitative PCR validation further confirmed the RNA-Seq results, showing that representative inflammatory genes, including *Nfkb1*, *Fcgr3*, *Irf1*, and *Gpnmb*, were markedly upregulated in the tau_{Tr} group but significantly suppressed upon NADPH treatment (Fig. 6G). Of the rev.DEG set, we also found key molecules for reported microglia subclusters^{12,13}, such as disease-associated microglia (DAM, *Cst7*, *Axl*, *Gpnmb*, *Apoe*, *Tyrobp*, *Itgax*), interferon-responsive (IFN, *Ifit2*) and MHC (*Cd74*, *H2-Ab1*, *H2-K1*, Fig. 6C). Our above analyses indicated a high degree of molecular similarity between the Tau_{Tr} model and the PS19 tau transgenic mouse model. To further confirm the cell type response of “rev.DEGs”, we analyzed a published single-nucleus RNA-Seq dataset from the hippocampus of PS19 mice¹⁸. Using the Seurat AddModuleScore function, we calculated “rev.DEG” activity scores across individual cells. This analysis revealed that “rev.DEG” activity was predominantly enriched in hippocampal microglia (Fig. 6H) and was significantly elevated in microglia from PS19 mice compared with wild-type controls (Fig. 6I). We next performed microglial subcluster analysis by reclustering microglia population. Microglia were classified into homeostatic, intermediate, disease-associated microglia (DAM), and interferon-responsive (IFN) subpopulations in the original study¹⁸. The proportion of intermediate microglia was markedly increased in PS19 mice, while DAM and IFN microglia were largely exclusive to the PS19 condition¹⁸. We found that “rev. DEG” activity was significantly higher in DAM and IFN microglia subclusters compared with homeostatic microglia (Fig. 6J). Collectively, these findings suggest that NADPH exerts neuroprotective effects by attenuating oxidative stress, excessive immune and inflammatory responses triggered by truncated tau, particularly through suppressing microglial activation and NF- κ B-associated inflammatory signaling.

We next used immunofluorescence and three-dimensional reconstruction to assess NADPH-induced morphological changes in microglia. Tau_{Tr} overexpression markedly activated microglia, as indicated by reduced process-process interactions and shortened process length, whereas NADPH treatment effectively reversed these alterations (Fig. 7A-C). To further characterize DAM, we performed co-immunostaining for Dectin-1 (encoded by *Clec7a*) and TREM2 together with Iba1 in the CA1 and DG regions. The numbers of Dectin-1 positive microglia and the fluorescence intensity of Trem2 were significantly increased in tau_{Tr}-overexpressing mice compared with controls, indicating that tau_{Tr} robustly induces the DAM phenotype. Notably, NADPH treatment markedly reduced the number of Dectin1-labeled microglia and TREM2 intensity (Fig. 7D-G). Taken together, these findings demonstrate

that NADPH not only normalizes microglial morphology but also suppresses the DAM phenotype induced by tau pathology *in vivo*.

Discussion

Our previous study has identified the high pathogenicity of a truncated tau spanning 151–391 residues in promoting tau aggregation and site-specific hyperphosphorylation, as well as seeding activity in the presence of AD P-tau⁸. In addition to validating these characteristics of tau_{Tr}, we further observed elevated ROS level and LDH release *in vitro* upon tau_{Tr} over-expression. To investigate its effects *in vivo*, we established a tau_{Tr} over-expression mouse model using an AAV-mediated deliver system. Immunofluorescence staining of AT8, pT217, and Iba1 revealed pronounced tau pathology and microglial activation in tau_{Tr} over-expression mice. To further explore the molecular alterations induced by tau_{Tr}, we perform RNA-Seq analysis and revealed a profound transcriptomic shift characterized by the downregulation of genes associated with synaptic function and axonal transport, alongside activation of inflammatory signaling pathway and oxidative stress response. PS19 transgenic mice are a widely used model of tauopathy in Alzheimer's disease research, expressing the human mutant MAPT (P301S). These mice develop progressive tau hyperphosphorylation, neurofibrillary tangle-like pathology, and neurodegeneration, making them a valuable model for studying tau-driven neurotoxicity and neuroinflammation²¹. Notably, approximately 55% of the upregulated genes and 30.9% of the downregulated genes significantly overlapped with those identified in the well-established PS19 transgenic mouse model, supporting the validity of the AAV-tau_{Tr} model in recapitulating key molecular features of human tauopathy.

Oxidative damage is closely associated with the pathogenesis and progression of AD, contributing to synaptic dysfunction, tau pathology, and neurodegeneration through excessive ROS production²². Accordingly, restoring redox homeostasis has emerged as a promising disease-modifying strategy for AD, in which NADPH serves as a central cellular reducing equivalent supporting major antioxidant systems and regulating ROS balance^{23,24}. As observed in our experiments, tau_{Tr} overexpression significantly increased intracellular ROS levels. Consistently, transcriptomic analysis of AAV-mediated tau_{Tr} mice revealed a coordinated upregulation of genes involved in ROS-generating pathways, including the NOX2 enzymatic complex (*Cybb*, *Cyba*, *Ncf1*, *Ncf2*, and *Hvcn1*), indicating an enhanced NOX2-dependent ROS production program. NOX2, the prototypical member of the NADPH oxidase family, has been increasingly recognized as a key contributor to the pathogenesis of AD^{25,26}. We found that NADPH treatment significantly reduced intracellular ROS levels in tau_{Tr}-overexpressing cells. This reduction was accompanied by a significant downregulation of NOX2 complex components, including *Cybb*, *Cyba* and *Ncf1*, suggesting that NADPH suppresses NOX2-dependent ROS amplification in tau pathology mouse model. Importantly, this restoration of redox balance was associated with a significant reduction in RIPA-insoluble tau species and attenuation of site-specific tau hyperphosphorylation. Consistently, immunofluorescence staining of AT8 and pT217 revealed a marked decrease in AT8 and pT217 tau-

positive neurons in the NADPH-treated group compared with tau_{Tr} mice, further indicating that NADPH alleviates tau pathological burden.

Microglial activation is increasingly recognized as a central determinant of AD progression, particularly in tauopathies where microglia actively shape both neuroinflammatory and tau propagation^{9,27–29}. Single-cell transcriptomic analyses have revealed that microglia undergo distinct transcriptional reprogramming into disease-associated states, including disease-associated microglia (DAM), interferon-responsive (IFN) microglia, and MHC-high antigen-presenting phenotypes in AD and mouse models^{10,12,18}. Our transcriptomic analyses indicate that tau_{Tr} induces a broad reprogramming of microglial gene expression, characterized by coordinated activation of *Nfkb1*, *Spi1*, *Stat1*, *Irf1*, *Rfx5*–associated transcriptional networks and the emergence of DAM, IFN, and MHC-high antigen-presenting signatures. These changes reflect a concerted shift in microglial state rather than isolated inflammatory gene induction. Importantly, NADPH treatment reversed the tau_{Tr} induced transcriptional program, suppressing disease-associated microglia states, such as DAM (*Cst7*, *Axl*, *Gpnmb*, *ApoE*, *Tyrobp*, *Itgax*). Transcription factor analyses consistently identified *Spi1*, *Irf1*, *Stat1*, *Rfx5*, and *Nfkb1* as central regulatory nodes underlying this state transition. Among these, NF-κB signaling has been shown to play a causal role in tau propagation and neurotoxicity by promoting microglial inflammatory activation and facilitating pathological tau spread in PS19 mice model³⁰. Consistent with this, our data demonstrate that NF-κB-associated gene programs are markedly attenuated following NADPH treatment, suggesting that modulation of this pathway is tightly linked to changes in microglial activation state. Cross-validation using a PS19 tauopathy single-nucleus RNA-seq dataset further confirmed that the NADPH-reversed gene signature was predominantly enriched in microglia, with significantly higher activity observed in DAM and IFN microglia subpopulations. This indicates that the observed transcriptional program is conserved across tauopathy models and reflects a fundamental feature of microglial responses to tau pathology. Dectin-1 (*Clec7a*) and Trem2 are well-established markers of disease-associated microglia (DAM) and play critical roles in microglial responses to neurodegeneration^{11,31,32}. Experimentally, co-immunostaining of Dectin-1 or Trem2 with Iba1 demonstrated a significant induction of the DAM phenotype in Tau_{Tr}-overexpressing mice, indicating that truncated tau promotes the transition of homeostatic microglia toward a neurodegeneration-associated state. Notably, NADPH treatment markedly reduced Dectin-1-positive microglia and TREM2 immunoreactivity, suggesting that NADPH suppresses pathological microglial activation and attenuates the DAM phenotype.

