

ALZHEIMER'S DISEASE

Inhaled xenon modulates microglia and ameliorates disease in mouse models of amyloidosis and tauopathy

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder. Anti-amyloid antibody treatments modestly slow disease progression in mild dementia due to AD. Emerging evidence shows that homeostatic dysregulation of the brain immune system, especially that orchestrated by microglia, plays an important role in disease onset and progression. Thus, a major question is how to modulate the phenotype and function of microglia to treat AD. Xenon (Xe) gas is a noble gas used in human patients as an anesthetic and a neuroprotectant used for treating brain injuries. Xe penetrates the blood-brain barrier, which could make it an effective therapeutic. To assess the effect of Xe on microglia and AD pathology, we designed a custom Xe inhalation chamber and treated several mouse models of AD with Xe gas. Xe treatment induced mouse microglia to adopt an intermediate activation state that we have termed pre-neurodegenerative microglia (pre-MGnD). This microglial phenotypic transition was observed in mouse models of acute neurodegeneration and amyloidosis (APP/PS1 and 5xFAD mice) and tauopathy (P301S mice). This microglial state enhanced amyloid plaque compaction and reduced dystrophic neurites in the APP/PS1 and 5xFAD mouse models. Moreover, Xe inhalation reduced brain atrophy and neuroinflammation and improved nest-building behavior in P301S mice. Mechanistically, Xe inhalation induced homeostatic brain microglia toward a pre-MGnD state through IFN- γ signaling that maintained the microglial phagocytic response in APP/PS1 and 5xFAD mice while suppressing the microglial proinflammatory phenotype in P301S mice. These results support the translation of Xe inhalation as an approach for treating AD.

INTRODUCTION

Alzheimer's disease (AD) and primary tauopathies such as progressive supranuclear palsy and frontotemporal dementia due to tauopathy cause progressive neurodegeneration, which ultimately leads to death. Currently, anti-amyloid antibody treatment only modestly slows disease progression in mild dementia due to AD (1). Thus, other therapies are needed to slow or stop the progression more effectively. Given that AD alone affects 50 million people worldwide, with a prediction to rise to 150 million by 2050 (2), there is a dire need for neuroprotective treatments that can minimize or attenuate the neurodegeneration and neuroinflammation observed in this disease. The mechanisms underlying AD are not fully understood.

However, microglia-mediated increased inflammation and oxidative stress, induced by abnormal protein accumulation of amyloid- β (A β) plaques and tau tangles, has been associated with AD progression. We identified that neurodegenerative microglia (MGnD) (3), also called disease-associated microglia (DAM) (4), are regulated by the reciprocal suppression of transforming growth factor- β (TGF β) and induction of apolipoprotein E (APOE) signaling in different neurodegenerative models, including mouse models of AD (3). Genome-wide association studies (GWASs) (5) identified microglia as the primary cell type in the central nervous system (CNS) whose gene expression pattern correlated with late-onset AD. However, no treatment that focuses on microglia modulation is available.

Various medical gases have been safely used for anesthesia and hyperbaric oxygen therapy (6). Xenon (Xe) gas is a noble gas and one of the least reactive chemical elements, with a complete set of valence electrons that make it stable and unreactive. Xe has been used as a general anesthetic since the mid-1900s, but its molecular targets remain obscure (7). Its effects on the glutamatergic neurotransmitter systems, especially N-methyl-D-aspartate (NMDA) receptors, are the most thoroughly studied. However, the mechanisms by which the potentially neuroprotective properties of Xe manifest are still unclear (7, 8). Excitatory glutamatergic neurotransmission through NMDA receptors is critical for synaptic plasticity and survival of neurons. However, excessive NMDA receptor activity can contribute to excitotoxicity and promote cell death. Some studies have shown that the function of NMDA receptor channels is correlated with neurotoxicity and degeneration observed in AD (9). Xe is an NMDA receptor

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antagonist (10) that can enter the brain parenchyma through the blood-brain barrier (BBB) (11), making it an effective CNS therapeutic. A number of in vivo and in vitro studies have examined the efficacy of Xe in models of traumatic brain injury (TBI), hypoxia, ischemic brain injury, and neuronal injury (12, 13). However, how Xe inhalation might affect amyloidosis, tau pathology, neurodegeneration, and neuroinflammation in AD has not been examined. Xe inhalation is being explored in clinical trials as a potential therapeutic in various neurological conditions and neurodegenerative diseases, but further understanding of the effects of Xe inhalation and molecular mechanisms is necessary (14, 15).

Our current study investigated the therapeutic potential of Xe inhalation for AD and tauopathy using various mouse models that simulated distinct amyloidosis and tauopathy features. The APP/PS1 (16) and 5x*FAD* (17) mice were used as models of amyloidosis with A β plaque accumulation, whereas P301S mice expressing APOE4 (TE4) were used to explore tau-induced neurodegeneration in the context of human APOE4 (18). To study human microglial responses, we used humanized 5x-MITRG mice engrafted with induced pluripotent stem cell (iPSC)-derived human microglia (19). An acute neurodegeneration model in which apoptotic neurons were injected into adult mouse brains (3) was used to examine Xe's effects on early microglial activation. Furthermore, to assess the role of MGnD in controlling A β accumulation and dystrophic neurites, we generated a *Clec7a*-Cre^{ERT2} mouse model capable of targeting *Clec7a*⁺ MGnD. To investigate how interferon- γ (IFN- γ) signaling might regulate the transition from homeostatic microglia to MGnD in response to Xe, we used *Cx3cr1*-Cre^{ERT2}:*Ifngr1*^{Flox} (*Ifngr1*-cKO) mice. Together, these mouse models provided a comprehensive understanding of Xe's impact on AD-like pathology, microglial modulation, and neuroimmune interactions.

RESULTS

Xe inhalation induces a microglia phagocytic response and suppresses the neuroinflammatory signature in mice with neurodegeneration

To determine whether Xe inhalation could modulate MGnD, we used an acute neurodegeneration mouse model to induce MGnD (3). Briefly, apoptotic neurons were injected into the cortices and hippocampi of naïve C57BL/6J mice, which induced the recruitment of microglia toward the site of injection and changed their morphology and phenotype from an M0 (homeostatic) (20) to an MGnD signature (3). Using this model, we tested four different concentrations of Xe, starting with 6, 12, 30, and 35% (fig. S1A). To treat mice with Xe, we constructed a special chamber that controlled the supply of Xe and oxygen (O₂). The closed-circuit system pumps recirculated gas through soda lime to absorb carbon dioxide (CO₂). CO₂ and O₂ concentrations were continuously monitored. The gas mixture was premixed in an outside container with excess pressure. When the O₂ concentration was reduced to below 18%, an additional gas mixture was supplied from the mixing container. The system was optimized to treat up to 12 mice simultaneously in one chamber. We used a cell sorter for microglia isolation, followed by our specific microglia antibody, anti-FCRLS (Fc receptor-like S) (fig. S1B) (3). When comparing different concentrations of Xe, the flow cytometry results indicated that the 30 and 35% concentrations promoted more neuron phagocytosis (fig. S1C). Transcriptomic characterization of phagocytic microglia showed that 30% Xe increased the expression of genes correlated with the IFN signature

(*Ifitm1*, *Ifit2*, and *Stat4*) and lysosomal and phagocytic functions (*P2ry10*, *Cd36*, and *Mrc1*) and decreased the expression of genes related to nuclear factor κ B (NF- κ B) responses such as *Il1a*, *Il6ra*, and *Jun* (fig. S1D). Ingenuity pathway analysis (IPA) indicated an up-regulation of peroxisome proliferator-activated receptor (PPAR) and retinoid X receptor (RXR) activity, myelination signaling, and anti-inflammatory interleukin-10 (IL-10) signaling. Moreover, senescence pathways associated with increased neuroinflammation—including Toll-like receptor (TLR), IL-8, and CXCR4 signaling—were down-regulated after Xe inhalation (30%) (fig. S1, E and F).

Xe is an inert gas, and its measurement is very difficult, often requiring specialized techniques. To address this, we developed a sensor capable of accurately detecting the concentration of Xe. We calibrated the sensor to detect the percentage of Xe in a binary mixture of Xe and O₂. Our testing has demonstrated that our sensor is reliable and accurate across a Xe concentration range from 0 to 100% (fig. S1, G to I). To determine how long the effects of Xe persist in microglia, we used APP/PS1 mice, an animal model with accumulation of A β and MGnD (3), which can be followed for more extended periods than the acute neurodegeneration mouse model (16). We treated 3-month-old APP/PS1 mice with a single dose of 30% Xe for 40 min. Animals were euthanized 3, 7, or 14 days after treatment, and isolated microglia were evaluated by flow cytometry and RNA sequencing (RNA-seq) (fig. S2A). We found that 7 days after treatment was the peak of the microglial response, with the highest number of differentially expressed genes (DEGs) (fig. S2B). IPA indicated CREB, IL-15, and nitric oxide as the major pathways induced by Xe at this time point (fig. S2, C to G). This kinetic evaluation showed that activation started in animals at 3 days after treatment, with the oxidative phosphorylation metabolism recovery at 14 days after treatment (fig. S2D). In addition, the evaluation of homeostatic and MGnD signatures showed that Xe inhalation induced expression of a subcluster of MGnD genes, such as *Gpnmb*, *Gas7*, *Clec7a*, *Axl*, and *Itgax*, but maintained expression of some homeostatic genes, as shown by the increased expression of *Tgfb2* and *Nr3c1* (fig. S2E). Upstream regulator IPA predicted the suppression of tumor necrosis factor (TNF) and the complement system (C3, IKZ1, and APP) and the up-regulation of TGF β (fig. S2F). Thus, Xe treatment suppressed inflammatory genes while maintaining up-regulation of genes related to phagocytic functions (fig. S2G). To validate the ability of Xe to enhance the microglial phagocytic response, we treated wild-type C57BL/6J mice with 30% Xe for 40 min. Seven days after inhalation, we injected fluorescent A β (1–42) into the cortex and hippocampus, and four hours later, we evaluated the phagocytic activity of microglia using flow cytometry (fig. S2, H and I). Our results demonstrated that Xe treatment significantly increased the percentage ($P = 0.0009$) of microglia that phagocytosed A β (fig. S2H) and the amount of A β that microglia could phagocytose ($P < 0.0001$) (fig. S2I).

Xe inhalation suppresses the microglia inflammatory phenotype associated with reduced AD pathology and neuritic dystrophy in APP/PS1 mice

To evaluate whether Xe inhalation has a protective immunomodulatory role in restoring homeostatic microglial functions in AD, we established a protocol to treat APP/PS1 mice. We initiated treatment with Xe at 2 months of age (at the onset of amyloid deposition) and continued weekly 40-min treatments for a total of 8 weeks. Notably, these APP/PS1 mice started developing amyloid plaques at 6 weeks

of age, with saturation around 8 months (3). In each group, we divided the animals into Xe (30%) treatment or atmospheric air inhalation, which served as the control group. We isolated FCRLS⁺ microglia by fluorescence-activated cell sorting (FACS) and performed RNA-seq analysis. Xe inhalation suppressed the classical MGnD genes *Clec7a*, *Clu*, *Lgals3*, and *Apoe*. Moreover, homeostatic genes were up-regulated, including *Nr3c1*, encoding a glucocorticoid receptor, and *Spi1* (PU.1), which is essential to maintain the homeostatic signature of microglia (Fig. 1A) (21). With a decrease in MGnD signature gene expression, IPA showed enrichment in pathways correlating with neuron protection and a reduction in pathways associated with inflammation and oxidative stress (Fig. 1, B and C). IPA and histopathology analysis showed that Xe inhalation enriched synaptogenesis genes in microglia, which decreased Lamp1⁺ dystrophic neurites ($P = 0.0026$) (Fig. 1, C to E). We also confirmed decreased expression of *Clec7a*⁺ ($P = 0.0319$), a surface marker of MGnD (3) and A β plaque accumulation ($P = 0.0452$) (Fig. 1, F and G). Moreover, the number and size of A β plaques presenting Lamp1⁺ dystrophic neurites decreased, particularly those of a larger size ($P = 0.0245$) (Fig. 1, H and I).

Although we showed that Xe inhalation suppressed MGnD and restored M0 microglia in acute and chronic neurodegeneration mouse models, we did not address whether Xe treatment directly modulated microglia. We previously found that granulocyte-macrophage colony-stimulating factor (GM-CSF) induced several MGnD molecules in adult microglia in vitro, including *Il1b* and *Clec7a* (20). To evaluate the direct effects of Xe on microglia, we cultured adult mouse microglia for 5 days with the following: (i) M-CSF (10 ng/ml) with human TGF β (50 ng/ml) to induce a homeostatic signature or (ii) GM-CSF (10 ng/ml) for MGnD differentiation (20). On day 6, all cytokines were removed after Xe exposure for 24 hours. RNA-seq analysis confirmed the ability of Xe to suppress the neuroinflammatory response directly in GM-CSF-induced MGnD-like microglia, including the suppression of *Il1b*, *Tnf*, *Nfkb1*, and *Ier3* (Fig. 1J). Moreover, Xe treatment suppressed the MGnD-related genes—including *Clec7a*, *Ccl2*, and *Tnf*—and induced the expression of regulators of M0 microglia, such as *Sall1* and *Tgfb β 2* (Fig. 1K).

Xe inhalation induces protective MGnD during early stages of AD in mice

The kinetics experiments showed increased expression of genes related to MGnD, including *Clec7a*, at 7 days after Xe treatment (fig. S2E). Histological analysis confirmed this increase at the protein level ($P = 0.0014$) (Fig. 2, A to C). To evaluate whether this microglial transition was beneficial for controlling A β accumulation and dystrophic neurites, we generated a *Clec7a*-Cre^{ERT2} mouse model capable of targeting *Clec7a*⁺ MGnD. Because *Clec7a* is specifically expressed in MGnD and not by any other neural cells in the brain (3), the Cre-recombinase will only be active in *Clec7a*⁺ MGnD, enabling us to track and deplete MGnD in AD mice specifically. To validate the specificity of our mouse model for targeting MGnD, we crossed *Clec7a*-Cre^{ERT2}:ROSA26^{TdTomato} mice with APP/PS1 mice. At 3 months of age, the animals were treated with 75 mg of tamoxifen/kg of body weight. One month later, the animals were euthanized, and their brains were evaluated. We found that TdTomato expression was specific to microglia coexpressing *Clec7a* associated with A β plaques (fig. S3, A and B). To confirm the MGnD signature

of these TdTomato⁺ cells, we quantified their cytokines by flow cytometry. We found that TdTomato⁺ microglia exhibited increased expression of major histocompatibility complex class II (MHC-II) ($P = 0.0271$), GM-CSF ($P < 0.0001$), and TNF α ($P < 0.0001$), whereas latency-associated peptide (LAP) (TGF β signaling) was decreased ($P < 0.0001$) (fig. S3C). RNA-seq analysis confirmed the increased expression of MGnD genes, including *Apoe*, *Spp1*, *Gas7*, and *Axl*, and reciprocal suppression of homeostatic genes, such as *Tmem119*, *P2ry12*, *Cx3cr1*, and *Csf1r* (fig. S3D). Gene Ontology (GO) analysis corroborated this profile, predicting increased glial cell differentiation, antigen presentation, and immune response (fig. S3E). Along with advanced age and the APOE4 genotype, females have a higher risk of developing late-onset AD compared with their male counterparts (22). In concordance with this, APP/PS1 female mice have increased soluble and insoluble A β compared with males (23). We observed decreased expression of *Clec7a*-TdTomato microglia in male mice at 4 months of age compared with females ($P < 0.0001$) (fig. S3, F and G). Histological analysis showed that this difference was associated with a decreased load of A β plaques and Lamp1⁺ dystrophic neurites in males at this time point (fig. S3H). Linear regression analysis confirmed that TdTomato⁺ microglia positively correlated with A β accumulation ($P < 0.0001$) (fig. S3I) and dystrophic neurites (fig. S3J) ($P < 0.0001$).

