DMTHB Ameliorates Memory Impairment in Alzheimer's Disease Mice Through Inhibition of IL-16 Signaling

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Abstract

Aim: Alzheimer's disease (AD) is one of the most prevalent neurodegenerative diseases. Growing evidence suggested that AD is associated with neuroinflammation, characterized with the chronic activation of microglial cells and astrocytes along with the subsequent excessive generation of the proinflammatory molecule. This study aimed to investigate the effect and molecular mechanism of IL-16 and Demethylenetetrahydroberberine (DMTHB) on Alzheimer's disease (AD).

Methods: AD mice model were made by intracranial injection of $A\beta_{25-35}$. Morris water maze (MWM) was applied to evaluate the capacity of learning and memory of mice. Moreover, concentrations of IL-6, IL-1β, and TNF-α in the brain tissue were measured by western blot. DMTHB-binding proteins were identified by mass spectrometry analysis. Pathological change and neuronal death were detected by HE staining and Nissl staining. The expressions of tau and Aβ in the brain tissue were measured by immunohistochemistry and Western blot.

Results: Our results showed that the cognition of AD mice was significantly improved after DMTHB administration. In addition, the content of p-Tau, Aβ and inflammatory cytokine (TNF-α IL-1β and IL-6) in the brains of AD mice were reduced by DMTHB treatment. The biotin-avidin system-based assay displayed that DMTHB can target and bind to inflammatory cytokine IL-16. Then, siRNA knockdown experiments demonstrated that interfering IL-16 expression can remarkably improve the cognitive ability and neuroinflammation in AD mice.

Conclusion: These results indicated that IL-16 is a potential therapeutic target for treatment of AD, and DMTHB has shown a potential natural medicine against AD through regulation of IL-16 signaling, including PI3K/Akt/GSK-3β/Tau pathway.

1. Introduction

Alzheimer Disease (AD) is one of the biggest health care challenges of the 21st century[1]. AD is a neurodegenerative disease characterized by two neuropathological hallmarks: extracellular deposition of amyloid plaques (Aβ) and intracellular neurofibrillary tangles (NFT)[2]. AD is a multi-factorial disease which is the result of interaction between genetic and environmental factors[3]. Within the last decade, neuroinflammation has shown to play a significant role in Alzheimer's disease (AD)[4, 5]. Much of knowledge regarding neuroinflammation is related to the pro-inflammatory cytokines, such as TNF-α, IL-1β and others, which are classically released by infiltrating immune cells within CNS during inflammatory CNS disorders[6, 7]. However, recent evidence indicates that CNS resident cells including astrocytes, microglia and neurons, also produce and release these cytokines[8]. Elevated levels of inflammatory cytokines are involved in disease initiation and progression of neuroinflammatory diseases including AD, Multiple Sclerosis (MS) and Parkinson's disease[9–11].

IL-16, an important inflammatory cytokine, was firstly identified as a T cell- derived cytokine that induces CD4-dependent migration and proliferation of immune cells[12]. Now, it was known that IL-16 is widely

expressed in a variety of immune and nonimmune cells[13, 14]. In addition to regulating the infiltration and differentiation of immune cells, IL-16 has also been reported to be with biological effects such as the inflammatory response and apoptosis[15, 16]. There is a close relationship between IL-16 and brain disorder[17]. In macrophages of AD patients, IL-16 level was significantly increased, as an expression of immune activation in response to the presence of β-amyloid deposits in AD patients[18, 19]. IL-16 is expressed by microglial cells and it might contribute to the recruitment of immune cells across the blood-brain barrier[19]. IL-16 is increased in AD patients, confirming that it may play an important role in the development and progression of AD. A few evidences displayed that IL-16 can stimulate the neuroinflammatory processes in AD and accelerate the progression of the disease. However, a previous study has shown that lymphocyte-derived IL-16 is neuroprotective against excitotoxicity. Interestingly, recent investigation indicated that recombinant IL-16 impairs neuronal excitability and synaptic activity in mouse primary hippocampal cultures in a CD4- and CD9-independent manner[20]. Therefore, the specific effect and mechanism of IL-16 on AD have not been fully defined. In this study, we focused on investigating the effect and molecular mechanism of IL-16 on AD in order to explore the possibility of IL-16 as a new therapeutic target against the neurodegenerative disease.

The currently approved treatments for AD are limited to cholinesterase inhibitors and memantine or the combination of these agents[21]. Therapies targeting amyloid β have been the focus for almost 30 years[22]. However, highly promising drugs recently failed to show clinical benefits in phase III trials. Currently, most anti-Tau agents in clinical trials are immunotherapies and they are in the early stages of clinical research[22]. The high failure rate of the therapies in development for AD stems in large part from the complex pathologic causes of the disease, as well as our incomplete understanding of the relationships among the numerous pathways involved in development of AD and subsequent neurodegeneration[23]. Thus, it has been a priority to develop new drug with the potential to change the progression of the disease.