Despite these findings, several limitations should be noted in this study. First, we employed an AAV-mediated overexpression system to model truncated tau *in vivo*, which, although efficient for inducing region-specific pathology, may not fully recapitulate the endogenous expression levels and physiological processing of truncated tau in AD. Second, while our transcriptome analyses identified *Spi1*- and *Nfkb1*-associated pathways as key regulatory nodes underlying NADPH-induced microglial reprogramming, the precise molecular mechanisms remain incompletely defined and require further validation using targeted genetic or pharmacological approaches.

Conclusion

Tau_{Tr} promotes tau pathology and pathological microglial activation accompanied by widespread genome-wide transcriptional reprogramming *in vivo*, characterized by activation of *Spi1*, *Nfkb1* associated inflammatory programs and disease-associated microglial states. Importantly, NADPH administration effectively disrupts this transcriptional and inflammatory cascade, suggesting that redox modulation can reprogram microglial state transitions and mitigate tau-induced neurodegeneration.

Materials and methods

Cell culture, plasmid construction, and drug treatment. HEK-293T and HeLa cells were cultured in DMEM (Sigma-Aldrich, Burlington, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific, Pittsburgh, PA, USA) at 37°C in a humidified atmosphere with 5% CO₂. The plasmids pCI/HA-tau_{FL} (encoding the full-length tau, 1-441) and pCI/HA-tau_{Tr} (encoding the truncated tau, 151–391) were constructed as previously described and sequence verified (GENEWIZ, Jiangsu, China). Cells were transfected with plasmids using X-tremeGENE™ HP (Roche, Madison, WI, USA) following the manufacture's instruction. To induce tau aggregation, AD P-tau was complexed with Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) and added 6 hours after transfection, then maintained for additional 42 hours. For NADPH treatment, cells were preconditioned with 0.2 or 2 μM NADPH (Sigma-Aldrich, Burlington, MA, USA) in saline (pH8.0) for 6 hours prior to exposure to AD P-tau.

Animal model construction and drug administration. Six-month-old female and male C57BL/6J mice were used for *in vivo* studies. AAV2/8-mCherry-P2A-HA-tau_{Tr} and AAV2/8-mCherry-P2A-HA driven by the synapsin-1 promotor (BrainVTA, Hubei, China) were stereotactically injected into the right hippocampus at the following coordinates relative to the bregma: AP-2.5 mm, ML + 2.0 mm, DV-1.8 mm (from the dura). For therapeutic evaluation, NADPH (7.5 mg/kg) in saline (pH8.0) was administered intravenously every three days via the tail vein 6 days before model establishment and then for additional 180 days.

AD P-tau preparation. Frozen cortical tissues from autopsy-confirmed Alzheimer's disease (AD) cases were obtained from the Brain Tissue Resource Center (McLean Hospital, Belmont, MA). AD P-tau was purified from cortical homogenates as the previous study⁸. Briefly, brain tissue was homogenized at 10% (w/v) in ice-cold lysis buffer containing Tris-HCl, sucrose, Mg²⁺, EDTA, and phosphatase/protease inhibitors. The homogenate was first subjected to differential centrifugation at 27,000 × g, followed by two rounds of ultracentrifugation at 235,000 × g to enrich insoluble Tau species. The final pellet enriched in AD P-tau was resuspended in saline and sonicated (5 s on/10 s off, 30 cycles) immediately prior to experimental use.

RIPA-insoluble tau fractionation. To assess tau aggregation, cultured cells were homogenized in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% sodium dodecyl sulfate with protease inhibitors) and incubated on ice for 20 minutes. Homogenates were subjected to ultracentrifugation at 65,000 × g for 40 min at 4°C. The supernatant was collected as

the RIPA-soluble fraction and the pellet was washed with RIPA buffer once. The pellet (as the RIPA-insoluble tau) was resuspended in 1×Laemmli buffer and sonicated using Fisher Sonic Dismembrator (Thermo Fisher Scientific, Pittsburgh, PA, USA) under the conditions: 2 second on, 5 seconds off, at 80% power for a cumulative sonication time for 10 minutes to achieve complete solubilization of the pellet.

Western blots analysis. After protein quantification using a BCA assay, equal amounts of protein from cell and tissue were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were incubated with primary antibodies against total Tau (HA), phospho-Tau, and NF-κB, followed by HRP-conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA, USA), and detected using enhanced chemiluminescence (Millipore, Burlington, MA, USA). The primary antibodies were listed below: NF-κB (80979-1-RR, Proteintech, Hubei, China), Anti-HA (H6908, Sigma-Aldrich, Burlington, MA, USA), Anti-pS262 (44-750G), Anti-pT231 (MN1040), Anti-pT217 (44-744), Anti-pS214 (44-742G), Anti-pT212 (44-740G), Anti-pT205 (44-738G), Anti-pS202 (PA5-37638), Anti-pS199 (44-734G) and AT8 (MN1020) were obtained from Thermo Fisher Scientific (Pittsburgh, PA, USA).

Immunofluorescence and Thioflavin T (ThT) staining. To visualize the tau aggregation in cultured cells, Hela cells overexpressing tau were treated with AD P-tau as described above. The cells were incubated with Thioflavin T (ThT) for 24 hours before cell harvest. Cultures were fixed with 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.5% Triton X-100 in PBS for 10 minutes, and blocked with 10% newborn goat serum in PBS for 1 hours at room temperature. Cells were then incubated with anti-HA primary antibody overnight at 4°C, and incubated with Alexa Fluor 555-conjugated secondary antibody (Thermo Fisher Scientific) for 2 h at room temperature. Following final washes, coverslips were mounted and imaged on a Nikon confocal microscope. Tau aggregation was quantified by scoring ThT-positive/HA-positive cells and normalizing to the total number of HA-positive (tau-expressing) cells.

For immunofluorescence staining of tau pathology in brain, mouse brain tissue was fixed in 4% paraformaldehyde and sectioned into 40 μm frozen slices. After blocking with 10% normal goat serum in PBS, brain sections were incubated overnight at 4°C with primary antibodies against Iba1, Dectin1 and TREM2 (microglia) and AT8 and pT217 (tau pathology). followed by incubation with species-matched fluorophore-conjugated secondary antibodies (Thermo Fisher Scientific, Pittsburgh, PA, USA) and DAPI for nuclear counterstaining. Fluorescent images were acquired using a Nikon confocal microscope, and signal intensities were quantified using ImageJ software.

Microglial morphology reconstruction. Microglial morphology was analyzed on Iba1-immunolabeled sections using Imaris software (version 11, Bitplane, Zurich, Switzerland). After immunofluorescence staining, confocal images were acquired as z-stacks under identical laser and detector settings. Z-stacks were used both to generate a representative 2D projection illustrating microglial branches and to perform 3D reconstruction. Individual microglia whose soma and processes were fully contained within the stack were reconstructed in 3D using the “Filaments” and “Surfaces” modules. The “Filaments” function was applied to trace microglial processes from the center of the soma and to quantify total process length.

For arborization complexity, Sholl analysis in Imaris was performed by constructing a series of concentric shells centered on the soma with a radius step of 1 μm ; the number of intersections between microglial branches and each shell was counted to generate Sholl curves, and intersection numbers were used as an index of microglial branching complexity.

LDH Cytotoxicity assay. The CyQUANT™LDH Cytotoxicity Assay Kit (Invitrogen, Carlsbad, CA, USA) was used to assess LDH cytotoxicity following the manufacturer's protocol. 50 μL of culture supernatant was transferred to a new 96-well plate and combined with 50 μL of LDH reaction mixture. Samples were incubated for 30 min at 37°C in the dark, and the reaction was terminated by adding stop solution. Absorbance was then recorded at 490 nm and 680 nm using a BioTek microplate reader, and LDH activity was determined after subtraction of background values.

Intracellular ROS detection. Intracellular ROS levels were measured using a fluorometric intracellular ROS assay kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Cells were incubated with the ROS detection reagent at 37°C for 1 hour, and fluorescence intensity was measured using a microplate reader with excitation/emission wavelengths of 490/525 nm.