To determine how specific ablation of *Clec7a*⁺ MGnD affected disease progression and pathology, we crossed APP/PS1:*Clec7a*-Cre^{ERT2} mice with ROSA26-DTR^{Flox} mice. In this mouse model, the diphtheria toxin receptor (DTR) was specifically expressed in MGnD, allowing for the ablation of this population upon administration of diphtheria toxin. We initiated treatment of APP/PS1:*Clec7a*-Cre^{ERT2}:ROSA-DTR^{Flox} mice with tamoxifen and diphtheria toxin at 2.5 months of age. The treatment consisted of 5 days of tamoxifen followed by 3 days of diphtheria toxin repeated three times in a total of 3.5 weeks. Flow cytometry showed reduced numbers of *Clec7a*⁺ microglia ($P = 0.0067$) (Fig. 2, D and E). Immunohistochemical staining for *Clec7a*⁺/Iba1⁺ microglia confirmed this reduction ($P = 0.0276$) (Fig. 2, F to H). Genetic ablation of *Clec7a*⁺ MGnD resulted in increased Lamp1⁺ dystrophic neurites ($P = 0.0380$) and APOE ($P = 0.0116$) accumulation associated with A β plaque deposition (Fig. 2, G and H). However, after 4 weeks of continuous treatment, the APP/PS1:*Clec7a*-Cre^{ERT2}:ROSA-DTR^{Flox} mice showed a decrease in survival, suggesting that *Clec7a*-Cre^{ERT2} might be affecting other cells. We found high expression of *Clec7a*-TdTomato in different tissues, specifically in the lung and gut tissues, even without disease (fig. S3, K and L), and more specifically in myeloid cells, such as monocytes and neutrophils (fig. S3M). Evaluation of *Clec7a* expression in different myeloid cells showed that 100% of alveolar macrophages expressed *Clec7a*-TdTomato (fig. S3N). To specifically address the role of *Clec7a*⁺ MGnD in AD mice, we transplanted microglia isolated from *Clec7a*-Cre^{ERT2}:DTR^{Flox} mice (2 months old) into 5xFAD:FIRE (CSF1r-KO) mice at postnatal stage P1 to P3, which lack microglia. For a control, heterozygous mice of the *fms*-intronic regulatory element (FIRE) genotype were used as recipients, because they maintain host microglia (24). At 2.5 months of age, we injected these mice with diphtheria toxin. We validated the *Clec7a*⁺ cell depletion by flow cytometry ($P = 0.0278$) (Fig. 2, I and J) and in situ ($P = 0.0010$) (Fig. 2, K and L). This microglial depletion increased dystrophic neurites ($P = 0.0261$) (Fig. 2, M and N), confirming that MGnD depletion was detrimental and hastened disease onset.

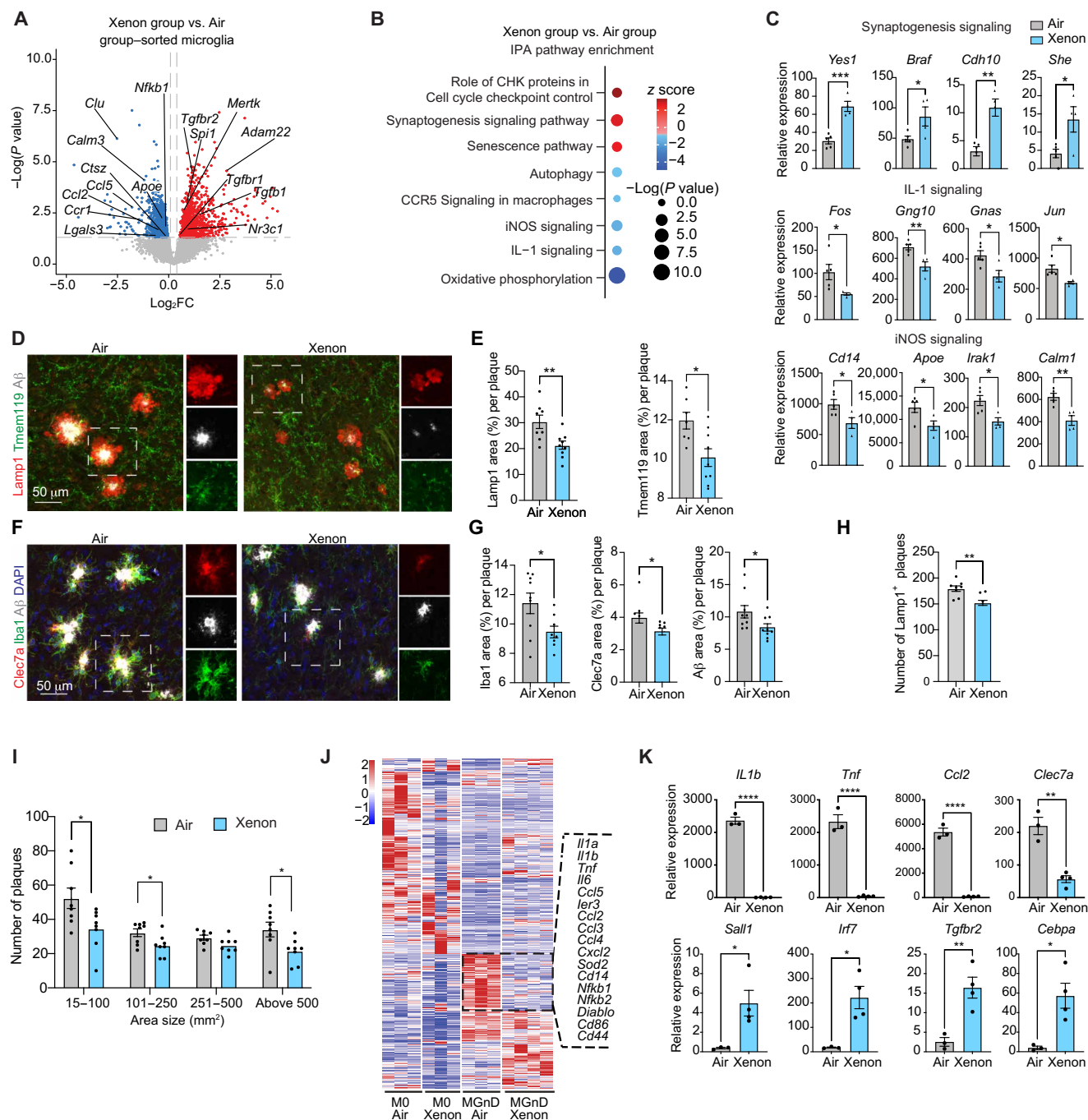
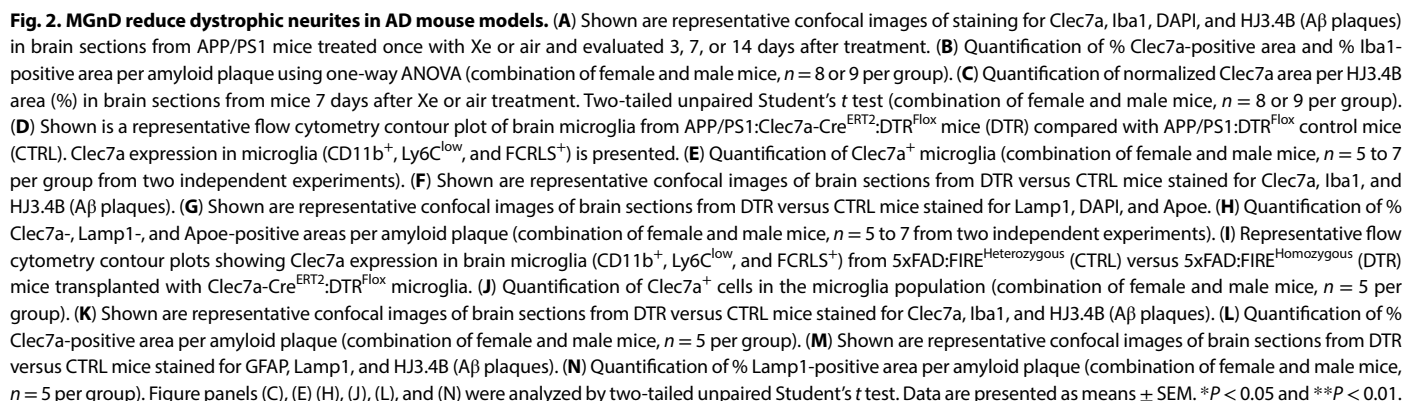


Fig. 1. Xe inhalation suppresses microglia with an inflammatory phenotype and reduces AD pathology and neuronal dystrophy in APP/PS1 mice. (A) Volcano plot of DEGs between microglia isolated from Xe-treated or air-treated APP/PS1 mice ($n = 5$ female mice per group). (B) Top-affected pathways from IPA of microglia from Xe versus air groups are shown. (C) Shown are normalized data for expression of key affected genes in microglia from the Xe versus air groups; genes affected included those associated with synaptogenesis, IL-1 signaling, and iNOS signaling pathways ($n = 5$ female mice per group). (D) Shown are representative confocal images of staining for Lamp1, Tmem119, and HJ3.4B (A β plaques) in brain sections from APP/PS1 mice treated with Xe or air. (E) Quantification of % Lamp1-positive area per amyloid plaque is shown ($n = 8$ or 9 female mice per group, from three independent experiments). (F) Shown are representative confocal images of staining for Clec7a, Iba1, 4',6-diamidino-2-phenylindole (DAPI), and HJ3.4B (A β plaques) in brain sections from APP/PS1 mice treated with Xe or air. (G) Quantification of % Clec7a-positive area, % Iba1-positive area, and % HJ3.4B (A β plaques)-positive area per amyloid plaque is presented ($n = 8$ or 9 female mice per group, from three independent experiments). (H) Quantification of the number of cortical A β plaques expressing Lamp1 in the brains of APP/PS1 mice exposed to Xe or air is shown ($n = 8$ or 9 female mice per group, from three independent experiments). (I) Quantification of the number of cortical A β plaques categorized by size in the brains of APP/PS1 mice exposed to Xe or air is shown ($n = 8$ or 9 female mice per group, from three independent experiments). (J) Heatmap of top DEGs affected by Xe inhalation; the dashed line represents the differences between the MGnD Xe versus MGnD air groups ($n = 3$ or 4 wells per group, $P \text{ value} < 0.05$). (K) Normalized data are shown for the expression of key affected genes associated with a homeostatic and neurodegenerative signature in MGnD ($n = 3$ or 4 wells per group). Data are presented as means $\pm$ SEM. Two-tailed unpaired Student's t test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.



Xe inhalation suppresses proinflammatory cytokines and promotes interferon responses in a pre-MGnD subset in APP/PS1 mice

To determine how Xe modulates microglia and its subtypes, we performed single-cell RNA-seq (scRNA-seq) of CD45⁺/CD11b⁺ myeloid

cells isolated from Xe-treated or air-treated APP/PS1 female mouse brains at 4 months of age. scRNA-seq revealed 13 unsupervised clusters (fig. S4, A and B), which were annotated according to microglia activation (M0, pre-MGnD, and MGnD), cell cycle, and border-associated macrophages (Fig. 3, A to D, and fig. S4C). Border-associated macrophages

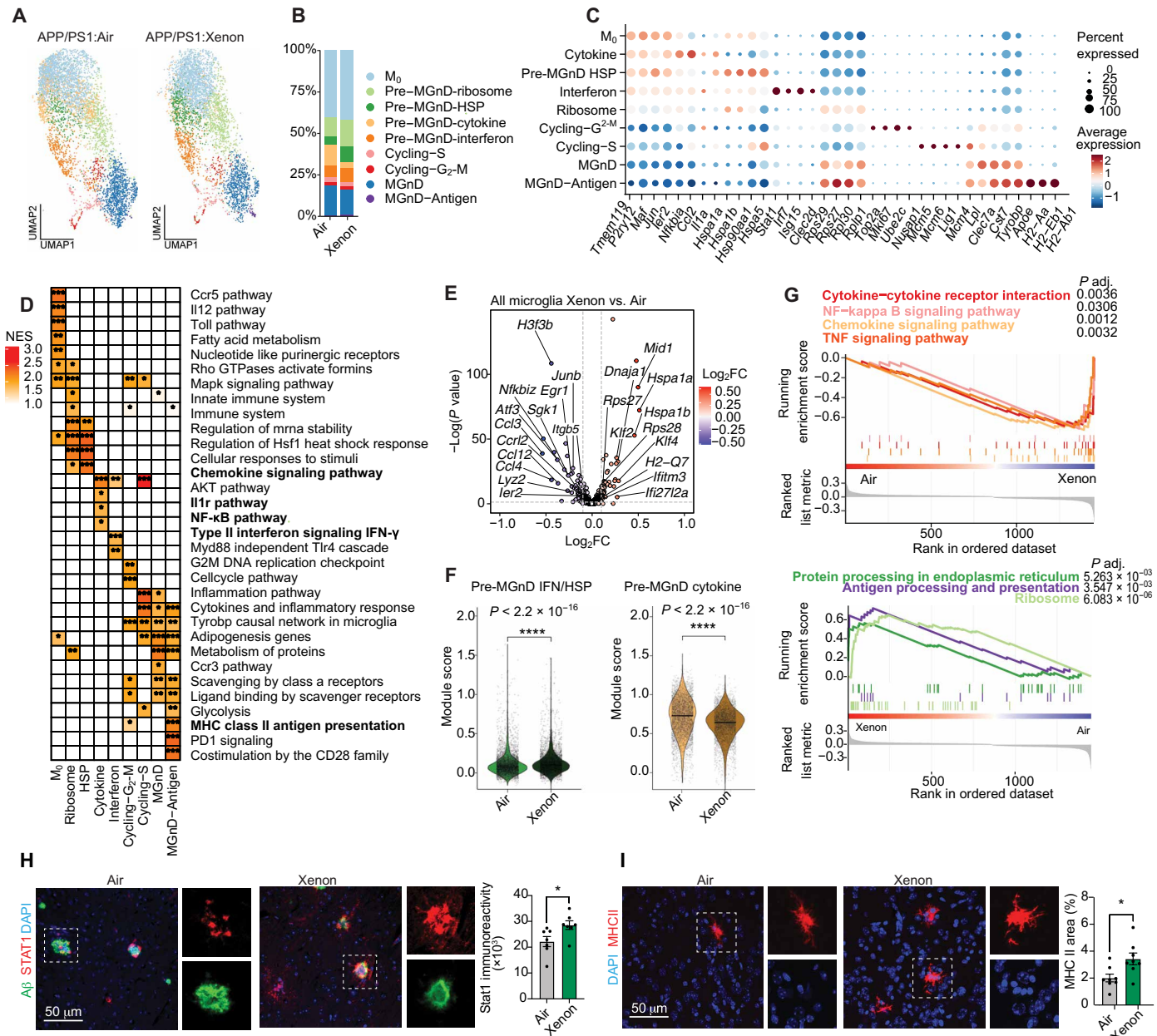


Fig. 3. Xe inhalation suppresses proinflammatory cytokine production and promotes an interferon response in subsets of pre-MGnD. (A) Shown is a UMAP of identified brain microglia cell states in APP/PS1 mice at 4 months of age after 2 months of weekly Xe or air treatment ($n = 2$ female mice per group). (B) Relative percentage of microglia subcluster states is shown. (C) Marker gene expression was determined for all microglia subcell states [$P < 0.05$, false discovery rate (FDR) corrected using Benjamini-Hochberg]. (D) KEGG pathway enrichment across all microglia subcluster states is shown (* $P < 0.05$, ** $P < 0.025$, and *** $P < 0.01$). (E) Volcano plot of DEGs from all microglia between Xe-treated and air-treated APP/PS1 mice is shown ($P < 0.05$). (F) Gene module enrichment for cytokine and interferon responses of pre-MGnD subsets is shown. (G) Gene set enrichment analysis (GSEA) pathway enrichment of DEGs for all microglia is shown ($P < 0.05$, FDR corrected using Benjamini-Hochberg). (H) Shown are representative confocal images of and quantification of Stat1 and H3.4B (A β) plaques in brain sections from APP/PS1 mice treated with Xe or air ($n = 8$ or 9 female mice per group, from three independent experiments). (I) Representative confocal images of and quantification of MHC-II in brain sections from APP/PS1 mice treated with Xe or air are shown ($n = 8$ or 9 female mice per group, from three independent experiments). Data are presented as means $\pm$ SEM. Two-tailed unpaired Student's t test, * $P < 0.05$.

were assigned on the basis of the established marker genes *Mrc1* and *Clec12a* (fig. S4B) (25, 26). Overall, pre-MGnD down-regulated M0 genes (*Tmem119* and *P2ry12*). Moreover, certain activation genes were up-regulated in comparison with M0 microglia: (i) pre-MGnD-cytokine had up-regulated *Ccl2*, *Il1a*, and *Nfkbia*; (ii) pre-MGnD-ribosome showed enrichment of ribosomal genes *Rps29*, *Rps27*, and *Rpl30*; (iii) pre-MGnD-interferon had up-regulated *Isg15*, *Stat1*, and *Ifit3*; and (iv) pre-MGnD-heat shock protein (HSP) was enriched for HSP genes (Fig. 3C). Pathway analysis further defined the microglia subsets (Fig. 3D). Xe treatment predominantly affected the proportions of the pre-MGnD populations (Fig. 3B). The pre-MGnD-cytokine phenotype was reduced, whereas the interferon, HSP, and ribosome pre-MGnD phenotypes were increased. M0 microglia increased marginally, whereas MGnD were reduced (Fig. 3B). Detailed investigation of the MGnD cluster revealed a small population showing enrichment of antigen-presenting genes, such as *H2-Aa*, *H2-Eb1*, and *H2-Ab1*, and MHC-II signaling, which only appeared after Xe treatment (Fig. 3, C and D). The change in microglia state proportions after Xe treatment was reflected in the overall transcriptomic shift, with up-regulation of interferon, HSP, and ribosome genes as well as antigen-presenting genes (*Hspa1a*, *Rps27*, *Ifi2712a*, and *H2-Q7*) and down-regulation of proinflammatory genes (*Ier2*, *Ccl3*, *Ccl4*, and *Ccr12*) and core NF- κ B signaling regulators (*Sgk1* and *Nfkbiz*) (Fig. 3E). Gene module analysis (top 50 enriched genes per cluster, adjusted $P < 0.05$) showed that the interferon and HSP pre-MGnD gene module was enriched, whereas the pre-MGnD-cytokine gene module was decreased after Xe inhalation (Fig. 3F). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment showed concordant down-regulation of cytokine signaling, NF- κ B signaling, and TNF α signaling. At the same time, protein processing, ribosome activity, and antigen presentation were up-regulated (Fig. 3G). To validate these populations in situ, we determined the expression of signal transducers and activators of transcription 1 (*Stat1*) (pre-MGnD-interferon) and MHC-II (MGnD-antigen) protein expression in the brains of APP/PS1 mice treated with Xe. We confirmed that Xe treatment increased the expression of *Stat1* ($P = 0.0285$) and MHC-II ($P = 0.0159$) (Fig. 3, H and I).