Demethylenetetrahydroberberine (DMTHB) is a derivative of natural medicine berberine and is synthesized from the demethylenation and reduction of berberine in our lab. It has demonstrated that oral bioavailability and biosafety of demethylenetetrahydroberberine (DMTHB) were higher than its precursors of berberine (BBR) and demethyleneberberine (DMB) [24].It has also been reported that the anti-inflammatory and antioxidant activities of DMTHB were stronger than BBR and DMB [24]. The modulation of inflammatory response and amelioration of oxidative stress confer an advantage to the prevention and treatment of AD[25]. In the present study, the avidin/biotin system was applied to explore the therapeutic targets of DMTHB against AD[26]. DMTHB was covalently coupled with biotin to form the bait to find the targets. Interestingly, our results shown that DMTHB targeted and bound to IL-16, and AD processes can be ameliorated by knockdown of IL-16 expression. DMTHB ameliorated memory impairment in AD mice through inhibiting IL-16 signaling. These findings may provide new potential therapeutic target and agent for the treatment of AD in the future.

2. Materials And Methods

2.1 Materials

DMEM medium was purchased from Gibco Life Technologies (Grand Island, NY, USA). Fetal bovine serum was purchased from Gibco Life Technologies (Grand Island, NY, USA). $A\beta_{25-35}$ was purchased from GL Biochem Ltd. (Shanghai, China). $A\beta_{25-35}$ peptide was dissolved in distilled water at the concentration of 1 mg/ml, filtered to remove bacteria and incubated at 37 °C for 4 days to form aggregated $A\beta_{25-35}$ peptide Transfection reagent was purchased from Engreen Biotechnology company (Beijing, China). PEI was purchased from Shanghai Qifa Experimental Reagent Co., Ltd. (Shanghai, China). DMTHB was synthesized in our laboratory.

2.2 Animals

Adult male C57BL/6 mice (8 weeks) were purchased from the College of Veterinary Medicine, Yangzhou University (Yangzhou, China). These mice were housed in temperature- and humidity-controlled rooms. The mice were maintained at a 12-hour light/dark cycle and had free access to rodent chow and tap water. All procedures were approved by the Institutional Animal Care and Use Committee at China Pharmaceutical University and adhered to the Jiangsu Provincial Guidelines for the use of experimental animals.

2.3 Experimental mouse models

The mice were divided into four groups (8 mice in each group): control, $A\beta_{25-35}$, $A\beta_{25-35}$ + DMTHB (50 mg/kg) and $A\beta_{25-35}$ + DMTHB (150 mg/kg) groups. DMTHB was intragastrically administered every day for three weeks. The intracranial injection of $A\beta_{25-35}$ was conducted at the 4th week after DMTHB treatment. All mice were anesthetized with pentobarbital sodium (50 mg/kg). Then, the anesthetized mice were fixed on the stereotaxic apparatus (RWD Life Science Co., Ltd., Shenzhen, China). Small burr holes were drilled on two sides of the skull (1.0 mm posterior to bregma and 1.5 mm lateral to the midline) to allow intracerebroventricular (icv) injection of $A\beta_{25-35}$ (2 nmol / 2 μl) / saline at the depth of 2 mm. Control injection speed 1μL/min and leave the syringe for 10 minutes to allow the brain to absorb the injection. Oral medication was continued with DMTHB daily until the end of 6th week of the experiment. The specific operation is shown in Figure 1.

2.4 IL-16 siRNA interference in mice

The mice were recovered for a week after the $A\beta_{25-35}$ injection. Then, siRNA was injected intracranially every four days for a total of 2 injections. siRNA sequence of IL-16 is 5'-CCU UGG GUU AGA AUU UCC GAC UGC A-3'[27], The siRNA concentration is 2μg/μl, and the injection volume is 2μl. The specific operation is shown in Figure 2.

2.4 Morris water maze (MWM)

MWM test was carried out at the 6th week after administration of DMTHB. The water temperature of MWM was controlled between 24 and 26 °C during the experiment. The round pool was divided into four quadrants and the target platform. The mice received two consecutive training trials daily for 5 days of the acquisition training session. If a mouse did not find the platform within a period of 60 s, it was gently guided to and remained on the platform for 10 s. The escape latency time and swimming speed were recorded. The probe test was performed on the sixth day. In this test session, the platform was removed from the pool and each rat was allowed to swim for 60 s in the maze. The number of crossing place of the platform and the time spent in the target quadrant were recorded.

2.5 Cell culture

PC12 or BV2 were cultured in Cells DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin in a 5% CO_2 incubator at 37°C. $A\beta_{25-35}$ (0.06 μM) or LPS (200ng/ml) were added to cells for 6h. Then DMTHB was subsequently added to cells at final concentration of 20 μM for 24 h of incubation. For siRNA experiments, siRNA was added to BV-2 cells at final concentration of 100 nM before half hour of LPS addition. For experiment of IL-16, 10 ng IL-16 was added to BV-2 cells and PC12 cells at final concentration of 10 ng/ml.

2.6 pull-down assay and mass spectrometry

The streptavidin magnetic beads were incubated at 30 °C for nearly 30 min with biotinylated DMTHB which was synthesized in our lab. Then, the biotinylated DMTHB-coupled beads complex was incubated with brain tissue lysate at 4°C for nearly 12 h, and separated from the supernatant on at magnetic stand. Subsequently, the samples were sent to the protein mass spectrometry technology platform (Fudan University, Shanghai, China) for testing and analysis.