RNA Extraction and RT-qPCR. Total RNA was isolated from the ipsilateral hippocampal tissues using the HP Total RNA Kit (Omega, Norcross, GA, USA). Following quality and concentration assessment, RNA was reverse-transcribed to synthesize cDNA using the HiScript II Q RTSuperMix for qPCR (Vazyme, Nanjing, JS, China). RT-qPCR was then carried out using gene-specific primers and SYBR Green fluorescence to determine the expression of target genes in a LightCycler® 96 Real-time PCR system (Roche, Indianapolis, IN) under the condition: 95°C 10 min, at 95°C for 10 sec and at 60°C for 30 sec for 45 cycles. Relative expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method and normalized to housekeeping gene, GAPDH. The primer sequences for qPCR were listed below:

Primer name	Forward	Reverse
Nfkb1	5'-ATGGCAGACGATGATCCCTAC-3'	5'-TGTTGACAGTGGTATTTCTGGTG-3'
Il1b	5'-GCAACTGTTCTGAACTCAACT-3'	5'-ATCTTTTGGGGTCCGTCAACT-3'
Il6	5'-CCAAGAGGTGAGTGCTTCCC-3'	5'-CTGTTGTTGAGACTCTCTCCCT-3'
Fcgr2b	5'-AGGGCCTCCATCTGGACTG-3'	5'-GTGGTTCTGGTAATCATGCTCTG-3'
Itgam	5'-ATGGACGCTGATGGCAATACC-3'	5'-TCCCCATTACGTCTCCCA-3'
Tgfb1	5'-CTCCCGTGGCTTCTAGTGC-3'	5'-GCCTTAGTTTGGACAGGATCTG-3'
Tnf	5'-CTGAACTTCGGGGTGATCGG-3'	5'-GGCTTGTCACCTCGAATTTTGAGA-3'
Gpnm1	5'-GCTGGTCTTCGGATGAAAATGA-3'	5'-CCACAAAGGTGATATTGGAACCC-3'
Irf1	5'-ATGCCAATCACTCGAATGCG-3'	5'-TTGTATCGGCCTGTGTGAATG-3'
Irf8	5'-CGGGGCTGATCTGGGAAAAT-3'	5'-CACAGCGTAACCTCGTCTTC-3'
Fcgr3	5'-CAGAATGCACACTCTGGAAGC-3'	5'-GGGTCCCTTCGCACATCAG-3'
Gbp2	5'-CTGCACTATGTGACGGAGCTA-3'	5'-GAGTCCACACAAAGGTTGGAAA-3'

Bulk RNA-Sequencing and RNA-Seq analysis. Total RNA was extracted from hippocampal tissues injected with AAV2/8-mCherry-P2A-HA or AAV2/8-mCherry-P2A-HA-tau_{Tr} with or without NADPH treatment for 8 weeks, and used for library preparation followed by paired-end sequencing on an Illumina platform. Low-quality reads were removed using fastp, and the remaining clean reads were aligned to the mouse reference genome (Gencode vM27) using the STAR aligner with default parameters. Gene-level read counts were quantified using featureCounts implemented in the Subread package. Differentially expressed genes (DEGs) were identified using the DESeq2 R package. Genes with an adjusted *P* value < 0.05 and an absolute log₂ fold change ≥ log₂(1.2) were considered as DEGs. Functional enrichment analyses of DEGs were performed using the clusterProfiler R package or the online tool Metascape. Gene set enrichment analysis (GSEA) was conducted using clusterProfiler and visualized with the GseaVis R package.

Analysis of a public RNA-Seq dataset. Because only a limited number of differentially expressed genes (DEGs) were detected between PS19 and wild-type (WT) mice at 4 months of age (4 m)¹⁸, we focused our analysis on the RNA-seq dataset from PS19 and WT mice at 9 months of age (9 m). Gene-level read count profiles of hippocampal tissues from PS19 and WT mice at 9 m were retrieved from the Gene Expression Omnibus (GEO) database under accession number GSE218728. Differential expression analysis and DEG identification were performed as described above. To assess the molecular similarity between PS19 mice and our AAV-mediated truncated tau mouse model, we evaluated the overlap of upregulated and downregulated genes between PS19 mice and truncated tau mice using Fisher's exact

test implemented in the GeneOverlap R package. The results were visualized using two-set Venn diagrams.

Analysis of a single-nucleus RNA-Seq (snRNA-Seq) dataset. Single-nucleus RNA-seq (snRNA-seq) datasets of hippocampal tissues from PS19 and wild-type (WT) mice at 9 months of age (9 m) were obtained from the Gene Expression Omnibus (GEO) database under accession number GSE218728. Data preprocessing, clustering, and cell-type annotation were performed as described in the original publication¹⁸. Gene set scores for individual nuclei were calculated using the AddModuleScore function implemented in Seurat v5.

Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). Data are expressed as mean $\pm$ standard error of the mean (SEM) based on at least three independent experiments. For comparisons between two groups, an unpaired two-tailed Student's t-test was applied to normally distributed data, whereas the Mann–Whitney U test was used for non-normally distributed data. For analyses involving more than two groups, one-way or two-way analysis of variance (ANOVA) was performed, followed by appropriate post hoc tests (Tukey's or Sidak's). Statistical significance was defined as $p < 0.05$.

Declarations

Ethics approval and consent to participate

All procedures involving mice were reviewed and approved by our Institutional Animal Care and Use Committee and were carried out according to guidelines of the National Institutes of Health.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

Funding

This work is supported by funds from National Natural Science Foundation of China (Grant No. 82171425), Nantong Natural Science Foundation (Grand No. JC2024023), the High-level Science and Technology Project Cultivation Fund for Provincial and Ministerial Projects of Nantong First People's Hospital (Grand No. YPYJJZD24003 and YPYJJZD24001), the Nantong University Special Research Fund

for Clinical Medicine (Grant No. 2024JQ011), the Large Research group project of King Khalid University (Grant No. R.G.P.2/125/47).

Author Contribution

N.J. and F.L. conceived and designed the study and provided overall supervision. J.D., R.Z. and R.W. performed the experiments. L.X. conducted bioinformatics and data analysis. L.X. and N.J. drafted the manuscript. S.Z., R.W., H.W., W.L., N.B., and J.S. contributed to data interpretation and participated in manuscript discussion and revision. N.J. and F.L. critically revised and finalized the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We thank the First People's Hospital of Nantong, Nantong University, the Neural Regeneration Co-innovation Center of Jiangsu Province, and the New York State Office for People With Developmental Disabilities for their support of this study.

Data Availability

The bulk RNA-Seq of hippocampus in mice generated in this study have deposited in the National Genomics Data Center (NGDC) under accession numbers PRJCA068375. All other data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chetelat G, Teunissen CE, Cummings J, van der Flier WM (2021) Alzheimer's disease. *Lancet* 397:1577–1590. 10.1016/S0140-6736(20)32205-4
2. Zhang Z, Song M, Liu X, Kang SS, Kwon IS, Duong DM, Seyfried NT, Hu WT, Liu Z, Wang JZ et al (2014) Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer's disease. *Nat Med* 20:1254–1262. 10.1038/nm.3700
3. Long JM, Holtzman DM (2019) Alzheimer Disease: An Update on Pathobiology and Treatment Strategies. *Cell* 179:312–339. 10.1016/j.cell.2019.09.001
4. Chu D, Yang X, Wang J, Zhou Y, Gu JH, Miao J, Wu F, Liu F (2024) Tau truncation in the pathogenesis of Alzheimer's disease: a narrative review. *Neural Regen Res* 19:1221–1232. 10.4103/1673-5374.385853
5. Cente M, Filipcik P, Pevalova M, Novak M (2006) Expression of a truncated tau protein induces oxidative stress in a rodent model of tauopathy. *Eur J Neurosci* 24:1085–1090. 10.1111/j.1460-9568.2006.04986.x