To investigate potential changes in microglia transition from M0 to MGnD after Xe treatment, we conducted a pseudotime trajectory analysis and identified two lineages of transition. Lineage-1 showed transition via the pre-MGnD-ribosome cluster, whereas Lineage-2 suggested microglia initially through the pre-MGnD-HSP state, followed by the pre-MGnD-interferon state toward the MGnD phenotype. We observed that Xe treatment accelerated Lineage-2 transition toward the pre-MGnD-interferon state with earlier up-regulation of *Stat1*. Lineage-1 demonstrated accelerated up-regulation of the MGnD gene *Clec7a* compared with Lineage-2. Furthermore, Xe treatment promoted Lineage-2 transition toward MGnD similar to Lineage-1 but with a final stage of MGnD-antigen (fig. S4, D and E). These results show that Xe treatment promoted a shift of microglia activation from neuroinflammatory cytokine activation toward interferon-mediated activation.

Xe inhalation enhances IFN- γ signaling and suppresses the inflammatory phenotype in human microglia in humanized 5xFAD mice

We next examined whether Xe treatment similarly affected human microglia. We selected human iPSC-derived microglia (iMG) expressing green fluorescent protein (GFP⁺) (27). iMG were generated using a method that produced >97% pure cultures that expressed

common microglial genes (fig. S5A) and exhibited high similarity to cultured adult and fetal human microglia (28). Similar to our experiment with mouse microglia cultures, Xe-treated iMG showed a decrease in the expression of proinflammatory genes correlated with neuroinflammation and reactive oxygen species, such as *IL3RA*, *CCL1*, *CSF2*, and *IRAK3* (fig. S5, B and C). IPA indicated enrichment in cholesterol biosynthesis pathways, including liver X receptor and PPAR-related responses, and the suppression of neuroinflammation by Xe treatment (fig. S5D).

To evaluate whether Xe inhalation could also modulate human microglia in vivo, we transplanted human iMG progenitor cells into early postnatal (P1) immunodeficient 5xFAD-MITRG (5x-MITRG) mice (19). Previous studies have shown that this approach leads to the widespread engraftment of human iMG, which exhibit morphological, functional, and transcriptional properties similar to human biopsy-derived ex vivo microglia (26, 29–31). Recent studies have shown that at 4 months of age, approximately 5% of human microglia have adopted an MGnD-like transcriptional signature and that by 6 months of age, when amyloid plaques are more prevalent, 12 to 15% of human microglia express an MGnD signature (27). Thus, we initiated Xe treatment (Xe, 30%; 40 min once a week) at 4 months of age and continued treatment once a week until 6 months of age. We confirmed that iMG progenitor cell transplantation led to robust forebrain engraftment of GFP-expressing human microglia (Fig. 4A), which were isolated by sorting for CD11b⁺/GFP⁺ cells (fig. S5E). Moreover, these transplants into transgenic 5x-MITRG mice allowed for close examination of interactions among human microglia, mouse microglia, and A β plaques (Fig. 4B). The evaluation of Xe's effects showed no differences in the percentage and numbers of engrafted iMG by flow cytometry ($P = 0.6729$; 0.9329) (fig. S5, F and G) and histology ($P = 0.4315$) (Fig. 4C). We found that Xe inhalation reduced A β plaque load ($P = 0.0338$) (Fig. 4, D and E) and dystrophic neurites in the cortex ($P = 0.0294$) (Fig. 4, F and G). In addition, it decreased the number and size of A β plaques in both the cortex ($P = 0.04938$) (Fig. 4H) and hippocampus ($P = 0.0070$; 0.0016) (Fig. 4I), including those containing dystrophic neurites ($P = 0.0083$; 0.0153) (Fig. 4, J and K). RNA-seq analysis showed that Xe suppressed MGnD-related genes, including *CLEC7A* and *ITGAM* in human microglia, whereas genes associated with microglial migration, including *CXCL10* and *ICAM1*, and IFN- γ signaling (*ISG15*, *EIF2K2*, and *IFI27*), were induced (Fig. 4L). IPA showed the role of RNA-activated protein kinase in interferon induction and antiviral response as the major pathway induced by Xe (Fig. 4M), which can be activated by IFN- γ (32). Furthermore, upstream regulator analysis predicted the activation of STAT1 as a microglial regulator (Fig. 4N), which was validated by protein quantification ($P = 0.0023$) (Fig. 4, O and P), aligning with mouse microglia single-cell analysis (Fig. 3C).

Astrocytes also respond to neurodegeneration and are associated with A β plaques in AD animal models (26). Moreover, reactive astrocytes were shown to play a beneficial role in limiting AD pathology. Astrocyte depletion in AD mice increases plaque load, synaptic dysfunction, and memory loss (33). To evaluate whether Xe affects astrocytes, we isolated astrocytes from 5x-MITRG mice and performed a Smartseq2 analysis. Our results showed that astrocytes suppressed the expression of genes associated with AD pathology (*App* and *Sod3*) and inflammation (*Ccl19*, *Casp8*, and *Il13ra1*) but maintained some activation signature with an increase in *Serpina3a* and *Gfap* (Fig. 4Q), which was confirmed by immunofluorescence analysis ($P = 0.0314$)

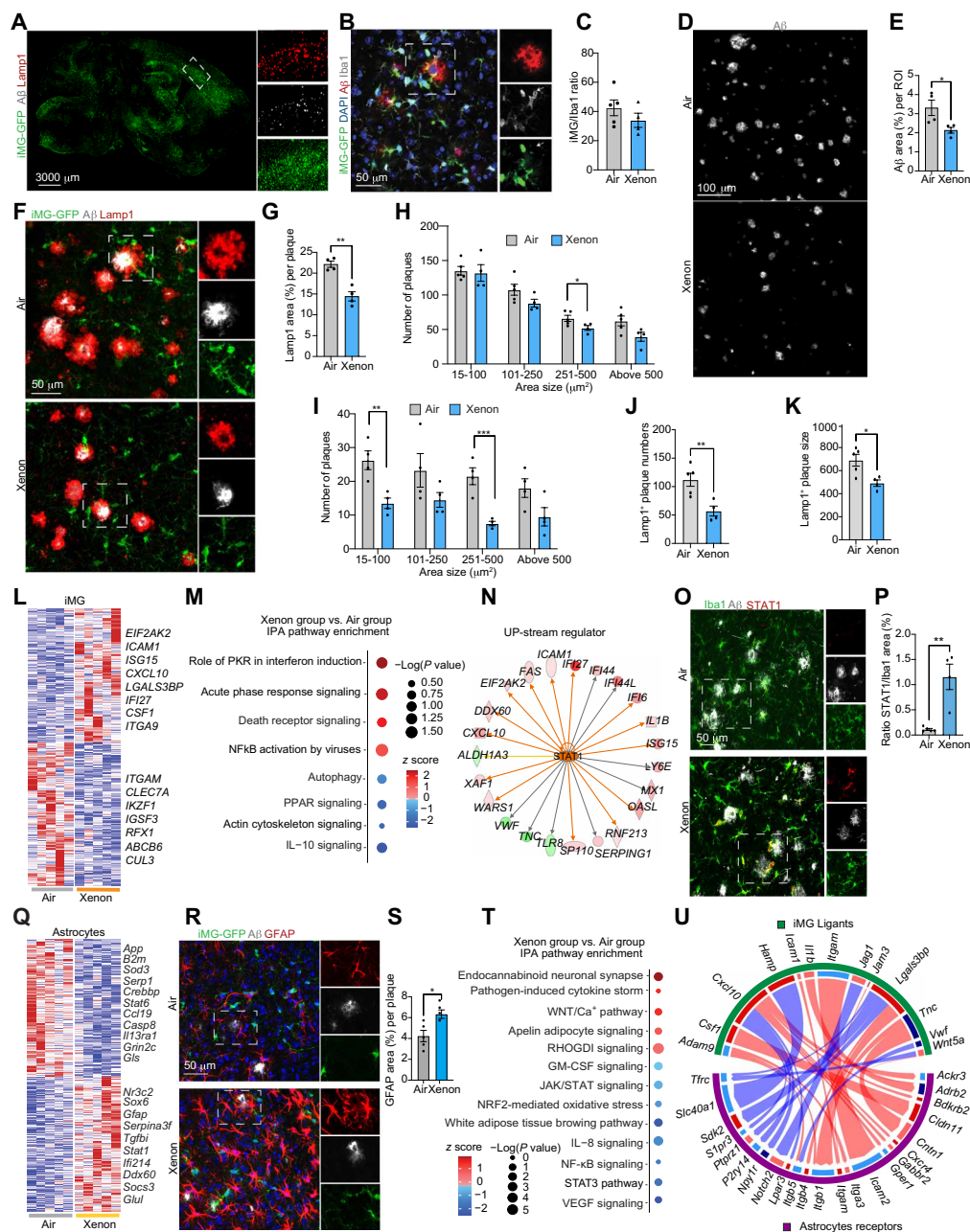


Fig. 4. Xe inhalation enhances IFN- γ signaling and suppresses an inflammatory phenotype in human microglia associated with AD pathology in 5x-MITRG mice.

(A) Representative image of human iMG distribution in the brains of 5x-MITRG mice at 6 months of age. White square indicates magnified region of interest (ROI) and colocalization of iMG with Lamp1 and 6E10 (A β plaque) staining. (B) Representative image of iMG and mouse microglia (GFP⁺ and Iba1⁺, white arrow) interacting with A β plaques (HJ3.4B) in the brains of 5x-MITRG mice at 6 months of age. (C) Percentage of iMG in relation to total microglia in the cortices of 5x-MITRG mice treated with Xe or air weekly for 2 months. (D) Representative confocal images of A β plaques (stained with HJ3.4B) in the cortices of 5x-MITRG mice treated with Xe or air. (E) Quantification of the size of HJ3.4B (A β plaques)-positive area per ROI, normalized per animal ($n = 4$ or 5 female mice per group). (F) Representative confocal images of cortical staining for Lamp1, A β plaques (HJ3.4B), and iMG in 5x-MITRG mice treated with Xe or air. (G) Quantification of % Lamp1-positive area per amyloid plaque normalized per animal ($n = 4$ or 5 female mice per group). (H) Quantification of the number of cortical A β plaques divided by size ($n = 5$ female mice per group). (I) Quantification of the number of hippocampal A β plaques divided by size ($n = 5$ female mice per group). (J) Number of hippocampal plaques expressing Lamp1 ($n = 5$ female mice per group). (K) Quantification of the size of hippocampal plaques expressing Lamp1 ($n = 5$ female mice per group). (L) Heatmap shows top DEGs in the iMG populations isolated from 5x-MITRG mice treated with Xe versus air ($n = 5$ female mice per group, $P < 0.05$). (M) Top-affected pathways from IPA using DEGs from the Xe versus air comparison of iMG populations isolated from 5x-MITRG mice. (N) Schematic showing STAT1 (center) as an upstream regulator in the IPA pathways. (O) Representative confocal images of staining for HJ3.4B (A β plaques), iMG, and Stat1 in the cortices of 5x-MITRG mice treated with Xe or air. (P) Quantification of % Stat1 area/Iba1⁺ cells normalized per animal ($n = 4$ or 5 female mice per group). (Q) Heatmap shows DEGs in astrocytes isolated from 5x-MITRG mice treated with Xe or air ($n = 5$ female mice, $P < 0.05$). (R) Representative confocal images of staining for GFAP, iMG, and A β plaques (HJ3.4B) in the cortices of 5x-MITRG mice treated with Xe or air. (S) Quantification of GFAP immunoreactivity per amyloid plaque normalized per animal ($n = 4$ or 5 female mice per group). (T) Top-affected pathways from IPA using DEGs from brain astrocytes isolated from 5x-MITRG mice treated with Xe or air ($n = 5$ female mice per group). (U) Circos plot illustrates prioritized cross-talk between microglia ligands and astrocyte receptors. Regulatory potential demonstrates increased (red) or reduced (blue) ligand-receptor cross-talk upon Xe inhalation. Data are means $\pm$ SEM. Two-tailed unpaired Student's t test. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

(Fig. 4, R and S). Similar to engrafted iMG, the IFN- γ response was up-regulated in astrocytes, with an increase in *Stat1*, *Ifi214*, and *Ddx60* gene expression (Fig. 4Q). IPA indicated a reactive state in astrocytes, including cytokine storm, WNT/calcium, and apelin adipose tissue pathways, but the suppression of NF- κ B, GM-CSF, and STAT3 signaling (Fig. 4T). To determine whether Xe inhalation affects microglia-astrocyte cross-talk, we analyzed ligand-receptor interactions. NichNetR analysis identified *CXCL10* and *LGALS3BP* as the top microglial ligands interacting with astrocyte receptors induced by Xe inhalation (Fig. 4U). *LGALS3BP* and *CXCL10* have been reported to be IFN-stimulated genes (34). These findings support the beneficial effect of Xe as a treatment for A β pathology by modulating microglia and astrocytes via induction of the IFN- γ response.

To further investigate whether the effect of Xe was microglia dependent, we depleted brain myeloid cells in 5xFAD mice using

PLX-5622 (Plexicon), a CSF1R inhibitor (35). We administered Plexicon in chow to 5xFAD mice at 4 months of age and continued treatment until the end of the experiment. One week after Plexicon administration, we initiated Xe treatment once a week for 8 weeks, following the same protocol as with 5x-MITRG mice. Flow cytometry analysis confirmed a fourfold reduction in total myeloid cells in the brain ($P < 0.0001$) (Fig. 5, A and B). Moreover, histopathology analysis confirmed decreased Iba1⁺ microglia surrounding A β plaques ($P = 0.0012$) (Fig. 5, C and D). Furthermore, Xe inhalation reduced A β plaque accumulation ($P = 0.0463$) and dystrophic neurites ($P = 0.0437$) in the cortices of these animals, an effect that was lost when brain myeloid cells were depleted ($P = 0.0210$) (Fig. 5, E and F). Despite its effects on the cortex, Xe treatment did not alter the soluble and insoluble A β concentration in the total mouse brain (Fig. 5, G and H).

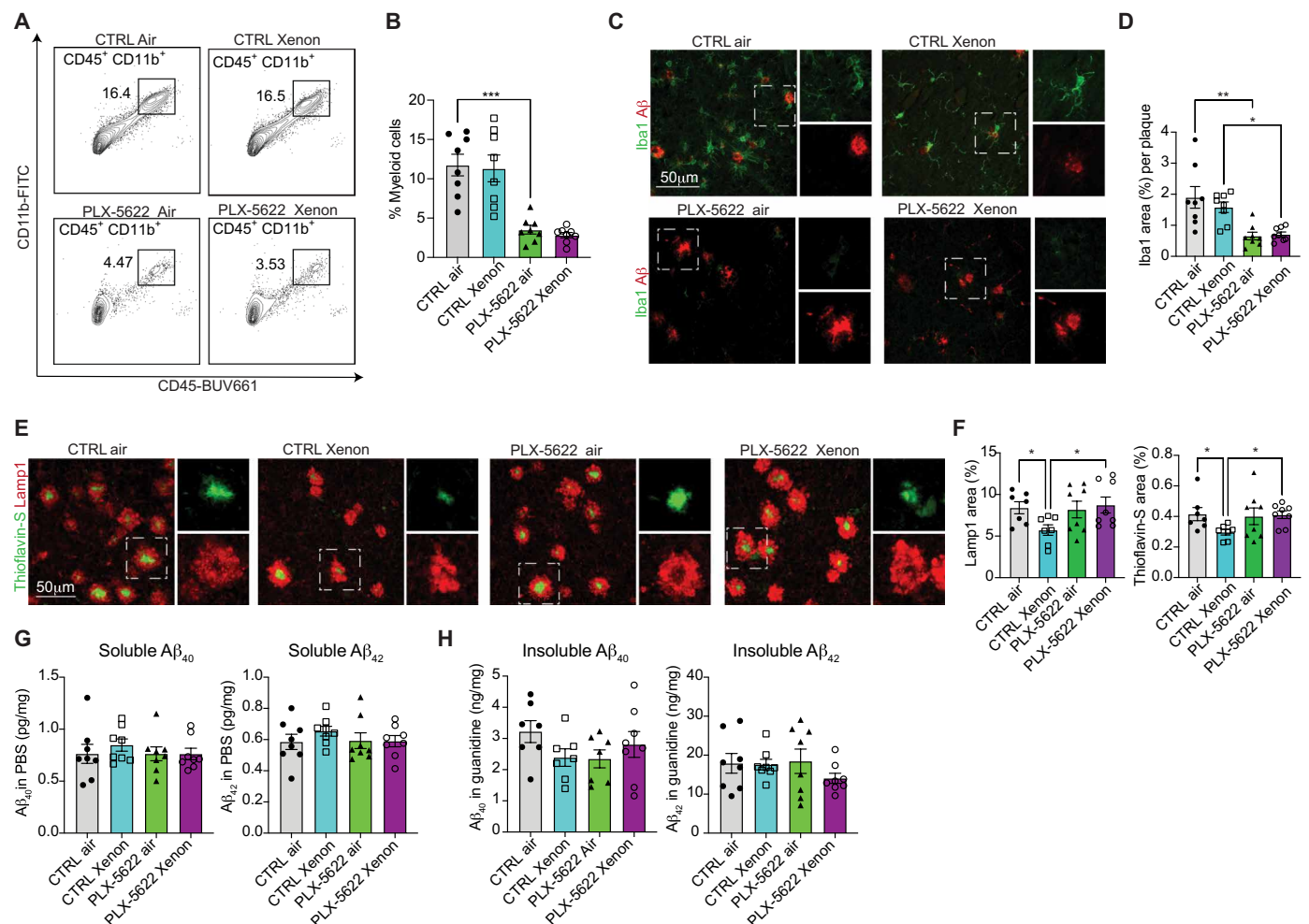


Fig. 5. Brain myeloid cells are necessary for the beneficial effect of Xe. (A) A representative flow cytometry contour plot shows the percentages of myeloid cells (CD45⁺ and CD11b⁺) in the brains of 5xFAD mice fed PLX-5622 (to deplete microglia) treated with Xe or air for 40 min weekly between 4 and 6 months of age. (B) Shown is the percentage of myeloid cells after PLX-5622 and Xe or air treatment. One-way ANOVA ($n = 8$ female mice per group). (C) Representative confocal images of staining for A β plaques (HJ3.4B) and Iba1 in the cortices of 5xFAD mice treated with PLX-5622 and Xe or air. (D) Quantification of % Iba1-positive area per amyloid plaque, normalized per animal ($n = 8$ female mice per group). (E) Representative confocal images of thioflavin-S (A β plaques) and Lamp1 staining in the cortices of 5xFAD mice treated with PLX-5622 and Xe or air. (F) Quantification of % Lamp1- and thioflavin-S (A β plaques)-positive areas per ROI, normalized per animal ($n = 8$ female mice per group), one-way ANOVA. (G and H) Quantification of soluble (G) and insoluble (H) A β ₄₀ and A β ₄₂ from total brains of 5xFAD mice treated with PLX-5622 and Xe or air ($n = 8$ female mice per group). Data are presented as means $\pm$ SEM. One-way ANOVA. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Blocking IFN- γ signaling impairs the Xe modulatory effect on microglia in an acute neurodegeneration mouse model