2.7 Histological examination and immunohistochemistry

The brain tissues were collected and fixed in 10% formalin, embedded in paraffin, cuted into 5μm section and were stained with hematoxylin and eosin (H&E), p-tau and Aβ in brain tissue was stained with immunohistochemistry detection (Servicebio Technology Co., Ltd., Wuhan, China). These stained sections were observed by a technician blinded to the experimental groups. Tissues were visualized in a Nikon Eclipse Ni-U microscope.

2.8 qRT-PCR

Total RNA was extracted from freshly isolated brain tissues and BV-12 macrophage cells using Trizol (10604ES60, Yeasen, China) following the kit's instructions. Isolated RNA was reverse-transcribed into cDNA with HiFiScript cDNA Synthesis Kit (1708891 Bio-rad, USA). The mRNA levels of CD86, MHC II, CD11b, CD16 and GAPDH were examined by qRT-PCR. The mRNA expression levels were displayed as fold change over that of the internal-control GAPDH. The following primer pairs were used: CD86, 5'- ACG ATG GAC CCC AGA TGC ACC A-3' and 5'- GCG TCT CCA CGG AAA CAG CA-3', MHC II, 5'- ACA GGA ATT

GTG TCC ACG GG -3’ and 5’- AAG GCC TGG GTC AGG GAT AA -3’, CD11b, 5’- GAG CAG CAC TGA GAT CCT GTT TAA -3’ and 5’- ATA CGA CTC CTG CCC TGG AA -3’, CD16, 5’- TGT GTG TCG TCG TAG ACG GT -3’ and 5’- TTC GCA CAT CAG TGT CAC CA -3’, GAPDH, 5’- AGG TCG GTG TGA ACG GAT TTG -3’ and 5’- TGT AGA CCA TGT AGT TGA GGT CA -3’,

2.9 Western blotting analysis

The mice brain tissue and BV-2 cells were washed three times with pre-cooled PBS. The total proteins of brain tissue were extracted using RIPA Lysis Buffer (20μL/mg tissue, Yeasen, China) following the manufacturer’s recommendations. Protein concentration was measured by BCA protein assay kit (B18020, Yeasen, China). Protein samples were separated by 10 % SDS-PAGE electrophoresis gels, then transferred to PVDF membranes, and incubated with 3% BSA for 90 minutes at room temperature. The membranes were washed in TBST buffer (0.1 % of Tween) for three times and incubated overnight at 4℃ with primary antibodies. IL-1β (ab200478, 1:1000), GSK 3β (ab32391, 1:1000) and AKT (ab38449, 1:1000) were purchased from Abcam, Bcl-2 (15071, 1:1000), IL-6 (12912, 1:1000), P-PI3K (17366, 1:1000), PI3K (4257, 1:1000), P-AKT (4060, 1:1000) and P-GSK (5558, 1:1000) were purchased from Cell Signaling Technology, Bax (WL01637 1:1000), Iba-1 (WL02406, 1:1000), Tau (WL03184, 1000), P-Tau (WL03540, 1000) and TNF-α (WL01581, 1:1000) were purchased from Wan Lei Biological Technology Co., Ltd. (Shenyang, China). GAPDH (60004, 1:10000) was purchased from proteintech (Chicago, USA). IL-16 (DF6600, 1:1000) was purchased from affinity. Before incubated with the second antibodies, these membranes should be washed in TBST buffer three times and fifteen minutes each time. Then the membranes were incubated with secondary antibodies conjugated with horseradish peroxidase (HRP) for 2h at room temperature. Proteins were visualized with normal or enhanced chemiluminescence detection kit (Tanon, China) and analyzed with Gel-pro Analyzer.

2.10 Statistical analysis

All data were represented as mean ± standard error of the mean (SEM). Student’s t-test and one-way ANOVA were applied to compare difference between two groups and multiple groups. Analyses were implemented by Graph Pad prism software (version5.0).

3. Results

3.1 DMTHB improves learning and memory in AD mice induced by $A\beta_{25-35}$.

The Morris water maze (MWM) is a classic experiment for behavioral assessments of the spatial learning and memory abilities of mice[28]. Mice from each group were subjected once daily to the MWM for a total of 6 days. Mice of model group had longer latency (about 41 seconds), which indicated that the spatial learning and memory of mice are significant decline. Meanwhile, the increased escape latency in the AD model mice was attenuated by DMTHB treatment (Figure 3A and 3B). However, there was no significant difference in swimming speed among the four groups of mice, indicating that the treatment with $A\beta_{25-35}$ and DMTHB did not affect the movement behavior of the mice (Figure 3C). The special exploration

capacity was investigated at day 6, the last experiment day. The time of escape latency in the AD model group were more than those in DMTHB treatment groups (Figure 3E). The number of crossing the place of the platform (Figure 3F) and the time spent in the target quadrant (Figure 3G) in the AD model group were less than those in the control group, and DMTHB-treated mice increased the crossing number and the swimming time in the target quadrant. Similarly, there was no difference in swimming speed among groups on the sixth day (Figure 3H). These results suggested that DMTHB can ameliorate $A\beta_{25-35}$-induced cognitive impairment.