6. Li S, Chen Y, Song T, Liu D, Wu R, Yang X, Wu Q, Lei L, Yu X, Zhang J et al (2026) Ganglioside sialylation modulates tau internalization and pathology spread. *Mol Psychiatry* 31:2561–2574. 10.1038/s41380-025-03394-2
7. Jin N, Gu J, Wu R, Chu D, Tung YC, Wegiel J, Wisniewski T, Gong CX, Iqbal K, Liu F (2022) Tau seeding activity in various regions of down syndrome brain assessed by two novel assays. *Acta Neuropathol Commun* 10:132. 10.1186/s40478-022-01436-2
8. Gu J, Xu W, Jin N, Li L, Zhou Y, Chu D, Gong CX, Iqbal K, Liu F (2020) Truncation of Tau selectively facilitates its pathological activities. *J Biol Chem* 295:13812–13828. 10.1074/jbc.RA120.012587
9. Leng F, Edison P (2021) Neuroinflammation and microglial activation in Alzheimer disease: where do we go from here? *Nat Rev Neurol* 17:157–172. 10.1038/s41582-020-00435-y
10. Sun N, Victor MB, Park YP, Xiong X, Scannail AN, Leary N, Prosper S, Viswanathan S, Luna X, Boix CA et al (2023) Human microglial state dynamics in Alzheimer's disease progression. *Cell* 186:4386–4403e4329. 10.1016/j.cell.2023.08.037
11. Ennerfelt H, Frost EL, Shapiro DA, Holliday C, Zengeler KE, Voithofer G, Bolte AC, Lammert CR, Kulas JA, Ulland TK, Lukens JR (2022) SYK coordinates neuroprotective microglial responses in neurodegenerative disease. *Cell* 185:4135–4152e4122. 10.1016/j.cell.2022.09.030
12. Chen Y, Colonna M (2021) Microglia in Alzheimer's disease at single-cell level. Are there common patterns in humans and mice? *J Exp Med* 218. 10.1084/jem.20202717
13. Deczkowska A, Keren-Shaul H, Weiner A, Colonna M, Schwartz M, Amit I (2018) Disease-Associated Microglia: A Universal Immune Sensor of Neurodegeneration. *Cell* 173:1073–1081. 10.1016/j.cell.2018.05.003
14. Du F, Yu Q, Kanaan NM, Yan SS (2022) Mitochondrial oxidative stress contributes to the pathological aggregation and accumulation of tau oligomers in Alzheimer's disease. *Hum Mol Genet* 31:2498–2507. 10.1093/hmg/ddab363
15. Hildebraunt AG, Roots I (1975) Reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent formation and breakdown of hydrogen peroxide during mixed function oxidation reactions in liver microsomes. *Arch Biochem Biophys* 171:385–397. 10.1016/0003-9861(75)90047-8
16. Li M, Zhou ZP, Sun M, Cao L, Chen J, Qin YY, Gu JH, Han F, Sheng R, Wu JC et al (2016) Reduced Nicotinamide Adenine Dinucleotide Phosphate, a Pentose Phosphate Pathway Product, Might Be a Novel Drug Candidate for Ischemic Stroke. *Stroke* 47:187–195. 10.1161/STROKEAHA.115.009687
17. Wang XX, Wang F, Mao GH, Wu JC, Li M, Han R, She J, Zhang R, Sheng R, Chen Z, Qin ZH (2022) NADPH is superior to NADH or edaravone in ameliorating metabolic disturbance and brain injury in ischemic stroke. *Acta Pharmacol Sin* 43:529–540. 10.1038/s41401-021-00705-5
18. Wang B, Martini-Stoica H, Qi C, Lu TC, Wang S, Xiong W, Qi Y, Xu Y, Sardiello M, Li H, Zheng H (2024) TFEB-vacuolar ATPase signaling regulates lysosomal function and microglial activation in tauopathy. *Nat Neurosci* 27:48–62. 10.1038/s41593-023-01494-2

19. Zhou JS, Zhu Z, Wu F, Zhou Y, Sheng R, Wu JC, Qin ZH (2019) NADPH ameliorates MPTP-induced dopaminergic neurodegeneration through inhibiting p38MAPK activation. *Acta Pharmacol Sin* 40:180–191. 10.1038/s41401-018-0003-0
20. Koju N, Qin ZH, Sheng R (2022) Reduced nicotinamide adenine dinucleotide phosphate in redox balance and diseases: a friend or foe? *Acta Pharmacol Sin* 43:1889–1904. 10.1038/s41401-021-00838-7
21. Bellucci A, Westwood AJ, Ingram E, Casamenti F, Goedert M, Spillantini MG (2004) Induction of inflammatory mediators and microglial activation in mice transgenic for mutant human P301S tau protein. *Am J Pathol* 165:1643–1652. 10.1016/S0002-9440(10)63421-9
22. Butterfield DA, Halliwell B (2019) Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. *Nat Rev Neurosci* 20:148–160. 10.1038/s41583-019-0132-6
23. Holmstrom KM, Finkel T (2014) Cellular mechanisms and physiological consequences of redox-dependent signalling. *Nat Rev Mol Cell Biol* 15:411–421. 10.1038/nrm3801
24. Ying W (2008) NAD⁺/NADH and NADP⁺/NADPH in cellular functions and cell death: regulation and biological consequences. *Antioxid Redox Signal* 10:179–206. 10.1089/ars.2007.1672
25. Dustin CM, Shiva SS, Vazquez A, Saeed A, Pascoal T, Cifuentes-Pagano E, Pagano PJ (2024) NOX2 in Alzheimer's and Parkinson's disease. *Redox Biol* 78:103433. 10.1016/j.redox.2024.103433
26. Wilkinson BL, Landreth GE (2006) The microglial NADPH oxidase complex as a source of oxidative stress in Alzheimer's disease. *J Neuroinflammation* 3. 10.1186/1742-2094-3-30
27. Hickman S, Izzy S, Sen P, Morsett L, Khoury E, J (2018) Microglia in neurodegeneration. *Nat Neurosci* 21:1359–1369. 10.1038/s41593-018-0242-x
28. Mathys H, Davila-Velderrain J, Peng Z, Gao F, Mohammadi S, Young JZ, Menon M, He L, Abdurrob F, Jiang X et al (2019) Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* 570:332–337. 10.1038/s41586-019-1195-2
29. Hansen DV, Hanson JE, Sheng M (2018) Microglia in Alzheimer's disease. *J Cell Biol* 217:459–472. 10.1083/jcb.201709069
30. Wang C, Fan L, Khawaja RR, Liu B, Zhan L, Kodama L, Chin M, Li Y, Le D, Zhou Y et al (2022) Microglial NF-kappaB drives tau spreading and toxicity in a mouse model of tauopathy. *Nat Commun* 13, 1969. 10.1038/s41467-022-29552-6
31. Keren-Shaul H, Spinrad A, Weiner A, Matcovitch-Natan O, Dvir-Szternfeld R, Ulland TK, David E, Baruch K, Lara-Astaiso D, Toth B et al (2017) A Unique Microglia Type Associated with Restricting Development of Alzheimer's Disease. *Cell* 169:1276–1290e1217. 10.1016/j.cell.2017.05.018
32. Yang S, Wang J, Cao Y, Zhang Y, Sun Z, Wan P, Pi M, Xiong Q, Shu X, Wang X, Xia Y (2025) Clec7a Signaling in Microglia Promotes Synapse Loss Associated with Tauopathy. *Int J Mol Sci* 26. 10.3390/ijms26072888