To identify possible mechanisms by which Xe modulates microglial responses in the context of AD, we reanalyzed our results to evaluate whether Xe affected glutamate ionotropic receptors (fig. S6A), which have been reported to be suppressed by Xe treatment (7). However, we did not find statistical differences in the expression of glutamate receptor genes in microglia from APP/PS1 (fig. S6B) and 5xFAD mice (fig. S6C), suggesting that Xe operates through a different mechanism.

Xe treatment consistently induced the IFN response in microglia across different mouse models (Figs. 3 and 4). Previous reports showed that higher concentrations of IFN- γ in plasma were associated with slower cognitive decline, less tau accumulation, and less neurodegeneration in patients with AD (36). Moreover, IFN- γ overexpression in 3xTg-AD mice diminished phospho-tau pathology (37). Because both mouse and human microglia showed augmentation of genes downstream of interferon signaling, we sought to investigate how IFN- γ signaling regulates the transition from M0 to MGnD. To address this question, we injected fluorescently labeled apoptotic neurons into the cortices and hippocampi of C57BL/6 mice previously treated with an IFN- γ neutralizing antibody or an immunoglobulin G (IgG) isotype control followed by Xe inhalation and microglia isolation (fig. S7A). IFN- γ quantification confirmed depletion of systemic cytokine ($P = 0.0009$) after IFN- γ neutralizing antibody treatment (fig. S7B). RNA-seq analysis showed that systemic blockade of IFN- γ before Xe treatment suppressed expression of microglial IFN- γ -related genes (*Cebpb*, *Cxcl9*, and *Akt2*) while increasing the proinflammatory response by up-regulation of *Ccr2*, *Ccr5*, *Irak2*, and *Mapk9* (fig. S7C). IFN- γ blockade impaired the up-regulation of genes associated with glial cell differentiation, cell motility, and protein maturation commonly induced by Xe inhalation (fig. S7D). IPA showed that IFN- γ blockade impaired the suppression of macrophage classical activation signaling and IL-15 production by Xe, whereas neuroinflammation signaling and IL-1-mediated inhibition of RXR function were increased (fig. S7E). These results suggest that IFN- γ signaling was necessary for Xe activity in the microglial response to phagocytosis of apoptotic neurons.

To confirm the IFN- γ effect exclusively in microglia, we repeated the experiment in Cx3cr1-Cre^{ERT2}*Ifngr1*^{Flox} (*Ifngr1*-cKO) mice treated with tamoxifen to delete *Ifngr1* 1 month before injection of apoptotic neurons. This approach limited the *Ifngr1* deletion to long-lived cells such as microglia and border-associated macrophages. Fourteen hours after Xe inhalation, phagocytic and nonphagocytic microglia were isolated for RNA-seq analysis. The suppression of *Ifngr1* was confirmed by gene expression ($P = 0.0006$) (Fig. 6A) associated with IFN- γ -related genes such as *Vamp3*, *Ifi208*, and *Eif5a* (Fig. 6, B and C). Approximately 70% of DEGs modulated by Xe were lost in *Ifngr1*-cKO mice, including the suppression of proinflammatory regulators, such as *Stat3*, *Nrp1*, and *Thr9*, and immune checkpoint genes, such as *Cd274* (programmed death-ligand 1, PD-L1) and *Inpp5d* (Fig. 6C) (38). We found that Xe-mediated IFN- γ signaling was responsible for regulation of leukocyte differentiation and mitochondrial organization, which contributed to control of the cellular response to TNF. At the same time, Xe-mediated IFN- γ signaling reduced NF- κ B activity, cytokine-mediated pathway signaling, and negative regulation of motility (Fig. 6, D and E). IPA revealed mitochondrial function as the most affected, followed by the induction of genes related to inflammation and pyroptosis (Fig. 6F).

These data show that IFN- γ signaling was essential for regulating the effects of Xe on microglia during acute neurodegeneration.

Given that accessing pathological changes in our acute mouse model was not feasible, we evaluated the effects of an IFN- γ blocker in mice with amyloid deposition. We treated APP/PS1 mice at 6 months of age once a week for 2 weeks with an IFN- γ -neutralizing antibody, administered 24 hours intraperitoneally before Xe exposure. After 14 days, we euthanized the animals and assessed microglial signatures and disease pathology. Our findings revealed that blocking IFN- γ increased the microglial proinflammatory response, as evidenced by the up-regulation of *Nfkb*, *Il1r1*, and *Cd14* (Fig. 6G), and pathways related to granzymes and TNFR1. This increase in microglial proinflammatory response was associated with the suppression of IFN- γ -related pathways, such as T helper 1 (T_H1) pathways, through an increase in suppressor of cytokine signaling 1 (SOCS1) (Fig. 6, H and I). Moreover, histological analysis showed increased A β plaque deposition ($P = 0.0497$) (Fig. 6, J and K) and dystrophic neurites ($P = 0.0469$) (Fig. 6, L and M). The blocking of IFN- γ signaling in response to acute Xe exposure decreased Clec7a⁺ microglia ($P = 0.0284$) (Fig. 6, N and O). This was confirmed by flow cytometry analysis ($P = 0.0097$) (Fig. 6, P and Q) and indicated the role of IFN- γ signaling to induce MGnD in the presence of amyloid plaques (39).

Xe inhalation increases IFN- γ production in CD8 T cells in mice with amyloidosis

Our findings suggest that Xe induced a pre-MGnD intermediate microglial state mediated by IFN- γ signaling. We recently showed that IFN- γ -responsive pre-MGnD restricted neurodegenerative pathology in an amyloidosis mouse model (39). As a systemic treatment, Xe is expected to affect various cell populations, including peripheral immune cells that may influence microglial polarization. Flow cytometry analysis of the APP/PS1 mouse brain revealed a small population of infiltrating immune cells, including T cells (fig. S8, A and B), one of the major sources of IFN- γ (40). However, Xe treatment did not alter the number of cells infiltrating the mouse brain, suggesting that the BBB was not directly affected (fig. S8C). To explore whether Xe can induce IFN- γ signaling in T cells, we conducted a kinetic experiment evaluating the splenic T cell signature in APP/PS1 mice at 3, 7, and 14 days after Xe inhalation (fig. S9A). We found that at 3 and 7 days after inhalation, several genes associated with IFN- γ signaling were increased, including *Stat1*, *Cd74*, *Sirpa*, *Capg*, and *Vamp8* (fig. S9, B and C). Intriguingly, the peak of T cell activation, as indicated by the number of DEGs, preceded that of microglial activation, which peaked at 3 days after inhalation (fig. S9D). IPA showed an increase in T_H1 and IFN- γ pathways, as well as in granzymes and GM-CSF, indicating an increase in the activation of these cells (fig. S9E). However, some pathways showed an opposite effect over time, with endothelial nitric oxide synthase (eNOS) and AMP-activated protein kinase (AMPK) signaling being suppressed and the regulation of IL-2 expression in activated and anergic T lymphocytes up-regulated at 7 days after inhalation (fig. S9E). Upstream regulator analysis indicated an increase in GM-CSF (CSF2) and suppression of SOCS1, corroborating the increase in IFN- γ signaling by Xe at 3 days after inhalation (fig. S9F). PD1 (PDCD1) was predicted to be up-regulated at 7 days after inhalation, correlating with the T cells' anergic response (fig. S9G).

To confirm an increased expression of IFN- γ in T cells during disease progression, we evaluated IFN- γ expression in T cells from

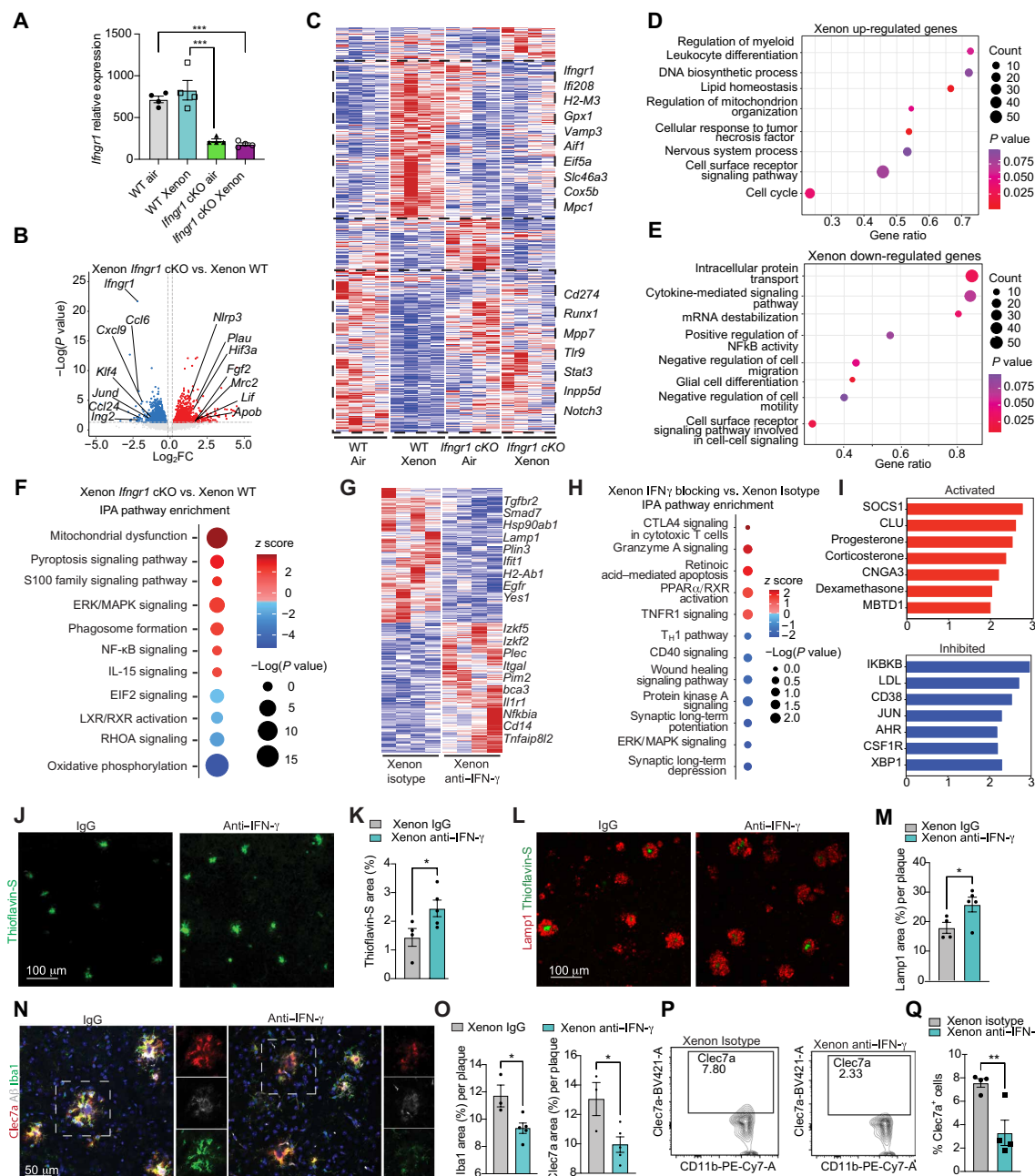


Fig. 6. Blocking IFN- γ signaling impairs the Xe modulatory effect in microglia. (A) Normalized counts of *Ifngr1* gene expression in brain microglia from wild type Cx3cr1-Cre^{WT}:*Ifngr1*^{Flox} mice [wild type (WT)] and *Ifngr1* conditional knockout (Cx3cr1-Cre^{ERT2}:*Ifngr1*^{Flox}) mice (*Ifngr1*-cKO). One-way ANOVA ($n = 4$ female mice per group). (B) Volcano plots of DEGs in phagocytic microglia from *Ifngr1*-cKO versus WT groups treated with Xe ($n = 4$ female mice per group). (C) Heatmap shows phagocytic microglia DEGs induced by Xe and maintained by IFN- γ signaling (black dashed lines). (D) GO pathways induced by Xe treatment. (E) GO pathways suppressed by Xe treatment. (F) Top-affected pathways from IPA based on *Ifngr1*-cKO versus WT groups treated with Xe. (G) Heatmap of gene expression in microglia isolated from the brains of APP/PS1 mice treated with IgG isotype control antibody or IFN- γ blocking antibody and Xe or air for 2 weeks. (H) Top-affected pathways from IPA of DEGs in brain microglia from APP/PS1 mice treated with IFN- γ blocking antibody or IgG isotype control antibody ($n = 4$ or 5 female mice per group). (I) Activated and inhibited upstream regulators predicted by IPA. (J) Representative confocal images of cortical thioflavin-S (A β plaques) staining in brain sections from APP/PS1 mice treated with IgG isotype control antibody or IFN- γ blocking antibody and Xe or air for 2 weeks. (K) Quantification of % thioflavin-S (A β plaques)-positive area per ROI, normalized per animal ($n = 4$ or 5 female mice per group). (L) Representative confocal images of cortical Lamp1 and thioflavin-S (A β plaques) staining in brain sections from APP/PS1 mice treated with IgG isotype control antibody or IFN- γ blocking antibody and Xe or air for 2 weeks. (M) Quantification of % Lamp1-positive area per amyloid plaque, normalized per animal ($n = 4$ or 5 female mice per group). (N) Representative confocal images of cortical Clec7a, A β plaques (HJ3.4B), and Iba1 staining in brain sections from APP/PS1 mice treated with IgG isotype control antibody or IFN- γ blocking antibody and Xe or air for 2 weeks. (O) Quantification of % Iba1- and Clec7a-positive areas per amyloid plaque, normalized per animal ($n = 4$ or 5 female mice per group). (P) Representative flow cytometry contour plot showing Clec7a expression in a microglia population (CD11b⁺, Ly6C^{low}, and FCRL5⁺) from the brains of APP/PS1 mice treated with IgG isotype control antibody or IFN- γ blocking antibody and Xe or air for 2 weeks. (Q) Quantification of Clec7a⁺ cells ($n = 4$ or 5 female mice per group). Data are presented as means $\pm$ SEM. Figure panels (K), (M), (O), and (Q) were analyzed by two-tailed unpaired Student's *t* test; panel (A) was analyzed by one-way ANOVA. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

the blood, spleens, and brains from 5xFAD mice after Xe treatment (fig. S9H). We found that Xe increased IFN- γ expression in CD8 ($P = 0.0289$) but not CD4 ($P = 0.3863$) T cells in the blood 3 days after Xe inhalation (fig. S9I); this was lost at 7 days after inhalation ($P = 0.1173$) (fig. S9J). This increase in IFN- γ expression was present in splenic CD8 T cells 2 months after Xe continuous treatment ($P = 0.0471$) (fig. S9K). Moreover, mouse brain evaluation showed an increase in the percentage of CD8 T cells that expressed IFN- γ ($P = 0.0221$) (fig. S9, L and M) and in the expression of IFN- γ by these cells ($P = 0.0201$) (fig. S9, N and O).