3.2 DMTHB protects neuron against Tau pathology and apoptosis in $A\beta_{25-35}$-induced mice.

Phosphorylated Tau and apoptosis of neuron are the important neuropathological characters of AD[29]. The results show that $A\beta_{25-35}$ induces hippocampus neuron deformation and apoptosis, and promote the expression of Bax and inhibit the expression of Bcl2 (Figure 4A and 4F). The ratio of Bax to Bcl2 was reduced after DMTHB treatment (Figure 4F). These data clearly indicated that DMTHB treatment significantly improved hippocampus neuron deformation and apoptosis. And DMTHB treatment reduced the levels of the phosphorylated Tau induced by $A\beta_{25-35}$ in hippocampus (Figure 4B). More research has shown that GSK-3β plays a key role in neuropathological features such as Tau protein hyperphosphorylation and neuronal loss in AD, and phosphorylated-GSK-3β is an inactive form, which regulated by PI3K and Akt. The results showed that PI3K phosphorylation was observed in the group of DMTHB (Figure 4C). In addition, the phosphorylation of Akt and GSK-3β is also increased in the group of DMTHB (Figure 4D and 4E). Together, these data suggest that DMTHB protects neurons by reducing the phosphorylation of Tau protein and neuronal apoptosis through activation of PI3K-Akt- GSK-3β signaling.

3.3 DMTHB targets IL-16 molecule in AD model mice

To explore the binding target molecule of DMTHB on the treatment of AD, a chemical proteomics method of biotin-avidin system was used in this experiment. In general, the drug actions were accomplished by the interactions among drug and target proteins. The captured proteins were identified by mass-spectrometric analysis, the results show that DMTHB can target and bind to IL-16 (Figure 5A). Recently, IL-16 is known as a pro-inflammatory factor and is at a high level during the pathogenesis of neuroinflammation[30]. Then, IL-16 was detected in brain tissue by western blotting in our lab, the results show that the level of IL-16 was significantly increased in AD model mice group, however, it was closer to the control group in DMTHB treatment groups (Figure 5B). In addition, the typical proinflammatory cytokines, TNF-α, IL-1β and IL-6, are also increased in model group and significantly reduced in DMTHB groups (Figure 5C, 5D and 5E). These results suggested that IL-16 may serve as a novel therapeutic target against AD, and DMTHB may ameliorate neuroinflammation by targeting IL-16.

3.4 IL-16 is an important factor for neuroinflammation in cells model

Microglia play dual roles in neuroinflammation[31]. Small interference RNA (siRNA) against IL-16 was transduced to BV-2 microglia to reduce the expression of IL-16 (Figure 6A).BV-2 microglia were treated by LPS for 24 h to polarize them into M1 phenotype. The IL-16, IL-1β, and TNF-α expression were

significantly enhanced in BV-2 microglia induced by LPS, compared with that of BV-2 microglia without LPS exposure. Transfection with IL-16 siRNA reduced the LPS–induced expression of IL-16, IL-1β, and TNF-α (Figure 6B, 6C and 6D). In addition, the expressions of CD86, MHC II, CD11b and CD16 induced by LPS were also decreased after transfection with IL-16 siRNA (Figure 6E). It can be concluded that transfection with IL-16 siRNA can block the transformation of BV-2 microglia to M1 phenotype. In addition, the results showed that IL16 can induce BV-2 microglia to produce excessive amounts of IL-1β and TNF-α. Thereby, DMTHB bound to IL-16 to reduce neuroinflammation (Figure 6F). Taken together, our data show that IL-16 has an important function in regulating neuroinflammation and blockade of IL-16 ameliorates neuroinflammation, which further confirmed that IL-16 is a powerful therapeutic target for the treatment of AD in cell model.

3.5 Blockade of IL-16 ameliorates the learning and memory of AD mice induced by $A\beta_{25-35}$

To further verify the relationship between IL-16 and AD in AD mice models induced by icv-$A\beta_{25-35}$, IL-16 siRNA was injected into the brain of AD mice to knockdown the expression of IL-16 (Figure S3). Morris water maze experiment showed that mice of $A\beta_{25-35}$ +NC siRNA group had longer latency. Meanwhile, escape latency was increased in $A\beta_{25-35}$ +IL-16 siRNA group (Figure 7A and 7B). These data demonstrated that IL-16 plays a negative rule in the spatial learning and memory of mice in AD mice. In addition, there was no significant difference in swimming speed among groups, indicating that knockdown of IL-16 did not affect the movement behavior of the mice (Figure 7C). The special exploration capacity was investigated at day 6, the last experiment day. The time of escape latency in the $A\beta_{25-35}$ +NC siRNA group were longer and slower than those in the other group (Figure 7F). The number of crossing the place of the platform (Figure 7E) and the time spent in the target quadrant (Figure 7G) in the $A\beta_{25-35}$ +NC siRNA group were shorter and faster than those in the control group, and the crossing number and the swimming time in the target quadrant was significantly improved after icv-LI-16 siRNA. Similarly, there was no difference in swimming speed among groups at the sixth day (Figure 7H). These results suggested that IL-16 siRNA could serve as therapeutic agent to improve $A\beta_{25-35}$-induced cognitive impairment.