Figures

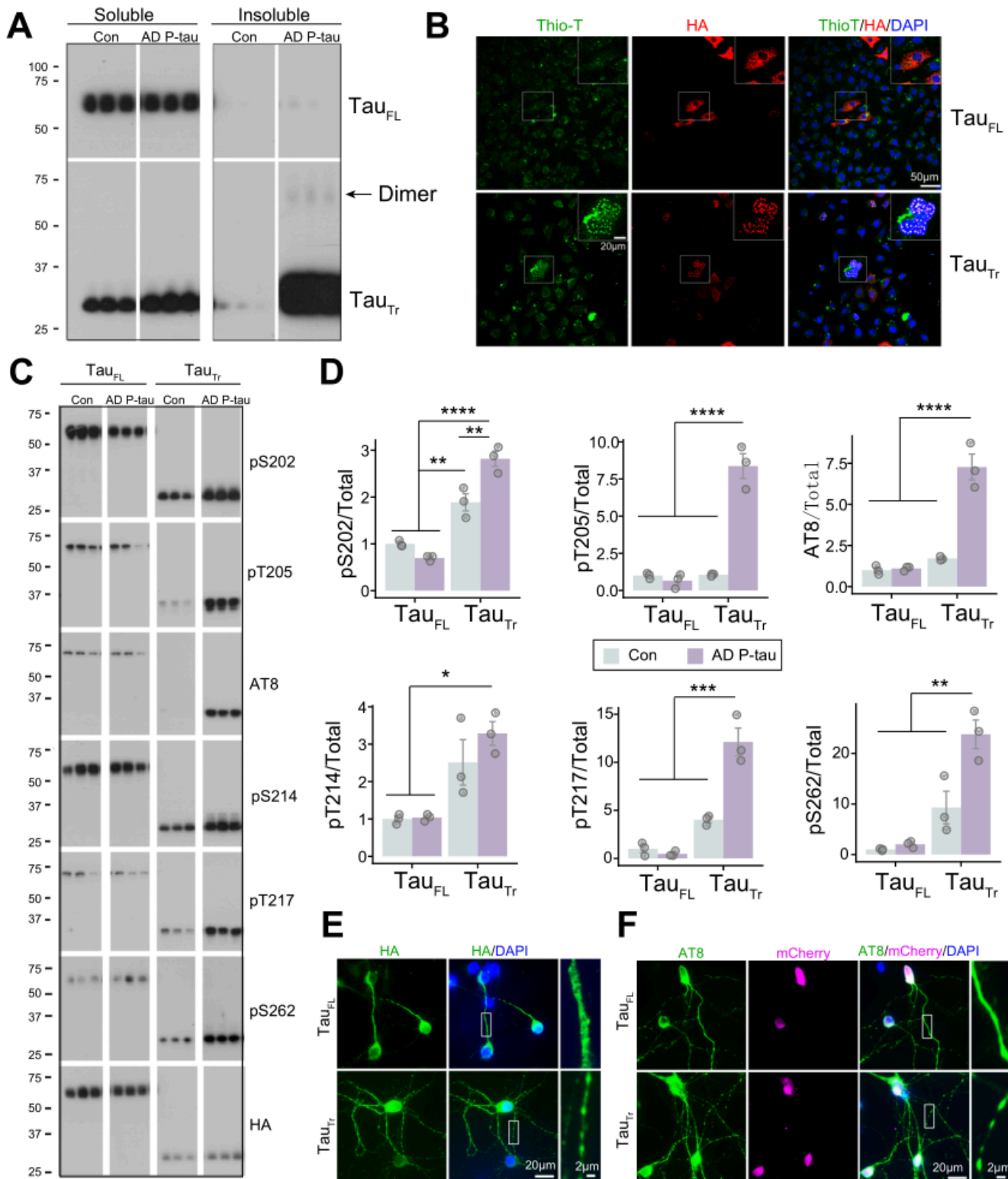


Figure 1

AD-derived P-tau induces aggregation and site-specific hyperphosphorylation of truncated tau. (A) HEK293T cells were transfected with HA-tagged full-length tau (tau_{FL}) or truncated tau (tau_{Tr}) for 6 h, followed by treatment with AD P-tau for 42 h. Cells were lysed in RIPA buffer, and RIPA-soluble and -insoluble tau fractions were analyzed by Western blots. (B) HeLa cells were treated as in (A), and tau aggregation was assessed by immunostaining with an anti-HA antibody together with Thioflavin T (ThT).

Hela cells overexpressing tau_{FL} were treated with AD O-tau for 120 h, Hela cells overexpressing tau_{FL} were treated with AD O-tau for 48 h. **(C, D)** AD P-tau induces site-specific hyperphosphorylation of Tau_{Tr}. Cells treated with AD P-tau as described above were lysed in Laemmli buffer, and phosphorylated tau and total tau (HA) were analyzed by Western blots. For each antibody, the results were obtained from the same membrane, and non-relevant lanes were removed. Data are presented as mean±s.e.m. (n=3), p<0.05; p<0.01; *p<0.001; **p<0.0001. **(E, F)** Representative immunofluorescence images showing HA **(E)** and AT8 **(F)** staining in primary neurons infected with AAV2/8-mCherry-P2A-HA-tau_{FL} or AAV2/8-mCherry-P2A-HA-tau_{Tr}.

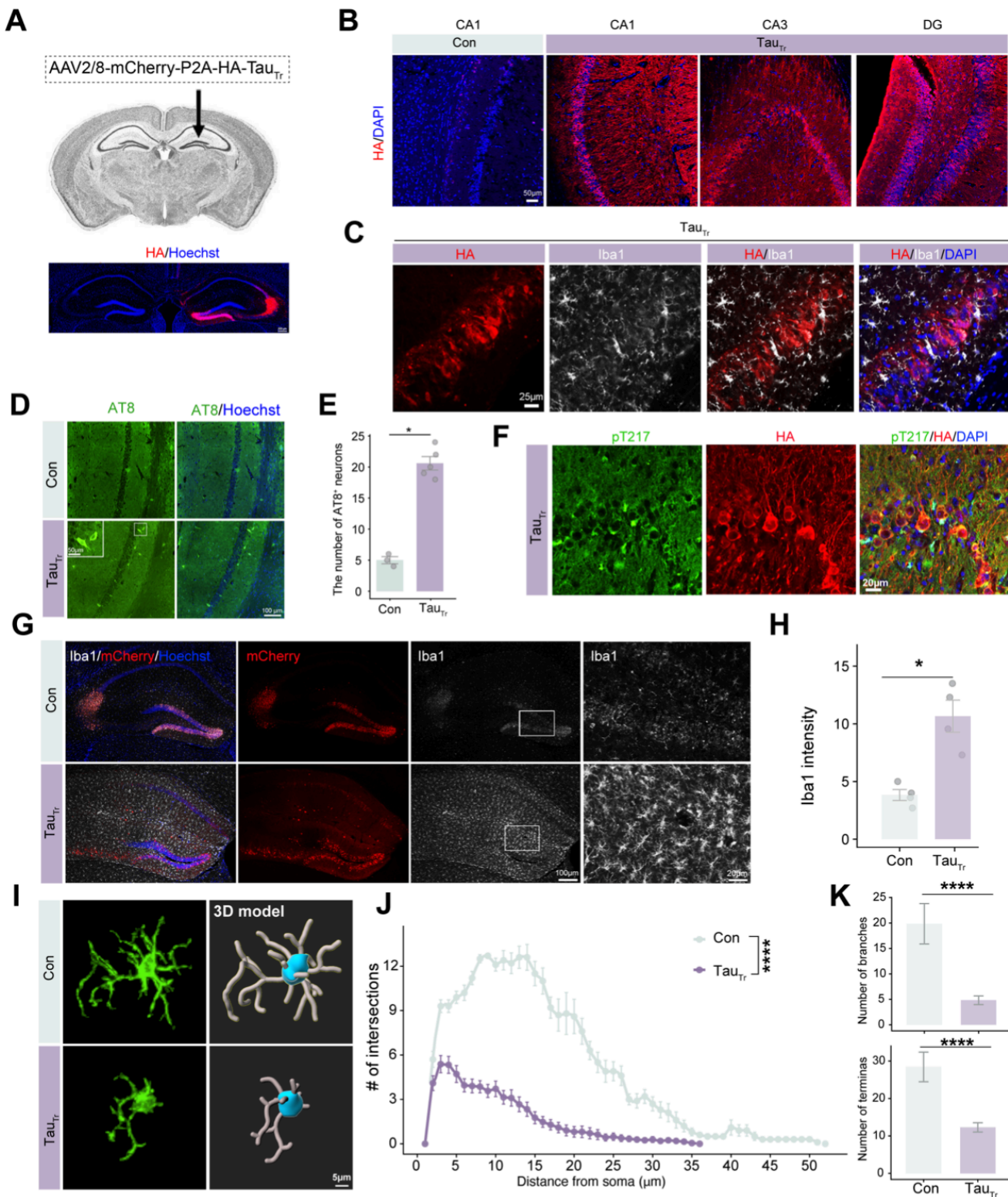


Figure 2

Overexpression of truncated tau induces tau pathology and microglia activation *in vivo*. (A) Schematic illustration of the experimental design. AAV2/8-mCherry-P2A-HA-tau_{Tr} was stereotactically injected into the hippocampus of wild-type mice. (B) Representative immunofluorescence images showing HA-tagged tau expression in CA1 region of the control (Con) group and the CA1, CA3 and DG regions in the tau_{Tr} group. (C) Representative immunofluorescence image of HA and Iba1 co-immunostaining in the

CA1 region in the tau_{tr} group. **(D)** Tau pathology was examined by immunofluorescence staining using the AT8 antibody. **(E)** Quantification of the number of AT8-positive neurons in panel B. **(F)** Representative immunofluorescence image showing phospho-Thr217 and HA in CA3 region in the tau_{tr} group. **(G, H)** Microglial activation in the hippocampus was assessed by Iba1 immunofluorescence staining **(G)**, and the fluorescence signal intensity of Iba1 was quantified **(H)**. **(I)** Representative 3D reconstructions of Iba1-positive microglia in the control and tau_{Tr}. **(J)** Sholl analysis showing the mean number of interactions as a function of the distance from the microglial soma in the two groups. **(K)** Quantification of the number of microglia branches and terminals in the two groups. Data are presented as mean ± s.e.m. n = 3-4; p < 0.05.