Xe inhalation reduces brain atrophy, preserves dentate gyrus layer thickness, and decreases astrogliosis in TE4 mice

We evaluated whether Xe inhalation could ameliorate tauopathy and associated neurodegeneration in the P301S tau human APOE4 knock-in mouse model (TE4) (18). Our primary question for these experiments was whether exposure to Xe compared with atmospheric air reduced neurodegeneration in TE4 mice. Starting at 6 months of age, when tau pathology begins to develop, until 9.5 months of age, when mice exhibit robust tau pathology, neurodegeneration, glial activation, and synapse loss, TE4 mice received weekly 40-min-long treatments with either 30% Xe or atmospheric air (control). In this experimental paradigm, APOE4 KI (E4) mice that received atmospheric air treatments were included as another control group. At 9.5 months of age, mice were euthanized, and tissue was harvested. We first assessed whether Xe inhalation affected tau and microglia-mediated neurodegeneration. It is known that TE4 mice develop marked hippocampal and entorhinal/piriform cortex atrophy and concomitant ventricular enlargement (18, 41). We found that Xe treatment resulted in a small but significant increase in the hippocampus volume ($P = 0.0210$) and a trend toward increased volume in the entorhinal/piriform cortex ($P = 0.0627$) (Fig. 7, A and B). Xe inhalation also resulted in a trend toward a reduction in ventricle volume compared with the atmospheric air-treated control groups ($P = 0.0572$) (Fig. 7, A and B). In addition, the dentate gyrus granular cell neuronal layer was significantly thicker in animals treated with Xe than in the control group ($P = 0.045$) (Fig. 7, C and D). We performed a nest-building behavior test (42) on these mice 2 days before brain tissue collection. We observed that TE4 mice that received Xe inhalation showed a trend toward better performance compared with mice that received control air inhalation (Fig. 7E). Dentate gyrus layer thickness was positively and significantly correlated with hippocampal volume in all TE4 mice that received either Xe treatment or the control air treatment ($P < 0.0001$) (Fig. 7F). For these experiments, data from E4 mice at 9.5 months of age that develop no neurodegeneration are shown (Fig. 7, A to R). However, the E4 mouse data were not compared directly with the TE4 mouse data in the statistical tests performed because the E4 mice developed no neurodegeneration (they are only shown as a reference).

At 9.5 months of age, P301S-tau transgenic mice displayed substantial tau pathology, including elevated insoluble tau, hyperphosphorylated tau, and neurofibrillary tangles. We next explored how Xe inhalation affected tau pathology in the TE4 mice. First, we assessed phosphorylated tau (p-tau) in the hippocampus and entorhinal/piriform cortex through AT8 antibody immunostaining. This antibody recognizes tau protein phosphorylated at both serine-202 and threonine-205. We did not find significant differences in the AT8 p-tau percent area covered in the hippocampus or entorhinal/piriform cortex (Fig. 7, G to I). Given that we did not observe significant changes in

the percent area covered by AT8 immunostaining, we were interested in next assessing a tau marker for disease-specific conformational modifications of tau, MC1 (43). MC1 percent areas covered in the hippocampi and entorhinal/piriform cortices were not changed between the Xe and control air inhalation groups (fig. S10). Around 9.5 months of age, p-tau immunoreactivity stained by AT8 could also be categorized into four major p-tau staining patterns, designated as type 1 to 4. These staining patterns also strongly correlated with brain atrophy and more advanced pathological tau, with type 1 associated with the most preserved brain tissue and type 4 associated with the greatest brain atrophy (18). When AT8 p-tau immunoreactivity was categorized into the four types, we observed that in mice that received Xe inhalation, there was an enrichment of type 1 and 2 patterns compared with the type 3 and 4 patterns. In contrast, TE4 mice that received the control air inhalation had a predominantly type 4 pattern associated with more advanced tau stages and neurodegeneration, although this was not significant (Fig. 7, J and K). In another study, the amount of astrogliosis correlated to brain volume in a mouse model of tauopathy and played an essential role in AD (18), so we next assessed whether Xe inhalation affected astrogliosis in the hippocampus and entorhinal/piriform cortex. We observed that Xe decreased astrocyte [glial fibrillary acidic protein positive (GFAP⁺)] percent volume covered in the hippocampus and entorhinal/piriform cortex ($P = 0.034$; 0.0495) (Fig. 7, L to N), suggesting decreased activation of astrocytes after Xe inhalation.

Xe inhalation and its effects on CD8⁺ T cells in the dentate gyri of TE4 mice

It has been shown that T cells are present in the brain parenchyma in a mouse model of tauopathy and in samples of brains from patients with AD, and their enrichment correlates to brain atrophy. This study showed evidence of interactions between T cells and disease-related microglia subgroups (44). Our RNA-seq analysis showed the suppression of expression of several genes related to proinflammatory responses by astrocytes and microglia. We evaluated whether Xe inhalation affected the number of CD8⁺ T cells in the dentate gyri of TE4 mice (Fig. 7O). There was a trend toward decreased CD8⁺ T cell numbers in the dentate gyri of mice given Xe inhalation compared with control mice receiving air ($P = 0.0971$) (Fig. 7P). In addition, we stained for fibrinogen, a blood-derived protein usually barred from entering the brain parenchyma (Fig. 7Q). In the hippocampus, we did not observe any significant changes in fibrinogen in the TE4 mice after Xe inhalation (Fig. 7R).

Xe inhalation leads to up-regulation of neuronal and synaptic genes and down-regulation of inflammatory genes in TE4 mice

To determine whether transcriptional changes were associated with Xe inhalation in TE4 mice, we performed RNA-seq analysis of hippocampal tissues from 9.5-month-old TE4 mice treated with either Xe or air. Principal components analysis (PCA) showed specific clustering among all three groups (E4, TE4 air, and TE4 Xe), with the TE4 Xe group having a signature more similar to the non-tau transgenic E4 mice than the TE4 air mice (Fig. 8A). We identified 4390 DEGs (adjusted $P < 0.05$) in the Xe inhalation group compared with the control air inhalation group, of which 1860 genes were up-regulated and 2530 were down-regulated. We generated a heatmap with the top 400 DEGs to visualize the significant effects. This analysis showed a suppression of expression for several genes related to proinflammatory responses (*C3*, *S100*, *B2m*, and *Apoe*) in astrocytes and microglia

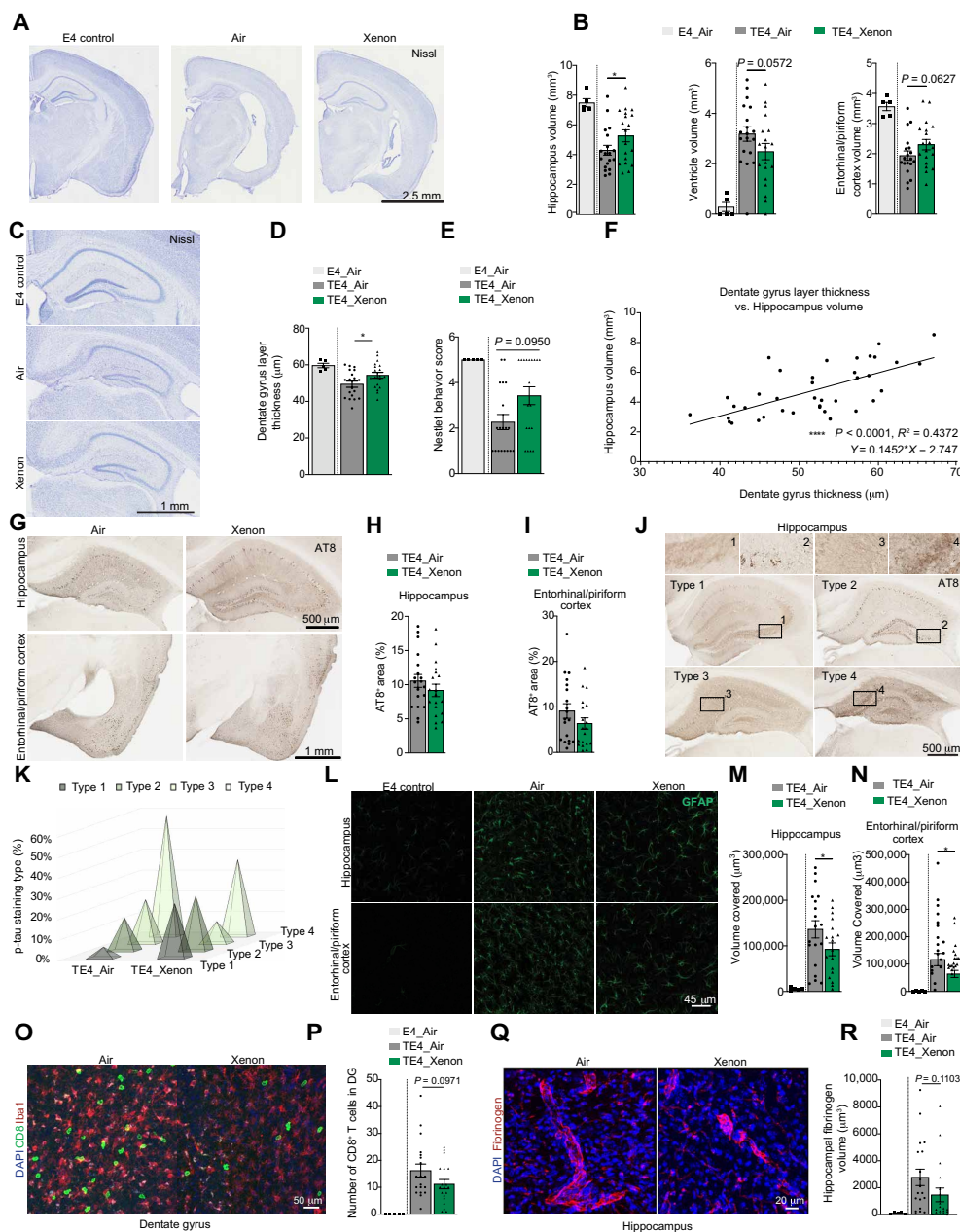


Fig. 7. Xe inhalation reduces mouse brain atrophy, rescues dentate gyrus layer thickness, and decreases GFAP⁺ astroglia. (A) Representative brain section images stained with cresyl violet Nissl from 9.5-month-old TE4 mice that received Xe gas or control air treatment for 40 min weekly from 6 to 9 months of age. Scale bar, 2.5 mm. (B) Quantification of hippocampus, ventricle, and entorhinal/piriform cortex volumes in the brains of E4 mice that received control air ($n = 5$), TE4 mice that received Xe gas ($n = 19$ per group), and TE4 mice that received control air ($n = 19$ per group). (C) Representative images of cresyl violet Nissl-stained mouse brain sections showing the dentate gyrus (DG) granule cell layers for the E4 air, TE4 air, and TE4 Xe groups. Scale bar, 1 mm. (D) Quantification of the DG granule cell layer thicknesses in the brains of mice from the three groups. (E) Quantification of nest building behavior in the nestlet test for the three groups. (F) Correlation between DG granule cell layer thicknesses and hippocampal volumes in TE4 mice exposed to Xe or air. (G) Representative images of brain sections stained with AT8 antibody for p-tau immunoreactivity from 9.5-month-old TE4 mice that received Xe or air treatment. Hippocampus (top images; scale bar, 500 μ m) and entorhinal/piriform cortex (bottom images; scale bar, 1 mm). (H and I) Shown is the AT8⁺ percent area in the hippocampi (H) and entorhinal/piriform cortices (I) of brains from TE4 mice that received Xe ($n = 19$ per group) or air ($n = 19$ per group). (J) Representative images of the four p-tau staining patterns with AT8 antibody in brain sections from 9.5-month-old TE4 mice that received air treatment. Scale bar, 500 μ m. (K) Quantification of AT8 p-tau staining pattern types in TE4 mice that received Xe ($n = 19$ per group) or air ($n = 19$ per group). (L) Representative images of GFAP staining in the hippocampi and entorhinal/piriform cortices of brains from 9.5-month-old TE4 mice that received Xe or air treatment. Scale bar, 45 μ m. (M) Quantification of the percent volumes of the hippocampi with GFAP⁺ staining in the brains of E4 mice that received air ($n = 5$ per group), TE4 mice that received Xe ($n = 19$ per group), and TE4 mice that received air ($n = 19$ per group). (N) Quantification of the percent volumes of entorhinal/piriform cortices with GFAP⁺ staining in the brains of E4 mice that received air ($n = 5$ per group), TE4 mice that received Xe ($n = 19$ per group), and TE4 mice that received air ($n = 19$ per group). (O) Representative images of CD8⁺ T cells in the DGs of 9.5-month-old TE4 mice that received Xe or air treatment. Scale bar, 50 μ m. (P) Quantification of the numbers of CD8⁺ T cells in the DGs of the E4 and TE4 groups. (Q) Representative images of fibrinogen staining in the hippocampi of 9.5-month-old TE4 mice that received Xe or air treatment. Scale bar, 20 μ m. (R) Quantification of fibrinogen volumes in the hippocampi of 9.5-month-old TE4 mice that received Xe or air treatment. Each dot represents one mouse. The statistical test used for analyses was a two-tailed Student's *t* test apart from (B), which used a one-tailed Student's *t* test, and (E) and (K), which used Fisher's exact test. * $P < 0.05$. Data are presented as means $\pm$ SEM.

(Fig. 8B). We found that some immune checkpoint genes showed reduced expression, including *Inpp5d* and *Havcr2*, suggesting a more active phenotype in hippocampal cells even with a decrease in inflammatory response (Fig. 8B). IPA highlighted an increase in the glutamate receptor and calcium signaling after Xe treatment along with suppression of neuroinflammation, production of IL-15, nitric oxide, and reactive oxygen species (ROS) (Fig. 8C). Upstream regulator analysis indicated the induction of BDNF and calmodulin and suppression of NF- κ B, IL-1, and TNF signaling (Fig. 8D).

Next, we analyzed the data via a weighted gene correlation network analysis (WGCNA), which revealed nine different clustered modules that identified the most critical genes and pathways in the TE4 mice after Xe treatment. We found four interesting modules that changed in opposite directions in TE4 mice that received Xe inhalation compared with those that received air (Fig. 8, E to H). The black module represented many GO biological processes involving lipid and fatty acid metabolic and catabolic functions; some top genes included *Hadhb*, *Hadhb*, and *Acadm* (Fig. 8E). The blue module represented many GO biological processes involving the inflammatory response (Fig. 8F). As predicted, this module correlated with disease development (TE4 air versus E4 air) and was suppressed in animals that received Xe treatment. The pink module included peptide and amide biosynthetic and ribosomal biogenesis GO pathways (Fig. 8G). The brown module indicated changes in synaptic and neuronal GO pathways (Fig. 8H); it was similarly induced in E4 mice and TE4 mice receiving Xe, indicating that Xe can restore neuronal function in TE4 mice. As indicated by the WGCNA, many neuronal and synaptic genes were up-regulated in mice that received Xe, and conversely, inflammatory genes were down-regulated (Fig. 8I). Notably, these genes included many GABA receptors, glutamate AMPA receptors, glutamate NMDA receptors, and synapse-associated genes such as *Snapt25* and *Stx1a*. Last, we found that the RNA expression of glutamate ionotropic receptor NMDA type subunit 1 (*Grin1*) and glutamate ionotropic receptor NMDA type subunit 2A (*Grin2A*) correlated positively with hippocampal volume in brain samples from TE4 mice ($P = 0.0204$; 0.002) (Fig. 8, J and K).

To investigate similarities in the Xe response between TE4 and APP/PS1 mouse models, we investigated the microglial genes modulated in TE4 mice by Xe inhalation. We found that Xe treatment suppressed several MGnD classical genes such as *Apoe*, *Itgax*, *Spp1*, *Lgals3*, *Axl*, and *Trem2* (Fig. 8L). Linear regression analysis indicated that 20% of DEGs were common between Xe-treated TE4 and APP/PS1 mice (Fig. 8M). Between the genes regulated in the same direction, we found an induction of *Grin2a*, *Mef2a*, *Adam22*, and *Nrxn3* expression (Fig. 8N), a presynaptic adhesion molecule that is a major regulator of synapse development and function (45). Xe inhalation also suppressed *Apoe*, *Trem2*, *Cd86*, *Plin2*, and *Cd14* gene expression in TE4 and APP/PS1 mice (Fig. 8N). Notably, CD14 is an accessory molecule that works with TLR4 to induce NF- κ B activation and induction of proinflammatory genes (46). The deletion of *Cd14* is associated with a decrease in the number of microglia and A β plaque burden in APP/PS1 mice (47). IPA indicated increased calcium signaling and antioxidant action of vitamin C and a suppression in IL-1 and ROS production as common pathways (Fig. 8O).