3.6 Blockade of IL-16 suppresses neuroinflammation in AD model mice

AD is characteristics of chronic neuroinflammation in the brain of AD mice, which includes multiple proinflammatory cytokines[32]. Western blotting analysis showed that IL-16 was obviously increased in brain of AD model mice, but reduced in IL-16 siRNA-transfected AD model mice (Figure 8A). Iba-1 is a biomarker of microglial activation and Iba-1^+ microglia as a marker of neuroinflammation. The expression of Iba-1 was significantly increased in AD model mice and injection of IL-16 siRNA inhibited the expression of Iba-1 (Figure 8B). In addition, other proinflammatory factors in the AD model were also significantly reduced after transfection of siRNA, such as IL-1β, IL-6 and TNF-α (Figure 8C, 8D and 8E). Taken together, the results clearly demonstrated that knockdown of IL-16 ameliorated neuroinflammation in AD model mice.

3.7 Blockade of IL-16 protects neuron against Tau tangles and apoptosis in $A\beta_{25-35}$-induced mice.

Hyperphosphorylation of tau and subsequent accumulation of tau tangles in brain are a major pathological hallmark of AD[33]. And PI3K/AKT/GSK-3β signaling pathway is well-known to be critical in Tau pathology[34]. The result shows the tau tangles in the model group is statistically higher than others group, but p-Tau content was significantly decreased by interference with IL-16 siRNA (Figure 9A). Western blot demonstrated that IL-16 siRNA ameliorated PI3K/Akt/GSK signaling, compared with NC siRNA (Figure 9B, 9C and 9D). Correspondingly, the content of Aβ oligomer in the brain of the model group is higher. compared with others group, and the content of Aβ oligomer is reduced after IL-16 interference (Figure 9E). Aβ oligomer and p-tau are important biomarkers in the AD samples. After $A\beta_{25-35}$ induction, the neurons were significantly impaired and were improved by IL-16 siRNA treatment (Figure 9F). Together, these data demonstrate that IL-16 siRNA suppresses Tau phosphorylation through the PI3K/Akt/GSK-3β pathway. In addition, IL-16 siRNA suppressed $A\beta_{25-35}$-induced apoptosis in brain of mice and enhanced protection of neuronal cells. The results indicated that IL-16 siRNA promoted the expression of Bcl2 and inhibited the expression of Bax (Figure 9G). Therefore, blockage of IL-16 expression can reduce neuronal cell apoptosis.

4. Discussion

Alzheimer's disease (AD) is a chronic neurodegenerative disease that is characterized by progressive memory loss and cognitive disorder[35]. Inflammation processes and cytokines play an important role in pathogenesis of AD. Damaged tissues and β-amyloid plaque are the main inflammation irritants in Alzheimer's patient brain[36]. In this study, it has been shown that DMTHB ameliorates memory impairment in Alzheimer's disease mice through inhibition of IL-16 signaling to reduce neuroinflammation. IL-16 could be a new potential target for the development of medicine against AD.

IL-16 was identified as a T cell-derived cytokine that induces CD_4-dependent migration and proliferation of immune cells,but we now know that IL-16 is also produced by other immune cells including B cells and monocytes[27]. Recent studies have proposed that IL-16 plays a role in AD, relapsing MS and experimental autoimmune encephalomyelitis[37]. IL16 is one of pro-inflammatory cytokines, which is observed in inflammation pathology around the β-amyloid plaque in AD patients[38]. However, there is a Neuronal IL-16 (NIL-16) which is selectively expressed in hippocampal and cerebellar neurons. The neuronal variant (NIL-16) exists as a longer splice variant of pro-IL-16, which is cleaved by caspase-3 and results in the release of mature NIL-16 with two extra PDZ domains in the N-terminal region of IL-16[39]. Furthermore, NIL-16 induces the upregulation of the transcription factor c-fos and enhances neurite outgrowth and interacts with neurotransmitter receptors and several ion channel proteins[40]. Interestingly, there are different functions on neurons between IL-16 and NIL-16. Therefore, this study focused on exploring the possibility of IL-16 as a new therapeutic target for development of AD drugs.

IL-16 plays a critical role in the pathogenesis of the onset of AD[19]. However, there is not any medicine targeting IL-16 to treat AD on the market in the current results. We have firstly to determine whether IL-16

could be a therapeutic target for treatment of AD. However, some researchers have used IL-16 antibodies to improve LPS-induced heart inflammation and block IL-16 expression to improve DSS-induced intestinal inflammation[41]. The above research shows that targeting IL-16 could disable the inflammatory response in body. It is possible that knockdown of IL-16 can improve the pathological features of AD and spatial memory and learning ability in AD mice. In this article, by using the siRNA approach, IL-16 siRNA was used to interfere with the expression of IL-16 in vivo and in vitro. The results show that the interference effect is obvious, so it is feasible to use siRNA to block the expression of IL-16 (Fig. 8A). We used IL-16 siRNA to locally block the expression of IL-16 in the brains of AD mice, and found that the spatial recognition and cognitive abilities of the AD mice were significantly improved (Fig. 7A). Besides, IL-16 siRNA can improve the inflammatory effect in LPS-induced BV-2 cells (Fig. 6). Thus, we can conclude that IL-16 siRNA can ameliorate AD-related pathological symptoms and suppresses tau phosphorylation through PI3K/Akt/GSK-3β pathway (Fig. 9). Therefore, IL-16 could be a potential therapeutic target for treatment of AD.