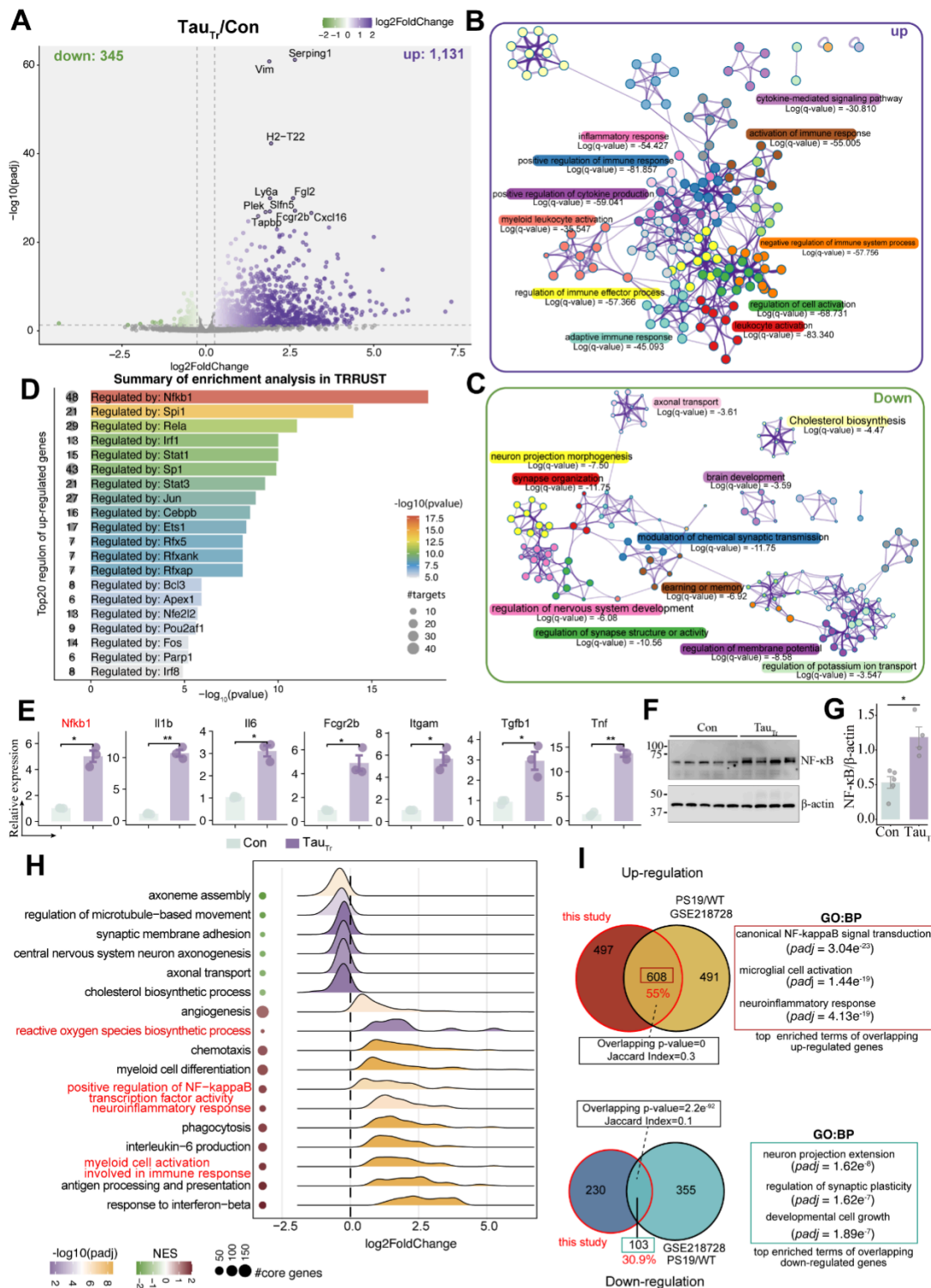


Figure 3

Inflammatory response is activated by AAV-mediated truncated tau as revealed by RNA-Seq analysis.

A, volcano plot showing differentially expressed genes (DEGs) between the Tau_{Tr} group and the control (Con) group. Functional enrichment of up-regulated genes (**B**) and down-regulated genes (**C**). each node represents a functional term and same node color represent similar function. The representative terms are shaded and labelled. **D**, top20 enriched regulators inferred from TRRUST database. **E**, qPCR

validation of seven representative genes, including Nfkb1. **F** Representative Western blots analysis showing elevated Nfkb1 protein levels in the TauTr group relative to controls. **G** Quantification of Nfkb1 protein levels. **H**, dot and ridge plots showing GSEA of representative terms and fold-change of core enrichment genes. **I**, left panel: venn diagram showing high similarity of up-regulated genes (upper) and down-regulated genes (bottom) from our AAV-mediated truncated tau model and PS19 mice (dataset was retrieved from GSE218728 accession) versus compared with the respective counterparts. Right panel: top enriched terms of shared up-regulated genes (upper) or down-regulated genes (bottom).

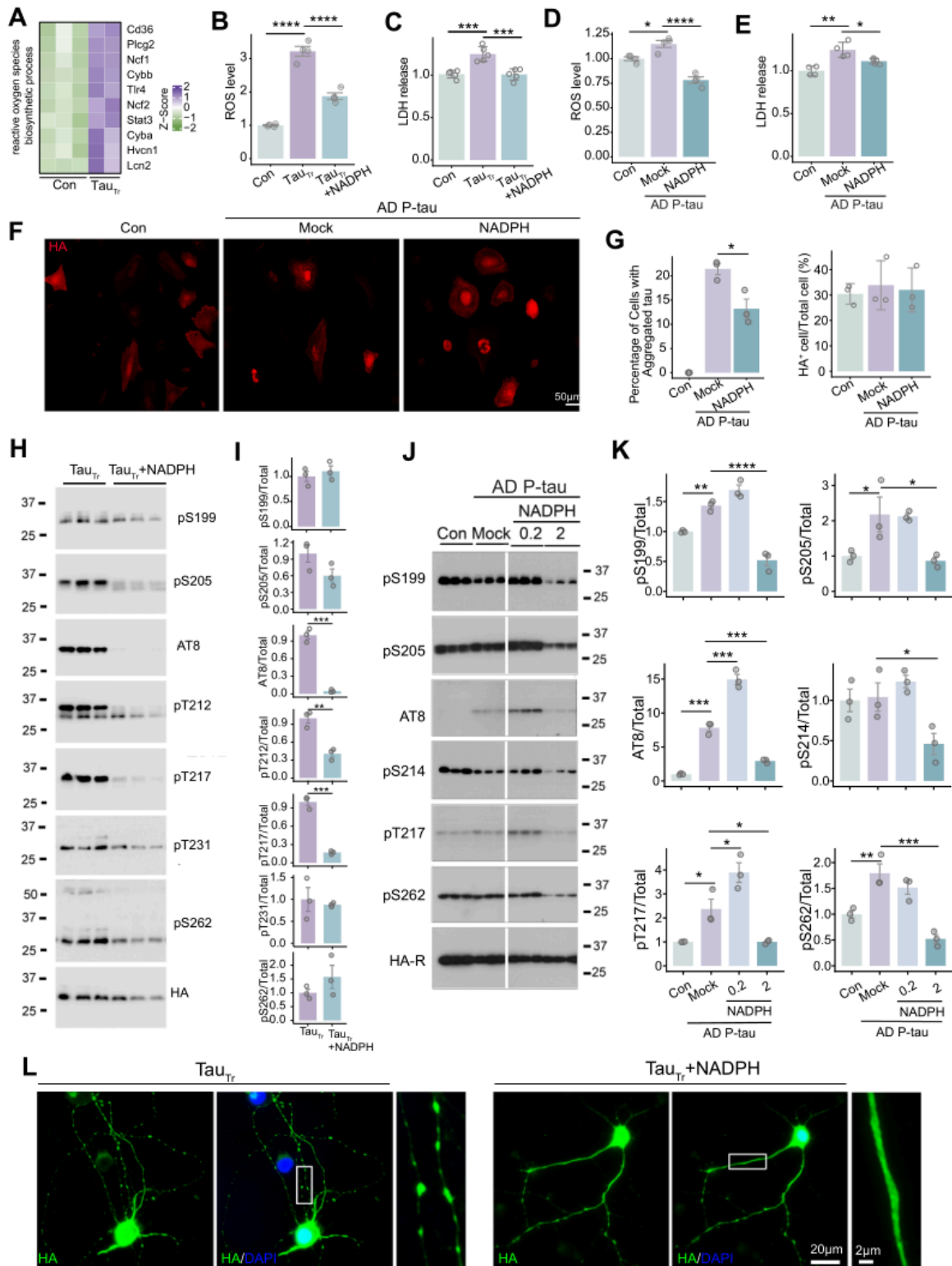


Figure 4

NADPH suppresses tau aggregation and site-specific hyperphosphorylation via reducing ROS. A.