DISCUSSION

Three classical hallmarks of Alzheimer's disease are A β deposition, tau tangles, and microglial activation (48). These abnormalities act

in concert to cause neuronal damage, which in turn results in the clinical features of the disease. Anti-amyloid therapy is the first disease-modifying treatment for AD approved by the US Food and Drug Administration (FDA) and has a modest effect in the early stages of the disease (1). Both amyloid and tau result in microglial activation, a crucial component of AD pathogenesis. Microglial dysregulation plays an important role in both the onset and progression of AD, and recent findings show that GWAS-associated genes are specifically or highly expressed in microglia (5). Dysregulated microglia acquire an MGnD/DAM phenotype in response to neurodegeneration (5). Although this phenotype may help to promote A β clearance, it is also a toxic phenotype that may result in neurotoxicity. An ideal microglial phenotype to be induced for AD treatment would be one in which microglia effectively ameliorate A β pathology without causing cytotoxicity. We recently identified a microglial phenotype termed "pre-MGnD" (39). This phenotype is mediated by IFN- γ signaling and is associated with restriction of A β plaques, reduction of dystrophic neurites, attenuation of plaque-associated synaptic degradation, and cognitive improvement (39). We have found that Xe inhalation directly promotes pre-MGnD cell subtypes via IFN- γ signaling in an acute neurodegeneration mouse model and in A β -depositing mice.

IFN- γ is a key cytokine involved in the immune response and is crucial in innate immunity (40). IFN- γ is a proinflammatory cytokine because it activates immune responses, promotes nitric oxide production and expression of antigen presentation genes, and improves the phagocytic response. However, under specific circumstances, IFN- γ can also exert anti-inflammatory effects to regulate and resolve immune responses, including suppressing TNF and IL-1 expression by synergistically inducing IL-10 and blocking NF- κ B (49, 50). We found that Xe could stimulate peripheral immune cells, particularly CD8 T cells, increasing IFN- γ expression in the circulation and the brain. This augmentation of IFN- γ expression appears to play a role in facilitating the microglia transition. Furthermore, pharmacological and genetic targeting of IFN- γ effectively abrogated the modulatory effect of Xe on microglia, increasing their proinflammatory response and accumulation of A β plaques and dystrophic neurites. Notably, Xe inhalation increased the MGnD gene signature, including the expression of *Clec7a*, *Itgax*, and *Gpnmb* while maintaining the expression of some homeostatic genes, including *Sall1*, *Tgfb2*, and *Nr3c1* (a glucocorticoid receptor that can suppress the inflammatory response by blocking NF- κ B activation) (51). In contrast, a pre-MGnD-cytokine cell subtype characterized by the induction of IL-1, TNF, chemokines, and NF- κ B pathways was reduced. It is known that IL-1 and TNF promote A β production and aggregation in the brain (51, 52), given that excessive TNF, IL-1, and ROS can directly damage neurons, leading to synaptic dysfunction, impaired neuronal communication, and, ultimately, neuronal death (52).

Xe may have potential therapeutic effects, particularly in relation to microglia modulation in AD, but has a complex mechanism of action. Xe's action as an NMDA receptor blocker (53) and its role in mitophagy (54) suggests it may affect specific neuronal and cellular processes. However, this previous analysis was made only within limited brain regions or for a short time period (3 days). This narrow focus might not fully account for the complexity of Xe's mechanism of action. The consistent effects of Xe on microglia modulation in AD animal models open up a potential therapeutic window, given that microglial function is a critical aspect of neuroinflammation and disease progression in AD. It will be essential to study Xe's actions

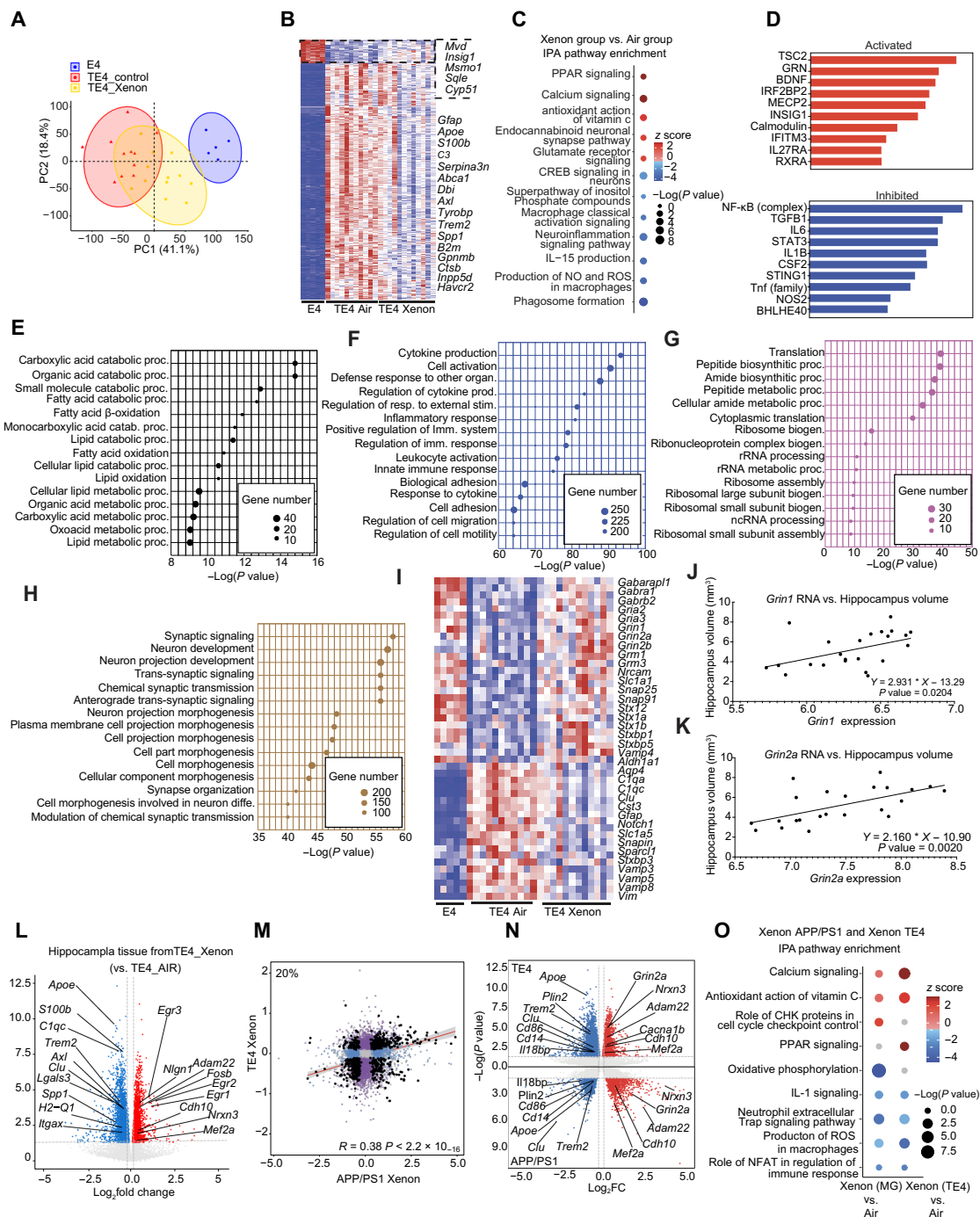


Fig. 8. Xe inhalation down-regulates inflammatory and oxidative stress-related genes in TE4 mice. (A) RNA was isolated from hippocampal tissues from 28 animals: Non-P301S-tau transgenic mice on an APOE4 background (E4 mice) that received air ($n = 5$ per group), P301S-tau transgenic mice on an APOE4 background (TE4 mice) that received Xe gas ($n = 12$), and TE4 mice that received air ($n = 11$). PCA indicates clusters for the three mouse groups. (B) Heatmap shows expression of the top 400 genes modulated by Xe inhalation. A total of 4390 DEGs were identified by comparing the TE4 control air versus TE4 Xe groups (2530 genes down-regulated in blue, and 1860 genes up-regulated in red). (C) Top-affected pathways from IPA based on TE4 Xe versus TE4 air groups. (D) Activated and inhibited upstream regulators predicted by IPA. (E) Black module within the WGCNA shows the top 15 GO biological pathways involved and the number of genes. (F) Blue module within the WGCNA shows the top 15 GO biological pathways involved and gene number. (G) Pink module within the WGCNA shows the top 15 GO biological pathways involved and gene number. (H) Brown module within the WGCNA shows the top 15 GO biological pathways involved and gene number. (I) Heatmap shows selected neuronal, synaptic, and astrocytic DEGs. (J and K) Correlations between *Grin1* and *Grin2a* RNA expression and hippocampal volumes in TE4 mice ($n = 23$). Each dot represents a mouse. (L) Volcano plots of DEGs from TE4 Xe versus TE4 air groups highlighting genes related to microglial response to Xe. (M) Correlation analysis between DEGs in brain microglia from TE4 and APP/PS1 mice exposed to Xe. (N) Combinatorial volcano plots correlating DEGs in brain microglia from TE4 and APP/PS1 mice. (O) Top-affected canonical pathways in APP/PS1 microglia Xe versus air groups and TE4 microglia Xe versus air groups. The statistical test used for (K) and (L) was a linear regression. PC, principal component.

across broader systems and time frames to understand its full potential and mechanisms, as well as its applicability for long-term treatment approaches.

In APP/PS1 mice, MGnD are crucial in reducing the amyloid burden by engulfing and degrading A β plaques, compacting them to limit A β toxicity (4), and sequestering toxic protein aggregates from surrounding neurons (55). APP/PS1 mice treated with Xe inhalation for 2 months demonstrated enhanced A β plaque compaction associated with reduced neurotoxicity and dystrophic neurites. Notably, the MGnD profile with increased *Clec7a* expression is directly related to A β accumulation (3), and reducing plaque burden can also suppress this signature. To evaluate whether the decrease in MGnD signaling at the end of Xe treatment resulted from reduced plaque burden or direct modulation by Xe, we conducted a short-term treatment demonstrating MGnD polarization by Xe. Using a *Clec7a-Cre^{ERT2}* mouse model designed to target MGnD specifically, we validated the significance of MGnD in reducing dystrophic neurites during disease onset.

To investigate how Xe inhalation affected tau pathology, we used the TE4 mouse model of tauopathy. We observed larger brain hippocampal volumes and decreased neuronal loss in the mouse dentate gyrus, which was associated with a trend toward improved nest-building behavior in TE4 mice that received Xe treatment compared with control animals exposed to air inhalation. Although we did not assess other behaviors in these mice, we have found that an improvement in nest-building behavior scores in TE4 mice correlates to performance on other cognitive tasks that assess aspects of memory, such as the Y-maze, and in contextual and auditory cued conditioning tests (44). Although Xe inhalation reduced hippocampal atrophy in the TE4 mice, these mice still exhibited considerable neurodegeneration. Xe inhalation did not affect p-tau accumulation or disease-specific conformational modification of tau or p-tau staining patterns. However, notably, we have previously shown that this mouse model with human APOE4 knocked in develops greater tau pathology than mouse models expressing other human ApoE isoforms or with ApoE knocked out (18). Thus, there may be additional effects from the presence of the APOE4 isoform contributing to tau pathology that Xe inhalation may not overcome that need to be defined by studying the effects of Xe on mice expressing different APOE isoforms or in mice lacking APOE.

Under basal conditions, astrocytes produce the majority of APOE in the CNS. However, upon specific insults such as brain injury or neurodegeneration, ApoE expression can also be strongly up-regulated in microglia (3). Recently, we showed that removal of APOE4 from astrocytes in a mouse model of tau pathology resulted in decreased neurodegeneration, p-tau pathology, and synaptic loss and microglial phagocytosis of synaptic elements, suggesting a key role for astrocytic ApoE in synaptic degeneration (41). We found that, unlike 5x-MITRG mice (19), Xe suppressed astrogliosis in TE4 mice (18). On the basis of our experience, these contradictory observations are likely the result of different experimental designs and comparisons. Despite both mouse models being treated with Xe at the same concentration, there were big differences between them, including variations in age, brain regions analyzed (cortex versus hippocampus), the presence of human microglia, and the absence of adaptive immune cells in the 5x-MITRG mouse model. In contrast, the P301S mice displayed a disease on the basis of tau phosphorylation and aggregation with marked neuronal loss, synaptic loss, and brain atrophy. Another important distinction is that our P301S mice were developed on a human APOE4 background

(18). A recent publication from our group demonstrated that APOE4 restrains microglia polarization to MGnD, resulting in decreased galectin-3 expression and, consequently, less astrocyte activation (39). Thus, it is possible that differences in the APOE background could modulate part of the differences in astrogliosis induced by Xe observed in the two different models.

Synapse loss has been well characterized in mouse models of tauopathies (56–58). TE4 mice that received control air inhalation showed down-regulation of many synaptic and neuronal genes. Conversely, TE4 mice receiving Xe inhalation showed up-regulation of similar genes and a gene expression profile similar to that of E4 control mice. In line with these findings, a previous study in a mouse model of TBI treated with Xe showed reduced astrogliosis (59). Our WGCNA revealed that Xe inhalation in TE4 mice down-regulated lipid and fatty acid genes, including *Plin2*, *Lamp1*, *Cd68*, *Lamp2*, *Grn*, and *Gpnm2*. We recently showed that TE4 mice developed abundant brain lysosomal damage and lipid accumulation (60). Our RNA-seq results indicated that pathways that were dysregulated in TE4 mice were reversed in the opposite direction to a certain extent after Xe inhalation.

We evaluated the similarities between TE4 and APP/PS1 mouse models after Xe inhalation. We found that Xe suppressed the expression of several MGnD classical genes such as *Itgax*, *Spp1*, *Lgals3*, *Axl*, and *Trem2*. It has been demonstrated that microglia-mediated damage, rather than direct pathological tau-induced neurotoxicity, is required for neurodegeneration in a tauopathy mouse model (61). These results indicate that Xe inhalation affected microglial pathways rather than directly affecting tau-induced neuronal damage.

To evaluate the effect of Xe inhalation on the modulation of human microglia in AD pathology, we used the humanized 5xFAD mouse model transplanted with human iMG (28). These cells have gene expression profiles and functional properties similar to those of primary human microglia (62) and have shown effectiveness in reducing A β burden and modulating amyloid pathology in the 5xFAD mouse model (63). We found that Xe inhalation reduced A β plaque load and dystrophic neurons even after these pathologies were well established. Furthermore, RNA-seq analysis showed that Xe suppressed genes associated with MGnD such as *Clec7a* and *CD9* (3) while inducing a gene expression signature indicative of IFN- γ signaling. In 5x-MITRG mice, astrocytes associated with human microglia showed enrichment for IFN- γ signaling, accompanied by the suppression of genes related to GM-CSF, IL-8, IL-6, and NF- κ B activation.

In summary, our findings demonstrate that inhaled Xe modulates microglia and ameliorates disease in mouse models of amyloidosis and tauopathy. Clinical testing of Xe treatment in healthy volunteers will start in early 2025.

MATERIALS AND METHODS

Study design

This study aimed to determine whether Xe inhalation could be used to treat mouse models of AD. To assess whether Xe inhalation could modulate brain microglia and to determine the optimal Xe concentration, we used an acute neurodegeneration mouse model in which fluorescent apoptotic mouse neurons were injected into the cortices and hippocampi of naïve C57BL/6J mice, which were then treated with Xe at concentrations ranging from 6 to 35%. The optimal dose and timing of Xe treatment resulting in microglia modulation was

also conducted in APP/PS1 mice and was determined to be 30%. To evaluate whether Xe inhalation had a protective immunomodulatory role in restoring microglial function in AD mouse models, we treated APP/PS1, P301S-APOE4 (TE4), and 5xFAD mice with 30% Xe in an inhalation chamber (fig. S1I). We performed scRNA-seq of brain myeloid cells from APP/PS1 mice to understand how Xe modulated microglia and its subtypes. To further investigate whether the effects of Xe were microglia dependent, we depleted brain myeloid cells in 5xFAD mice using PLX-5622. We assessed the importance of MGnD for AD pathology using our Clec7a-Cre^{ERT2} mice. In addition, we transplanted human iMG into 5x-MITRG mice to evaluate whether Xe inhalation could modulate human microglia. Last, the role of IFN- γ in modulating microglial function in response to Xe treatment was investigated using neutralizing antibodies against IFN- γ and transgenic (Cx3Cr1-Cre^{ERT2}; *Ifngr*^{Flox}) mice. Data collection and analysis were not performed blinded. Outliers were removed using GraphPad Outlier Calculator, which uses the robust regression and outlier removal test (ROUT). All animal procedures and experiments were performed under guidelines approved by the Institutional Animal Care and Use Committee (IACUC) at Brigham and Women's Hospital, Harvard Medical School, University of California, Irvine, and Washington University School of Medicine.

Mice

C57BL/6-J (stock no. 000664), B6-5xFAD (stock no. 034848-JAX), Cx3cr1-Cre^{ERT2} (stock no. 021160), *Ifngr*^{Flox} (stock no. 025394), ROSA26-DTR^{Flox} (stock no. 007900), ROSA26:TdTomato^{Flox} (stock no. 007914), P301S-tau transgenic mice (stock no 008169), and Csf1r (Δ FIRE/ Δ FIRE) (stock no. 007914) mice were purchased from Jackson Laboratory. APOE4 (E4) mice were generated by replacing the mouse genomic sequence from the translation initiation codon in exon 2 to the termination codon in exon 4 with its human counterparts flanked by loxP sites (64). APP/PS1 (16) mice were provided by M. Jucker (University of Tübingen). 5xFAD-MITRG mice (28, 62, 65) were generated and xenotransplanted by M.B.-J.'s laboratory (University of California Irvine). Clec7a-Cre^{ERT2} mice were developed in O.B.'s laboratory.