Polypharmacology has emerged as an essential paradigm for modern drug discovery process. Ample evidence suggests that agents capable of modulating multiple targets in a selective manner may offer better therapeutic efficacy than single-targeted agents. AD is a multifactorial disease related to complex pathophysiological characteristics, including protein aggregation, oxidative stress and neuroinflammation, which finally lead to the loss of neuronal functions and cognitive impairments[42]. There is still no effective drug treatment of AD. DMTHB is an isoquinoline alkaloid with catechin structure, which is a derivative of natural products berberine and demethyleneberberine and maintains the characteristics of polypharmacology and multiple targets. Our previous study has reported that DMTHB has antioxidant, anti-inflammatory, anti-apoptotic properties. In this study, our data indicate that DMTHB can target and bind to IL-16 through pull-down assay and mass spectrometry analysis. In addition, DMTHB can also decrease IL-16 expression (Fig. 5B), this may result from the features of DMTHB multiple targets. Therefore, it has confirmed that DMTHB ameliorates memory impairment in Alzheimer's disease mice through inhibition of IL-16 signaling, including PI3K/Akt/GSK-3β/Tau pathway (Fig. 4 and Fig. 9). In addition, the results indicate that excessive IL16 will induce nerve cells to increase Aβ expression and activate the PI3K/Akt/GSK-3β/Tau pathway (Fig.S5). By the way, we also found that DMTHB can bind other therapeutic targets, including Dopamine receptor, Coronin-2A and C-Jun-amino-terminal kinase-interacting protein. However, there are literature reports that IL-16 is associated with AD, but has not been explored in depth. Therefore, this study focused on exploring the specific molecular pharmacological mechanism of IL-16 in the treatment of AD. Finally, IL-16 was identified as new therapeutic target and DMTHB is a potential medicine for the treatment of AD in the future.

Abbreviations

AD, Alzheimer's Disease; DMTHB, demethylenetetrahydroberberine; DMB, demethyleneberberine; MS, Multiple Sclerosis; PD, Parkinson's disease; IL-16, Interleukin-16; TNF-α, tumor necrosis factor-α; IL-1β, Interleukin-1β; IL-6, Interleukin-6; MWM, Morris water maze; qRT-PCR, quantitative Real-time PCR; PBS,

phosphate-buffered saline; LDH, Lactate dehydrogenase; ROS, reactive oxygen species; TBST, Tris-buffered saline with Tween 20. BSA, bovine serum albumin.

Declarations

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Authors' contributions

Participated in research design: Yuanqiang. Zhang, Yubin. Zhang.

Conducted experiments: Yuanqiang Zhang, Dongqing Liu, Xutao Yao

Performed data analysis: Yuanqiang Zhang, Dongqing Liu.

Wrote or contributed to the writing of the manuscript: Yuanqiang. Zhang, Yubin. Zhang.

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Availability of data and materials

All data generated and materials supporting the conclusion of the study are included within the article and its supplementary information files

Ethics approval and consent to participate

All procedures were approved by the Institutional Animal Care and Use Committee at China Pharmaceutical University and adhered to the Jiangsu Provincial Guidelines for the use of experimental animals.

Consent for publication

Not applicable

Competing interests

All authors declare that there are no conflicts of interest.

References

1. Avila J, Perry G: **A Multilevel View of the Development of Alzheimer's Disease**. *Neuroscience* 2021, **457**:283-293. DOI: 10.1016/j.neuroscience.2020.11.015
2. Knopman DS, Amieva H, Petersen RC, Chetelat G, Holtzman DM, Hyman BT, Nixon RA, Jones DT: **Alzheimer disease**. *Nat Rev Dis Primers* 2021, **7**(1):33. DOI: 10.1038/s41572-021-00269-y
3. Huang Y, Sun X, Jiang H, Yu S, Robins C, Armstrong MJ, Li R, Mei Z, Shi X, Gerasimov ES *et al*: **A machine learning approach to brain epigenetic analysis reveals kinases associated with Alzheimer's disease**. *Nat Commun* 2021, **12**(1):4472. DOI: 10.1038/s41467-021-24710-8
4. Dekens DW, Eisel ULM, Gouweleeuw L, Schoemaker RG, De Deyn PP, Naude PJW: **Lipocalin 2 as a link between ageing, risk factor conditions and age-related brain diseases**. *Ageing Res Rev* 2021, **70**:101414. DOI: 10.1016/j.arr.2021.101414
5. Hartnell IJ, Blum D, Nicoll JAR, Dorothee G, Boche D: **Glial cells and adaptive immunity. in frontotemporal dementia with tau pathology**. *Brain* 2021, **144**(3):724-745. DOI: 10.1093/brain/awaa457
6. Startin CM, Ashton NJ, Hamburg S, Hithersay R, Wiseman FK, Mok KY, Hardy J, Lleo A, Lovestone S, Parnetti L *et al*: **Plasma biomarkers for amyloid, tau, and cytokines in Down syndrome and sporadic Alzheimer's disease**. *Alzheimers Res Ther* 2019, **11**(1):26. DOI: 10.1186/s13195-019-0477-0
7. Nakandakari S, Munoz VR, Kuga GK, Gaspar RC, Sant'Ana MR, Pavan ICB, da Silva LGS, Morelli AP, Simabuco FM, da Silva ASR *et al*: **Short-term high-fat diet modulates several inflammatory, ER stress, and apoptosis markers in the hippocampus of young mice**. *Brain Behav Immun* 2019, **79**:284-293. DOI: 10.1016/j.bbi.2019.02.016
8. Rahimifard M, Maqbool F, Moeini-Nodeh S, Niaz K, Abdollahi M, Braidy N, Nabavi SM, Nabavi SF: **Targeting the TLR4 signaling pathway by polyphenols: A novel therapeutic strategy for neuroinflammation**. *Ageing Res Rev* 2017, **36**:11-19. DOI: 10.1016/j.arr.2017.02.004
9. Willette AA, Pappas C, Hoth N, Wang Q, Klinedinst B, Willette SA, Larsen B, Pollpeter A, Li T, Le S *et al*: **Inflammation, negative affect, and amyloid burden in Alzheimer's disease: Insights from the kynurenine pathway**. *Brain Behav Immun* 2021, **95**:216-225. DOI: 10.1016/j.bbi.2021.03.019
10. Wang A, Rojas O, Lee D, Gommerman JL: **Regulation of neuroinflammation by B cells. and plasma cells**. *Immunol Rev* 2021, **299**(1):45-60. DOI: 10.1111/imr.12929
11. Hirsch EC, Hunot S: **Neuroinflammation in Parkinson's disease: a target for. neuroprotection?** *Lancet Neurol* 2009, **8**(4):382-397. DOI: 10.1016/S1474-4422(09)70062-6
12. Cruikshank W, Little F: **Interleukin-16: the ins and outs of regulating T-cell activation**.*Crit Rev Immunol* 2008, **28**(6):467-483. DOI: 10.1615/critrevimmunol.v28.i6.10
13. Jana M, Pahan K: **IL-12 p40 homodimer, but not IL-12 p70, induces the expression. of IL-16 in microglia and macrophages**. *Mol Immunol* 2009, **46**(5):773-783. DOI: 10.1016/j.molimm.2008.10.033