Expression of DEGs in **Fig. 3A** associated with reactive oxygen species biosynthetic process. **(B)** HEK-293T cells were transfected with pCI-neo (Con), pCI/HA-tau_{Tr} and treated with 2 μ M NADPH for 48 h. The intracellular ROS levels were measured using a ROS assay kit. **(C)** Quantification of LDH release in the culture supernatant of different groups. **(D)** HEK-293T/HA-tau_{Tr} cells were pretreated with 2 μ M NADPH for 6 hr, followed by co-treatment with AD P-tau for 42 hr. The intracellular ROS levels were measured. **(E)** Quantification of LDH release in the culture supernatant of different groups. **(F, G)** HeLa cells were transfected with pCI/HA-tau_{Tr}, treated with 2 μ M NADPH for 6 h, and subsequently co-treated with AD P-tau for 42 h. Overexpressed tau and tau aggregates were detected by immunofluorescence staining using an anti-HA antibody **(F)**. The percentage of cells containing tau aggregates relative to total HA-positive cells was quantified **(G)**. **(H, I)** HEK-293T cells were treated as in **(B)** and lysed in Laemmli buffer, Western blots were used to check tau phosphorylation **(H)**. Phosphorylation at individual sites was normalized to total tau (HA) **(I)**. **(J, K)** HEK293T cells were treated as in **(D)**, and total cellular proteins were collected. Tau phosphorylation levels were analyzed by Western blots **(J)**, and the phosphorylation levels at individual sites were quantified as ratios relative to total tau (HA) **(K)**. For each antibody, the results were obtained from the same membrane, and non-relevant lanes were removed. **(L)** Representative immunostaining images of HA in primary neurons from the tau_{Tr} and tau_{Tr} with NADPH treatment. Data are presented as mean $\pm$ s.e.m. (n = 3). p < 0.05; p < 0.01; *p < 0.001; **p < 0.0001.

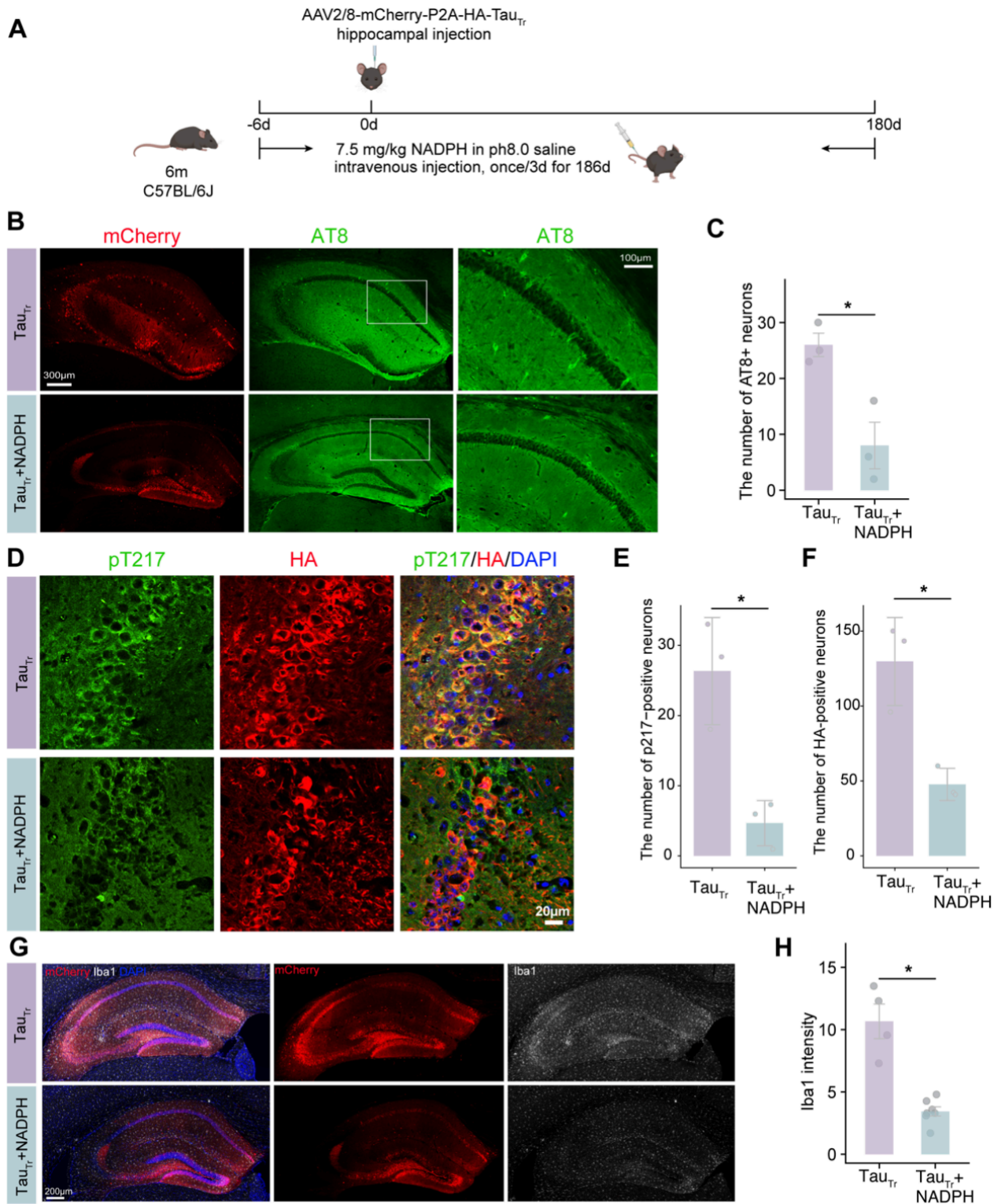


Figure 5

NADPH suppresses tau pathology and microglial activation *in vivo*. (A) Schematic illustration of the experimental workflow. (B, C) Tau pathology was evaluated by immunofluorescence staining using the AT8 antibody (B), and the number of AT8-positive neurons was quantified (C). (D-F) Tau pathology was evaluated by immunofluorescence staining using the phospho-Thr217 tau and HA antibody (D), and the number of pT217-positive neurons (E) and the number of HA-positive neurons (F). (G, H) Microglial

activation in the hippocampus was assessed by Iba1 immunofluorescence staining (G), and Iba1 fluorescence intensity was quantified (H). Data are presented as mean $\pm$ s.e.m. $p < 0.05$.