Xe inhalation

A closed-circuit gas inhalation system provided by General Biophysics (www.generalbiophysics.com) was used to administer either the Xe experimental or air control inhalation treatment. This system included an airtight sealed mouse chamber with a pump, allowing for continuous gas recirculation, gas concentration sensors, and a CO₂ absorber (fig. S1I). In this work, we used two versions of this system. The first accommodated up to six mice per treatment in the airtight chamber with a 4-liter volume. It had CO₂ and O₂ gas monitors, a recirculation pump, and a CO₂ absorber. The gas mixture was prepared in a separate container by hyperbaric mixing and then was delivered to the treatment chamber, creating the required average concentrations of Xe and oxygen. The second and more advanced version of the system accommodated up to 12 mice per treatment, and, in addition to the features of the first version, this one was equipped with a proprietary Xe gas concentration monitor (www.generalbiophysics.com, model A). This allowed complete control of the gas concentrations to be established when the gases were supplied to the treatment chamber directly from the gas cylinders through the flow meters. In both chambers, O₂ and CO₂ concentrations were continuously monitored. A CO₂ absorbent helped to

maintain the CO₂ concentration between 600 and 1500 parts per million (ppm) in the first chamber with an average of 900 ppm and 600 and 900 ppm in the second chamber. The pure Xe gas was provided by General Biophysics and was produced by Linde Gas & Equipment Inc. The minimum Xe gas purity was 99.99%. The oxygen was purchased from Airgas with 100% purity.

Statistical analysis

All results are reported as mean $\pm$ SEM, and all statistical analyses were conducted in Prism 9 (GraphPad). Gaussian distribution was evaluated using the D'Agostino-Pearson normality test. Statistical analysis between two groups was performed using a Student's *t* test under a normal distribution. Statistical analysis between two groups with categorical data was performed using Fisher's exact test. Comparisons among multiple groups were analyzed by one-way analysis of variance (ANOVA) with Tukey's multiple comparison correction. Correlations were assessed with linear regressions. *P* value less than 0.05 (*P* < 0.05) was considered a statistically significant difference. ns, *P* > 0.05; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001. The number of mice per group (*n*) used for each experiment can be found in the figure legends.

Supplementary Materials

The PDF file includes:

Materials and Methods
Tables S1 to S3
Figs. S1 to S10
Legends for data files S1 to S3
References (66–90)

Other Supplementary Material for this manuscript includes the following:

Data files S1 to S3
MDAR Reproducibility Checklist

REFERENCES AND NOTES

1. C. H. van Dyck, C. J. Swanson, P. Aisen, R. J. Bateman, C. Chen, M. Gee, M. Kanekiyo, D. Li, L. Reyderman, S. Cohen, L. Froelich, S. Katayama, M. Sabbagh, B. Vellas, D. Watson, S. Dhabha, M. Irizarry, L. D. Kramer, T. Iwatsubo, Lecanemab in early Alzheimer's disease. *N. Engl. J. Med.* **388**, 9–21 (2023).
2. C. S. Kruse, M. E. Mileski, R. Wilkinson, B. Hock, R. Samson, T. Castillo, Leveraging technology to diagnose Alzheimer's disease: A systematic review and meta-analysis. *Healthcare* **11**, 3013 (2023).
3. S. Krasemann, C. Madore, R. Cialic, C. Baufeld, N. Calcagno, R. El Fatimy, L. Beckers, E. O'Loughlin, Y. Xu, Z. Fanek, D. J. Greco, S. T. Smith, G. Twest, Z. Humulock, T. Zrzavy, P. Conde-Sanroman, M. Gacias, Z. Weng, H. Chen, E. Tjon, F. Mazaheri, K. Hartmann, A. Madi, J. D. Ulrich, M. Glatzel, A. Worthmann, J. Heeren, B. Budnik, C. Lemere, T. Ikezu, F. L. Heppner, V. Litvak, D. M. Holtzman, H. Lassmann, H. L. Weiner, J. Ochando, C. Haass, O. Butovsky, The TREM2-APOE pathway drives the transcriptional phenotype of dysfunctional microglia in neurodegenerative diseases. *Immunity* **47**, 566–581.e9 (2017).
4. H. Keren-Shaul, A. Spinrad, A. Weiner, O. Matcovitch-Natan, R. Dvir-Szternfeld, T. K. Ulland, E. David, K. Baruch, D. Lara-Astaiso, B. Toth, S. Itzkovitch, M. Colonna, M. Schwartz, I. Amit, A unique microglia type associated with restricting development of Alzheimer's disease. *Cell* **169**, 1276–1290.e17 (2017).
5. I. E. Jansen, J. E. Savage, K. Watanabe, J. Bryois, D. M. Williams, S. Steinberg, J. Sealock, I. K. Karlsson, S. Hagg, L. Athanasiu, N. Voyle, P. Proitsi, A. Witoelar, S. Stringer, D. Aarsland, I. S. Almdahl, F. Andersen, S. Bergh, F. Bettella, S. Bjornsson, A. Braekhus, G. Brathen, C. de Leeuw, R. S. Desikan, S. Djurovic, L. Dumitrescu, T. Fladby, T. J. Hohman, P. V. Jonsson, S. J. Kiddle, A. Rongve, I. Saltvedt, S. B. Sando, G. Selbaek, M. Shoaib, N. G. Skene, J. Snaedal, E. Stordal, I. D. Ulstein, Y. Wang, L. R. White, J. Hardy, J. Hjerling-Leffler, P. F. Sullivan, W. M. van der Flier, R. Dobson, L. K. Davis, H. Stefansson, K. Stefansson, N. L. Pedersen, S. Ripke, O. A. Andreassen, D. Posthuma, Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer's disease risk. *Nat. Genet.* **51**, 404–413 (2019).
6. W. Liu, N. Khatibi, A. Sridharan, J. H. Zhang, Application of medical gases in the field of neurobiology. *Med. Gas Res.* **1**, 13 (2011).

7. D. A. Winkler, A. Thornton, G. Farjot, I. Katz, The diverse biological properties of the chemically inert noble gases. *Pharmacol. Ther.* **160**, 44–64 (2016).
8. J. Lavaur, M. Lemaire, J. Pype, D. Le Nogue, E. C. Hirsch, P. P. Michel, Neuroprotective and neurorestorative potential of xenon. *Cell Death Dis.* **7**, e2182 (2016).
9. J. Lewerenz, P. Maher, Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? *Front. Neurosci.* **9**, 469 (2015).
10. N. P. Franks, R. Dickinson, S. L. de Sousa, A. C. Hall, W. R. Lieb, How does xenon produce anaesthesia? *Nature* **396**, 324 (1998).
11. J. H. Baumert, F. Falter, D. Eletr, K. E. Hecker, M. Reyle-Hahn, R. Rossaint, Xenon anaesthesia may preserve cardiovascular function in patients with heart failure. *Acta Anaesthesiol. Scand.* **49**, 743–749 (2005).
12. H. M. Homi, N. Yokoo, D. Ma, D. S. Warner, N. P. Franks, M. Maze, H. P. Grocott, The neuroprotective effect of xenon administration during transient middle cerebral artery occlusion in mice. *Anesthesiology* **99**, 876–881 (2003).
13. J. Dingley, J. Tooley, H. Porter, M. Thoresen, Xenon provides short-term neuroprotection in neonatal rats when administered after hypoxia-ischemia. *Stroke* **37**, 501–506 (2006).
14. A. Dobrovolsky, T. E. Ichim, D. Ma, S. Kesari, V. Bogin, Xenon in the treatment of panic disorder: An open label study. *J. Transl. Med.* **15**, 137 (2017).
15. L. S.-C. Law, E. A.-G. Lo, T. J. Gan, Xenon anesthesia: A systematic review and meta-analysis of randomized controlled trials. *Anesth. Analg.* **122**, 678–697 (2016).
16. R. Radde, T. Bolmont, S. A. Kaeser, J. Coomaraswamy, D. Lindau, L. Stoltze, M. E. Calhoun, F. Jaggi, H. Wolburg, S. Gengler, C. Haass, B. Ghetti, C. Czech, C. Holscher, P. M. Mathews, M. Jucker, A β 42-driven cerebral amyloidosis in transgenic mice reveals early and robust pathology. *EMBO Rep.* **7**, 940–946 (2006).
17. H. Oakley, S. L. Cole, S. Logan, E. Maus, P. Shao, J. Craft, A. Guillozet-Bongaarts, M. Ohno, J. Disterhoft, L. Van Eldik, R. Berry, R. Vassar, Intraneuronal β -amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: Potential factors in amyloid plaque formation. *J. Neurosci.* **26**, 10129–10140 (2006).
18. Y. Shi, K. Yamada, S. A. Liddell, S. T. Smith, L. Zhao, W. Luo, R. M. Tsai, S. Spina, L. T. Grinberg, J. C. Rojas, G. Gallardo, K. Wang, J. Roh, G. Robinson, M. B. Finn, H. Jiang, P. M. Sullivan, C. Baufeld, M. W. Wood, C. Sutphen, L. McCue, C. Xiong, J. L. Del-Aguila, J. C. Morris, C. Cruchaga, Alzheimer's Disease Neuroimaging Initiative, A. M. Fagan, B. L. Miller, A. L. Boxer, W. W. Seeley, O. Butovsky, B. A. Barres, S. M. Paul, D. M. Holtzman, ApoE4 markedly exacerbates tau-mediated neurodegeneration in a mouse model of tauopathy. *Nature* **549**, 523–527 (2017).
19. A. Rongvaux, T. Willinger, J. Martinek, T. Strowig, S. V. Gearty, L. L. Teichmann, Y. Saito, F. Marches, S. Halene, A. K. Palucka, M. G. Manz, R. A. Flavell, Development and function of human innate immune cells in a humanized mouse model. *Nat. Biotechnol.* **32**, 364–372 (2014).
20. O. Butovsky, M. P. Jedrychowski, C. S. Moore, R. Cialic, A. J. Lanser, G. Gabriely, T. Koeglspenger, B. Dake, P. M. Wu, C. E. Doykan, F. Fanek, L. Liu, Z. Chen, J. D. Rothstein, R. M. Ransohoff, S. P. Gygi, J. P. Antel, H. L. Weiner, Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nat. Neurosci.* **17**, 131–143 (2014).
21. D. Gosselin, V. M. Link, C. E. Romanoski, G. J. Fonseca, D. Z. Eichenfield, N. J. Spann, J. D. Stender, H. B. Chun, H. Garner, F. Geissmann, C. K. Glass, Environment drives selection and function of enhancers controlling tissue-specific macrophage identities. *Cell* **159**, 1327–1340 (2014).
22. O. Scheyer, A. Rahman, H. Hristov, C. Berkowitz, R. S. Isaacson, R. D. Brinton, L. Mosconi, Female sex and Alzheimer's risk: The menopause connection. *J. Prev. Alzheimers Dis.* **5**, 225–230 (2018).
23. X. Li, Y. Feng, W. Wu, J. Zhao, C. Fu, Y. Li, Y. Ding, B. Wu, Y. Gong, G. Yang, X. Zhou, Sex differences between APPswePS1dE9 mice in A- β accumulation and pancreatic islet function during the development of Alzheimer's disease. *Lab. Anim.* **50**, 275–285 (2016).
24. S. Kiani Shabestari, S. Morabito, E. P. Danhash, A. McQuade, J. R. Sanchez, E. Miyoshi, J. P. Chadarevian, C. Claes, M. A. Coburn, J. Hasselmann, J. Hidalgo, K. N. Tran, A. C. Martini, W. Chang Rothermich, J. Pascual, E. Head, D. A. Hume, C. Pridans, H. Davtyan, V. Swarup, M. Blurton-Jones, Absence of microglia promotes diverse pathologies and early lethality in Alzheimer's disease mice. *Cell Rep.* **39**, 110961 (2022).
25. M. Frosch, L. Amann, M. Prinz, CNS-associated macrophages shape the inflammatory response in a mouse model of Parkinson's disease. *Nat. Commun.* **14**, 3753 (2023).
26. N. Habib, C. McCabe, S. Medina, M. Varshavsky, D. Kitsberg, R. Dvir-Szternfeld, G. Green, D. Dionne, L. Nguyen, J. L. Marshall, F. Chen, F. Zhang, T. Kaplan, A. Regev, M. Schwartz, Disease-associated astrocytes in Alzheimer's disease and aging. *Nat. Neurosci.* **23**, 701–706 (2020).
27. J. Hasselmann, M. A. Coburn, W. England, D. X. Figueroa Velez, S. Kiani Shabestari, C. H. Tu, A. McQuade, M. Kolahdouzan, K. Echeverria, C. Claes, T. Nakayama, R. Azevedo, N. G. Coufal, C. Z. Han, B. J. Cummings, H. Davtyan, C. K. Glass, L. M. Healy, S. P. Gandhi, R. C. Spital, M. Blurton-Jones, Development of a chimeric model to study and manipulate human microglia in vivo. *Neuron* **103**, 1016–1033.e10 (2019).
28. E. M. Abud, R. N. Ramirez, E. S. Martinez, L. M. Healy, C. H. H. Nguyen, S. A. Newman, A. V. Yeromin, V. M. Scarfone, S. E. Marsh, C. Fimbres, C. A. Caraway, G. M. Fote, A. M. Madany, A. Agrawal, R. Kayed, K. H. Glyls, M. D. Cahalan, B. J. Cummings, J. P. Antel, A. Mortazavi, M. J. Carson, W. W. Poon, M. Blurton-Jones, iPSC-derived human microglia-like cells to study neurological diseases. *Neuron* **94**, 278–293.e9 (2017).
29. R. Mancuso, J. Van Den Daele, N. Fattorelli, L. Wolfs, S. Balusu, O. Burton, A. Liston, A. Agarswal, S. Fourn, S. Poovathingal, A. Arranz-Mendiguer, C. Sala Frigerio, C. Claes, L. Serneels, T. Theys, V. H. Perry, C. Verfaillie, M. Fiers, B. De Strooper, Stem-cell-derived human microglia transplanted in mouse brain to study human disease. *Nat. Neurosci.* **22**, 2111–2116 (2019).
30. D. S. Svoboda, M. I. Barrasa, J. Shu, R. Rietjens, S. Zhang, M. Mitalipova, P. Berube, D. Fu, L. D. Shultz, G. W. Bell, R. Jaenisch, Human iPSC-derived microglia assume a primary microglia-like state after transplantation into the neonatal mouse brain. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 25293–25303 (2019).
31. J. Hasselmann, M. Blurton-Jones, Human iPSC-derived microglia: A growing toolset to study the brain's innate immune cells. *Glia* **68**, 721–739 (2020).
32. Y. Ben-Asouli, Y. Banai, Y. Pel-Or, A. Shir, R. Kaempfer, Human interferon- γ mRNA autoregulates its translation through a pseudoknot that activates the interferon-inducible protein kinase PKR. *Cell* **108**, 221–232 (2002).
33. L. Katsouri, A. M. Birch, A. W. J. Renziehausen, C. Zach, Y. Aman, H. Steeds, A. Bonsu, E. O. C. Palmer, N. Mirzaei, M. Ries, M. Sastre, Ablation of reactive astrocytes exacerbates disease pathology in a model of Alzheimer's disease. *Glia* **68**, 1017–1030 (2020).
34. A. D. Luster, J. C. Unkeless, J. V. Ravetch, γ -interferon transcriptionally regulates an early-response gene containing homology to platelet proteins. *Nature* **315**, 672–676 (1985).
35. E. Spangenberg, P. L. Severson, L. A. Hohsfield, J. Crapser, J. Zhang, E. A. Burton, Y. Zhang, W. Spevak, J. Lin, N. Y. Phan, G. Habets, A. Rymar, G. Tsang, J. Walters, M. Nespi, P. Singh, S. Broome, P. Ibrahim, C. Zhang, G. Bollag, B. L. West, K. N. Green, Sustained microglial depletion with CSF1R inhibitor impairs parenchymal plaque development in an Alzheimer's disease model. *Nat. Commun.* **10**, 3758 (2019).
36. H. S. Yang, C. Zhang, B. C. Carlyle, S. Y. Zhen, B. A. Trombetta, A. P. Schultz, J. J. Pruzin, C. D. Fitzpatrick, W. W. Yau, D. R. Kim, D. M. Rentz, S. E. Arnold, K. A. Johnson, R. A. Sperling, J. P. Chhatwal, R. E. Tanzi, Plasma IL-12/IFN- γ axis predicts cognitive trajectories in cognitively unimpaired older adults. *Alzheimers Dement.* **18**, 645–653 (2022).
37. M. A. Mastrangelo, K. L. Sudol, W. C. Narrow, W. J. Bowers, Interferon- γ differentially affects Alzheimer's disease pathologies and induces neurogenesis in triple transgenic-AD mice. *Am. J. Pathol.* **175**, 2076–2088 (2009).
38. M. Zych, A. Roszczyk, M. Kniolek, F. Dabrowski, R. Zagozdzon, Differences in immune checkpoints expression (TIM-3 and PD-1) on T cells in women with recurrent miscarriages-preliminary studies. *J. Clin. Med.* **10**, 4182 (2021).
39. Z. Yin, S. Herron, S. Silveira, K. Kleemann, C. Gauthier, D. Mallah, Y. Cheng, M. A. Margeta, K. M. Pitts, J. L. Barry, A. Subramanian, H. Shorey, W. Brandao, A. Durao, J. C. Delpech, C. Madore, M. Jedrychowski, A. K. Ajay, G. Murugaiyan, S. W. Hersh, S. Ikezu, T. Ikezu, O. Butovsky, Identification of a protective microglial state mediated by miR-155 and interferon- γ signaling in a mouse model of Alzheimer's disease. *Nat. Neurosci.* **26**, 1196–1207 (2023).
40. G. Tau, P. Rothman, Biologic functions of the IFN- γ receptors. *Allergy* **54**, 1233–1251 (1999).
41. C. Wang, M. Xiong, M. Gratuze, X. Bao, Y. Shi, P. S. Andhey, M. Manis, C. Schroeder, Z. Yin, C. Madore, O. Butovsky, M. Artymov, J. D. Ulrich, D. M. Holtzman, Selective removal of astrocytic APOE4 strongly protects against tau-mediated neurodegeneration and decreases synaptic phagocytosis by microglia. *Neuron* **109**, 1657–1674.e7 (2021).
42. R. M. Deacon, Assessing nest building in mice. *Nat. Protoc.* **1**, 1117–1119 (2006).
43. G. A. Jicha, R. Bowser, I. G. Kazam, P. Davies, Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J. Neurosci. Res.* **48**, 128–132 (1997).
44. X. Chen, M. Firulyova, M. Manis, J. Herz, I. Smirnov, E. Aladyeva, C. Wang, X. Bao, M. B. Finn, H. Hu, I. Shchukina, M. W. Kim, C. M. Yuede, J. Kipnis, M. N. Artymov, J. D. Ulrich, D. M. Holtzman, Microglia-mediated T cell infiltration drives neurodegeneration in tauopathy. *Nature* **615**, 668–677 (2023).
45. M. Uchigashima, A. Cheung, J. Suh, M. Watanabe, K. Futai, Differential expression of neurexin genes in the mouse brain. *J. Comp. Neurol.* **527**, 1940–1965 (2019).
46. R. L. Delude, M. J. Fenton, R. Savedra Jr., P. Y. Perera, S. N. Vogel, R. Thieringer, D. T. Golenbock, CD14-mediated translocation of nuclear factor-kappa B induced by lipopolysaccharide does not require tyrosine kinase activity. *J. Biol. Chem.* **269**, 22253–22260 (1994).
47. E. G. Reed-Geaghan, Q. W. Reed, P. E. Cramer, G. E. Landreth, Deletion of CD14 attenuates Alzheimer's disease pathology by influencing the brain's inflammatory milieu. *J. Neurosci.* **30**, 15369–15373 (2010).
48. A. Serrano-Pozo, M. P. Frosch, E. Masliah, B. T. Hyman, Neuropathological alterations in Alzheimer disease. *Cold Spring Harb. Perspect. Med.* **1**, a006189 (2011).