14. Liu S, Lei Z, Li J, Wang L, Jia R, Liu Z, Jiang C, Gao Y, Liu M, Kuang L *et al*: **Interleukin 16. contributes to gammaherpesvirus pathogenesis by inhibiting viral reactivation**. *PLoS Pathog* 2020, **16**(7):e1008701. DOI: 10.1371/journal.ppat.1008701
15. Chibowska K, Baranowska-Bosiacka I, Falkowska A, Gutowska I, Goschorska M, Chlubek. D: **Effect of Lead (Pb) on Inflammatory Processes in the Brain**. *Int J Mol Sci* 2016, **17**(12). DOI: 10.3390/ijms17122140
16. Wilson KC, Center DM, Cruikshank WW: **The effect of interleukin-16 and its precursor. on T lymphocyte activation and growth**. *Growth Factors* 2004, **22**(2):97-104. DOI: 10.1080/08977190410001704679
17. Skundric DS: **Basic Approaches in Therapy of Multiple Sclerosis (MS) and Related. Diseases: Current Achievement and Prospective**. *Cent Nerv Syst Agents Med Chem* 2018, **18**(1):21-31. DOI: 10.2174/1871524916666161018102254
18. Liu Y, Zhou R, Sulman EP, Scheurer ME, Boehling N, Armstrong GN, Tsavachidis S, Liang.FW, Etzel CJ, Conrad CA *et al*: **Genetic Modulation of Neurocognitive Function in Glioma Patients**. *Clin Cancer Res* 2015, **21**(14):3340-3346. DOI: 10.1158/1078-0432.CCR-15-0168
19. Anvar NE, Saliminejad K, Ohadi M, Kamali K, Daneshmand P, Khorshid HR: **Association. between polymorphisms in Interleukin-16 gene and risk of late-onset Alzheimer's disease**. *J Neurol Sci* 2015, **358**(1-2):324-327. DOI: 10.1016/j.jns.2015.09.344
20. Hridi SU, Franssen A, Jiang HR, Bushell TJ: **Interleukin-16 inhibits sodium channel. function and GluA1 phosphorylation via CD4- and CD9-independent mechanisms to reduce hippocampal neuronal excitability and synaptic activity**. *Mol Cell Neurosci* 2019, **95**:71-78. DOI: 10.1016/j.mcn.2019.01.002
21. Cummings JL, Tong G, Ballard C: **Treatment Combinations for Alzheimer's Disease: Current and Future Pharmacotherapy Options**. *J Alzheimers Dis* 2019, **67**(3):779-794. DOI: 10.3233/JAD-180766
22. Vaz M, Silvestre S: **Alzheimer's disease: Recent treatment strategies**. *Eur J Pharmacol.* 2020, **887**:173554. DOI: 10.1016/j.ejphar.2020.173554
23. Mangialasche F, Solomon A, Winblad B, Mecocci P, Kivipelto M: **Alzheimer's disease: clinical trials and drug development**. *Lancet Neurol* 2010, **9**(7):702-716. DOI: 10.1016/S1474-4422(10)70119-8
24. Zhang Y, Wen J, Liu D, Qiu Z, Zhu Q, Li R, Zhang Y: **Demethylenetetrahydroberberine. alleviates nonalcoholic fatty liver disease by inhibiting the NLRP3 inflammasome and oxidative stress in mice**. *Life Sci* 2021, **281**:119778. DOI: 10.1016/j.lfs.2021.119778
25. Di Bona D, Scapagnini G, Candore G, Castiglia L, Colonna-Romano G, Duro G, Nuzzo D,Iemolo F, Lio D, Pellicano M *et al*: **Immune-inflammatory responses and oxidative stress in Alzheimer's disease: therapeutic implications**. *Curr Pharm Des* 2010, **16**(6):684-691. DOI: 10.2174/138161210790883769
26. Lesch HP, Kaikkonen MU, Pikkarainen JT, Yla-Herttuala S: **Avidin-biotin technology in.targeted therapy**. *Expert Opin Drug Deliv* 2010, **7**(5):551-564. DOI: 10.1517/17425241003677749
27. Yang HY, Kim J, Kim SH, Choe CH, Jang YS: **Pro-IL-16 is associated with MHC class II-mediated negative regulation of mouse resting B cell activation through MAP kinases, NF-kappaB and Skp2-dependent p27kip regulation**. *Scand J Immunol* 2013, **77**(3):177-186. DOI: 10.1111/sji.12026