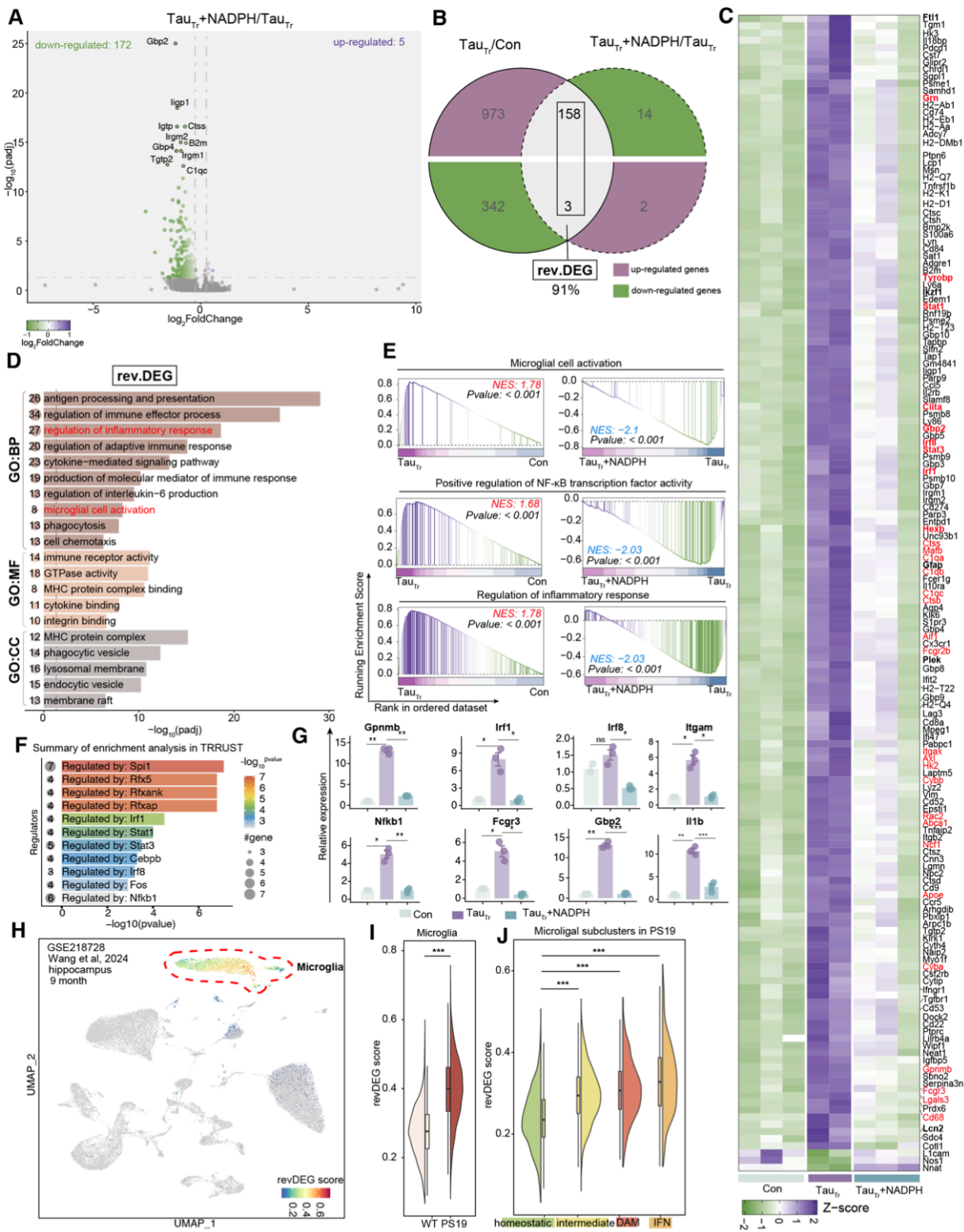


Figure 6

NADPH suppresses the inflammatory response induced by AAV-mediated truncated tau as revealed by RNA-Seq analysis. A, volcano plot showing differentially expressed genes (DEGs) between the

Tau_{Tr}+NADPH group and the tau_{Tr} group. **B**, venn diagram showing that 91% of DEGs in tau_{Tr}+NADPH versus tau_{Tr} were reversed relative to DEGs in tau_{Tr} versus Con, defined as reversed DEGs (rev.DEGs). **C**, Heatmap showing the expression patterns of rev.DEG across three experiential conditions, Con, tau_{Tr}, and tau_{Tr}+NADPH. **D**, functional enrichment analysis of rev.DEG. **E**, Gene set enrichment analysis (GSEA) plot showing enrichment of genes involved in terms of microglial cell activity, positive regulation of NK-κB transcription factor activity and regulation of inflammatory response. **F**, summary enrichment analysis in TRRUST. **G**, qPCR validation of six representative genes, including *Nfkb1*. **H**, revDEG activity is highly enriched in microglia, as inferred from a snRNA-seq dataset of hippocampal tissues from PS19 and wild-type (WT) mice at 9 m of age. **I**, revDEG activity is significantly higher in microglia from PS19 mice compared with WT mice. **J**, revDEG activity is significantly higher in microglia subclusters, including disease-associated microglia (DAM), compared with homeostatic microglia in PS19 microglia. IFN, interferon-response microglia.

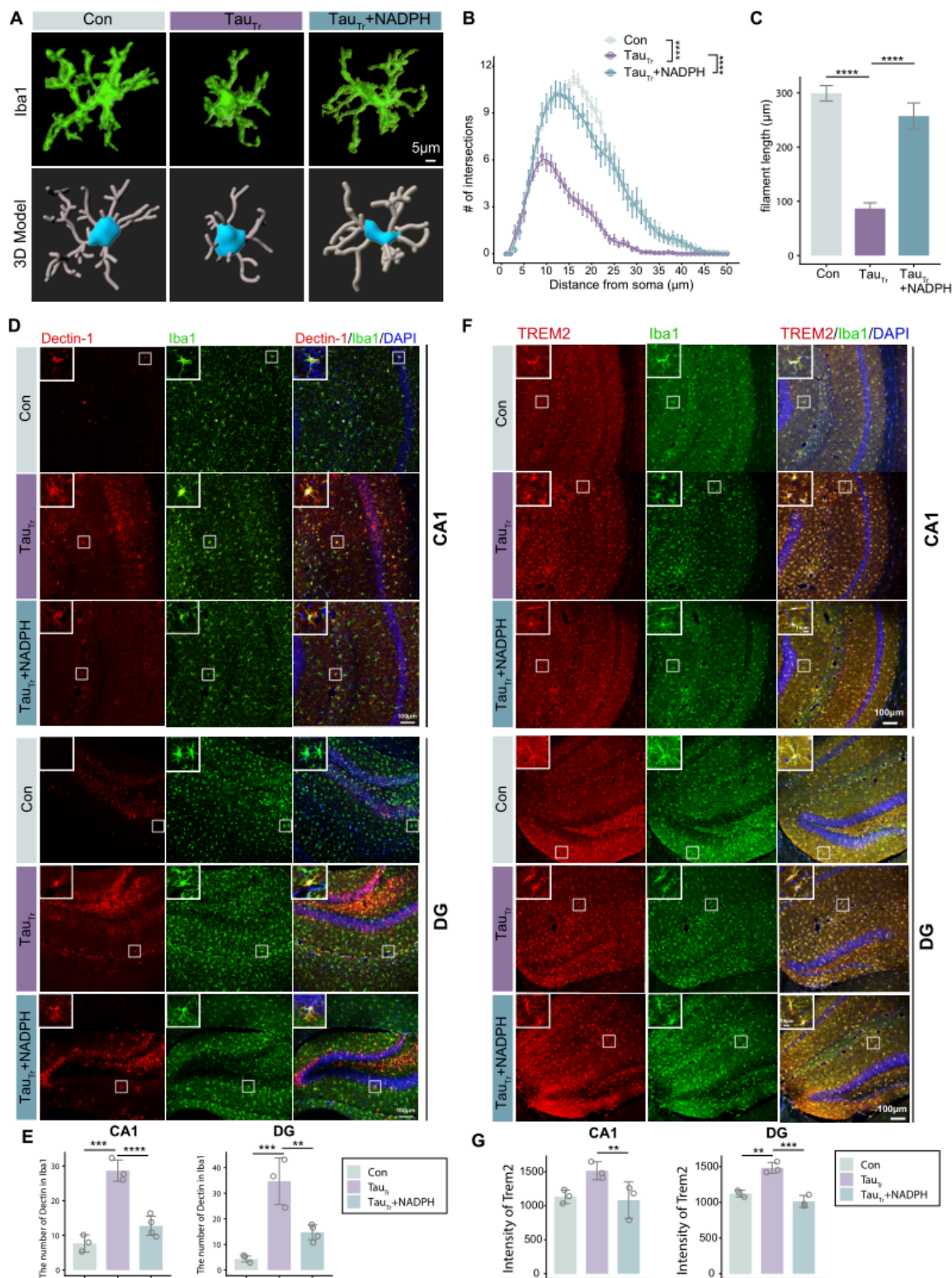


Figure 7

NADPH attenuates the disease-associated microglial phenotype induced by AAV-mediated truncated tau. (A) Representative 3D reconstructions of Iba1-positive microglia in the control, τ_{Tr} and τ_{Tr} +NADPH. (B) Sholl analysis showing the mean number of interactions as a function of the distance from the microglial soma in the three groups. (C) Quantification of total filament length of microglia in the three groups. (D, E) Representative immunostaining images of Dectin-1 and Iba1 in CA1 and DG brain

regions in three groups (**D**) and quantification the number of Dectin-1 (**E**). (**F, G**) Representative immunostaining images of Trem2 and Iba1 in CA1 and DG brain regions in three groups (**F**) and quantification the Trem2 intensity (**G**). Data are presented as mean $\pm$ s.e.m. (n = 3-4). *p < 0.05; **p < 0.01.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryfile.pdf](#)