49. T. Eigenbrod, K. A. Bode, A. H. Dalpke, Early inhibition of IL-1 β expression by IFN- γ is mediated by impaired binding of NF- κ B to the IL-1 β promoter but is independent of nitric oxide. *J. Immunol.* **190**, 6533–6541 (2013).
50. Y. Yanagawa, K. Iwabuchi, K. Onoé, Co-operative action of interleukin-10 and interferon- γ to regulate dendritic cell functions. *Immunology* **127**, 345–353 (2009).
51. R. I. Scheinman, P. C. Cogswell, A. K. Lofquist, A. S. Baldwin Jr., Role of transcriptional activation of I κ B α in mediation of immunosuppression by glucocorticoids. *Science* **270**, 283–286 (1995).
52. R. Fischer, O. Maier, Interrelation of oxidative stress and inflammation in neurodegenerative disease: Role of TNF. *Oxid. Med. Cell. Longev.* **2015**, 610813 (2015).
53. Y. H. Sanjoud, At least three xenon binding sites in the glycine binding domain of the N-methyl D-aspartate receptor. *Arch. Biochem. Biophys.* **724**, 109265 (2022).
54. H. Lv, J. Huang, X. Zhang, Z. He, J. Zhang, W. Chen, Xenon ameliorates chronic post-surgical pain by regulating mitophagy in microglia and rats mediated by PINK1/Parkin pathway. *PeerJ* **12**, e16855 (2024).
55. Y. Huang, K. E. Happonen, P. G. Burrola, C. O'Connor, N. Hah, L. Huang, A. Nimmerjahn, G. Lemke, Microglia use TAM receptors to detect and engulf amyloid β plaques. *Nat. Immunol.* **22**, 586–594 (2021).
56. Y. Yoshiyama, M. Higuchi, B. Zhang, S. M. Huang, N. Iwata, T. C. Saido, J. Maeda, T. Suhara, J. Q. Trojanowski, V. M. Lee, Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* **53**, 337–351 (2007).
57. N. A. Hoffmann, M. M. Dorostkar, S. Blumenstock, M. Goedert, J. Herms, Impaired plasticity of cortical dendritic spines in P301S tau transgenic mice. *Acta Neuropathol. Commun.* **1**, 82 (2013).
58. K. J. Kopeikina, M. Polydoro, H. C. Tai, E. Yaeger, G. A. Carlson, R. Pittstick, B. T. Hyman, T. L. Spire-Jones, Synaptic alterations in the rTg4510 mouse model of tauopathy. *J. Comp. Neurol.* **521**, 1334–1353 (2013).
59. R. Campos-Pires, T. Hirnet, F. Valeo, B. E. Ong, K. Radyushkin, J. Aldhoun, J. Saville, C. J. Edge, N. P. Franks, S. C. Thal, R. Dickinson, Xenon improves long-term cognitive function, reduces neuronal loss and chronic neuroinflammation, and improves survival after traumatic brain injury in mice. *Br. J. Anaesth.* **123**, 60–73 (2019).
60. M. Gratuze, J. C. M. Schlachetzki, R. D'Oliveira Albanus, N. Jain, B. Novotny, L. Brase, L. Rodriguez, C. Mansel, M. Kipnis, S. O'Brien, M. P. Pasillas, C. Lee, M. Manis, M. Colonna, O. Harari, C. K. Glass, J. D. Ulrich, D. M. Holtzman, TREM2-independent microglial responses promotes tau-mediated neurodegeneration in the presence of ApoE4. *Neuron* **111**, 202–219.e7 (2023).
61. Y. Shi, M. Manis, J. Long, K. Wang, P. M. Sullivan, J. Remolina Serrano, R. Hoyle, D. M. Holtzman, Microglia drive APOE-dependent neurodegeneration in a tauopathy mouse model. *J. Exp. Med.* **216**, 2546–2561 (2019).
62. A. McQuade, M. Coburn, C. H. Tu, J. Hasselmann, H. Davtyan, M. Blurton-Jones, Development and validation of a simplified method to generate human microglia from pluripotent stem cells. *Mol. Neurodegener.* **13**, 67 (2018).
63. A. McQuade, Y. J. Kang, J. Hasselmann, A. Jairaman, A. Sotelo, M. Coburn, S. K. Shabestari, J. P. Chadarevian, G. Fote, C. H. Tu, E. Danhash, J. Silva, E. Martinez, C. Cotman, G. A. Prieto, L. M. Thompson, J. S. Steffan, I. Smith, H. Davtyan, M. Cahalan, H. Cho, M. Blurton-Jones, Gene expression and functional deficits underlie TREM2-knockout microglia responses in human models of Alzheimer's disease. *Nat. Commun.* **11**, 5370 (2020).
64. T. V. Huynh, C. Wang, A. C. Tran, G. T. Tabor, T. E. Mahan, C. M. Francis, M. B. Finn, R. Spellman, M. Manis, R. E. Tanzi, J. D. Ulrich, D. M. Holtzman, Lack of hepatic apoE does not influence early A β deposition: Observations from a new APOE knock-in model. *Mol. Neurodegener.* **14**, 37 (2019).
65. A. Grubman, T. H. VandeKolk, J. Schroder, G. Sun, J. Hatwell-Humble, J. Chan, M. Oksanen, S. Lehtonen, C. Hunt, J. E. Koistinaho, S. K. Nilsson, J. M. Haynes, C. W. Pouton, J. M. Polo, A CX3CR1 reporter hESC Line facilitates integrative analysis of in-vitro-derived microglia and improved microglia identity upon neuron-glia co-culture. *Stem Cell Rep.* **14**, 1018–1032 (2020).
66. Z. Yin, N. Rosenzweig, K. L. Kleemann, X. Zhang, W. Brandao, M. A. Margeta, C. Schroeder, K. N. Sivanathan, S. Silveira, C. Gauthier, D. Mallah, K. M. Pitts, A. A. Durao, S. Herron, H. Shorey, Y. Cheng, J. L. Barry, R. K. Krishnan, S. Wakelin, J. Rhee, A. Yung, M. Aronchik, C. Wang, N. Jain, X. Bao, E. Gerrits, N. Brouwer, A. Deik, D. G. Tenen, T. Ikezu, N. G. Santander, G. L. McKinsey, C. Baufeld, D. Sheppard, S. Krasemann, R. Nowarski, B. J. L. Eggen, C. Clish, R. E. Tanzi, C. Madore, T. D. Arnold, D. M. Holtzman, O. Butovsky, APOE4 impairs the microglial response in Alzheimer's disease by inducing TGF β -mediated checkpoints. *Nat. Immunol.* **24**, 1839–1853 (2023).
67. S. Brioschi, J. A. Belk, V. Peng, M. Molgora, P. F. Rodriguez, K. M. Nguyen, S. Wang, S. Du, W. L. Wang, G. E. Grajales-Reyes, J. M. Ponce, C. M. Yuede, Q. Li, J. M. Baer, D. G. DeNardo, S. Gillfillan, M. Cella, A. T. Satpathy, M. Colonna, A Cre-deleter specific for embryo-derived brain macrophages reveals distinct features of microglia and border macrophages. *Immunity* **56**, 1027–1045.e8 (2023).
68. M. C. G. Miranda, R. P. Oliveira, L. Torres, S. L. F. Aguiar, N. Pinheiro-Rosa, L. Lemos, M. A. Guimaraes, D. Reis, T. Silveira, E. Ferreira, T. G. Moreira, D. C. Cara, T. U. Maioli, B. L. Kelsall, D. Carlos, A. M. C. Faria, Frontline science: Abnormalities in the gut mucosa of non-obese diabetic mice precede the onset of type 1 diabetes. *J. Leukoc. Biol.* **106**, 513–529 (2019).
69. A. W. Bero, P. Yan, J. H. Roh, J. R. Cirrito, F. R. Stewart, M. E. Raichle, J. M. Lee, D. M. Holtzman, Neuronal activity regulates the regional vulnerability to amyloid- β deposition. *Nat. Neurosci.* **14**, 750–756 (2011).
70. F. Liao, T. J. Zhang, H. Jiang, K. B. Lefton, G. O. Robinson, R. Vassar, P. M. Sullivan, D. M. Holtzman, Murine versus human apolipoprotein E4: Differential facilitation of and co-localization in cerebral amyloid angiopathy and amyloid plaques in APP transgenic mouse models. *Acta Neuropathol. Commun.* **3**, 70 (2015).
71. A. Litvinchuk, J. H. Suh, J. L. Guo, K. Lin, S. S. Davis, N. Bien-Ly, E. Tycksen, G. T. Tabor, J. Remolina Serrano, M. Manis, X. Bao, C. Lee, M. Bosch, E. J. Perez, C. M. Yuede, A. G. Cashikar, J. D. Ulrich, G. Di Paolo, D. M. Holtzman, Amelioration of Tau and ApoE4-linked glial lipid accumulation and neurodegeneration with an LXR agonist. *Neuron* **112**, 384–403.e8 (2024).
72. S. Picelli, A. K. Bjorklund, O. R. Faridani, S. Sagasser, G. Winberg, R. Sandberg, Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nat. Methods* **10**, 1096–1098 (2013).
73. R. Patro, G. Duggal, M. I. Love, R. A. Irizarry, C. Kingsford, Salmon provides fast and bias-aware quantification of transcript expression. *Nat. Methods* **14**, 417–419 (2017).
74. P. Ewels, M. Magnusson, S. Lundin, M. Kaller, MultiQC: Summarize analysis results for multiple tools and samples in a single report. *Bioinformatics* **32**, 3047–3048 (2016).
75. M. I. Love, W. Huber, S. Anders, Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
76. A. Dobin, C. A. Davis, F. Schlesinger, J. Drenkow, C. Zaleski, S. Jha, P. Batut, M. Chaisson, T. R. Gingeras, STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
77. Y. Liao, G. K. Smyth, W. Shi, featureCounts: An efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923–930 (2014).
78. L. Wang, S. Wang, W. Li, RSeQC: Quality control of RNA-seq experiments. *Bioinformatics* **28**, 2184–2185 (2012).
79. M. D. Robinson, D. J. McCarthy, G. K. Smyth, edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139–140 (2010).
80. M. E. Ritchie, B. Phipson, D. Wu, Y. Hu, C. W. Law, W. Shi, G. K. Smyth, limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* **43**, e47 (2015).
81. R. Liu, A. Z. Holik, S. Su, N. Jansz, K. Chen, H. S. Leong, M. E. Blewitt, M. L. Asselin-Labat, G. K. Smyth, M. E. Ritchie, Why weight? Modelling sample and observational level variability improves power in RNA-seq analyses. *Nucleic Acids Res.* **43**, e97 (2015).
82. P. Langfelder, S. Horvath, WGCNA: An R package for weighted correlation network analysis. *BMC Bioinformatics* **9**, 559 (2008).
83. G. Yu, L.-G. Wang, Y. Han, Q.-Y. He, clusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS* **16**, 284–287 (2012).
84. A. Kramer, J. Green, J. Pollard Jr., S. Tugendreich, Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics* **30**, 523–530 (2014).
85. A. Subramanian, P. Tamayo, V. K. Mootha, S. Mukherjee, B. L. Ebert, M. A. Gillette, A. Paulovich, S. L. Pomeroy, T. R. Golub, E. S. Lander, J. P. Mesirov, Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 15545–15550 (2005).
86. R. Browaeys, W. Saelens, Y. Saeys, NicheNet: Modeling intercellular communication by linking ligands to target genes. *Nat. Methods* **17**, 159–162 (2020).
87. Y. Hao, S. Hao, E. Andersen-Nissen, W. M. Mauck III, S. Zheng, A. Butler, M. J. Lee, A. J. Wilk, C. Darby, M. Zager, P. Hoffman, M. Stoeckius, E. Papalexi, E. P. Mimitou, J. Jain, A. Srivastava, T. Stuart, L. M. Fleming, B. Yeung, A. J. Rogers, J. M. McElrath, C. A. Blish, R. Gottardo, P. Smibert, R. Satija, Integrated analysis of multimodal single-cell data. *Cell* **184**, 3573–3587.e29 (2021).
88. P.-L. Germain, A. Lun, C. Garcia Meixide, W. Macnair, M. D. Robinson, Doublet identification in single-cell sequencing data using scDblFinder. *F1000Res* **10**, 979 (2021).
89. L. Zappia, A. Oshlack, Clustering trees: A visualization for evaluating clusterings at multiple resolutions. *Gigascience* **7**, giy083 (2018).
90. H. Van Hove, L. Martens, I. Scheyltjens, K. De Vlaminck, A. R. Pombo Antunes, S. De Prijck, N. Vandamme, S. De Schepper, G. Van Isterdael, C. L. Scott, J. Aerts, G. Berx, G. E. Boeckxstaens, R. E. Vandenbroucke, L. Vereecke, D. Moechars, M. Guillemins, J. A. Van Ginderachter, Y. Saeys, K. Movahedi, A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. *Nat. Neurosci.* **22**, 1021–1035 (2019).

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Competing interests: O.B., I.I., H.L.W., Z.Y., and W.B. are coinventors on patent no. 17/914,061 held by Brigham and Women's Hospital and General Biophysics for the use of Xe to treat neurodegenerative diseases. O.B. is the cofounder and is on the scientific advisory board of Glial Therapeutics; collaborates with GSK and Regulus Therapeutics; has research funding from Sanofi and GSK; and consults for/has received honoraria from UCB, Camp4, Ono Pharma USA, and General Biophysics. D.M.H. cofounded and is on the scientific advisory board of C2N Diagnostics. D.M.H. is on the scientific advisory board of Denali, Genentech, and Cajal Neurosciences and consults for Asteroid Therapeutics. M.B.-J. cofounded and is on the scientific advisory board of NovoGlia Inc. I.I. is the founder and CEO of General Biophysics LLC. The other authors declare that they have no competing interests. **Data and materials availability:** All data associated with this study are in the paper or the Supplementary Materials. Sequence data and metadata, including fastq files used in the analysis, can be found in GEO with the accession number GSE277025. The code for analysis and generating the figures is available on Zenodo at DOI 10.5281/zenodo.14335083. APOE4 floxed knockin mice (E4 and TE4) are available from D.M.H. under a materials transfer agreement (MTA). Clec7a-Cre^{ERT2} mice are available from Cure Alzheimer's Fund under an MTA; requests should be directed to O.B. and Cure Alzheimer's Fund (grants@curealz.org).

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