28. Lee HJ, Choi TI, Kim YM, Lee S, Han B, Bak IS, Moon SA, Yu DY, Shin KS, Kwon YK *et al*: **Regulation of habenular G-protein gamma 8 on learning and memory via modulation of the central acetylcholine system**. *Mol Psychiatry* 2020. DOI: 10.1038/s41380-020-00893-2
29. Zhao J, Fu Y, Yamazaki Y, Ren Y, Davis MD, Liu CC, Lu W, Wang X, Chen K, Cherukuri Y. *et al*: **APOE4 exacerbates synapse loss and neurodegeneration in Alzheimer's disease patient iPSC-derived cerebral organoids**. *Nat Commun* 2020, **11**(1):5540. DOI: 10.1038/s41467-020-19264-0
30. Lepennetier G, Hracsko Z, Unger M, Van Griensven M, Grummel V, Krumbholz M, Berthele A, Hemmer B, Kowarik MC: **Cytokine and immune cell profiling in the cerebrospinal fluid of patients with neuro-inflammatory diseases**. *J Neuroinflammation* 2019, **16**(1):219. DOI: 10.1186/s12974-019-1601-6
31. Cheng J, Dong Y, Ma J, Pan R, Liao Y, Kong X, Li X, Li S, Chen P, Wang L *et al*: **Microglial. Calhm2 regulates neuroinflammation and contributes to Alzheimer's disease pathology**. *Sci Adv* 2021, **7**(35). DOI: 10.1126/sciadv.abe3600
32. Cai X, Zhang K, Xie X, Zhu X, Feng J, Jin Z, Zhang H, Tian M, Chen H: **Self-assembly. hollow manganese Prussian white nanocapsules attenuate Tau-related neuropathology and cognitive decline**. *Biomaterials* 2020, **231**:119678. DOI: 10.1016/j.biomaterials.2019.119678
33. Hampel H, Cummings J, Blennow K, Gao P, Jack CR, Jr., Vergallo A: **Developing the. ATX(N) classification for use across the Alzheimer disease continuum**. *Nat Rev Neurol* 2021, **17**(9):580-589. DOI: 10.1038/s41582-021-00520-w
34. Yoon JH, Lee N, Youn K, Jo MR, Kim HR, Lee DS, Ho CT, Jun M: **Dieckol Ameliorates. Abeta Production via PI3K/Akt/GSK-3beta Regulated APP Processing in SweAPP N2a Cell**. *Mar Drugs* 2021, **19**(3). DOI: 10.3390/md19030152
35. Maestu F, de Haan W, Busche MA, DeFelipe J: **Neuronal excitation/inhibition. imbalance: core element of a translational perspective on Alzheimer pathophysiology**. *Ageing Res Rev* 2021, **69**:101372. DOI: 10.1016/j.arr.2021.101372
36. Wyss-Coray T, Lin C, Yan F, Yu GQ, Rohde M, McConlogue L, Masliah E, Mucke L: **TGF-beta1 promotes microglial amyloid-beta clearance and reduces plaque burden in transgenic mice**. *Nat Med* 2001, **7**(5):612-618. DOI: 10.1038/87945
37. Skundric DS, Cruikshank WW, Drulovic J: **Role of IL-16 in CD4+ T cell-mediated. regulation of relapsing multiple sclerosis**. *J Neuroinflammation* 2015, **12**:78. DOI: 10.1186/s12974-015-0292-x
38. Rakic S, Hung YMA, Smith M, So D, Tayler HM, Varney W, Wild J, Harris S, Holmes C, Love S *et al*: **Systemic infection modifies the neuroinflammatory response in late stage Alzheimer's disease**. *Acta Neuropathol Commun* 2018, **6**(1):88. DOI: 10.1186/s40478-018-0592-3
39. Fenster CP, Fenster SD, Leahy HP, Kurschner C, Blundon JA: **Modulation of Kv4.2 K+. currents by neuronal interleukin-16, a PDZ domain-containing protein expressed in the hippocampus and cerebellum**. *Brain Res* 2007, **1162**:19-31. DOI: 10.1016/j.brainres.2007.05.051
40. Fenster CP, Chisnell HK, Fry CR, Fenster SD: **The role of CD4-dependent signaling in interleukin-16 induced c-Fos expression and facilitation of neurite outgrowth in cerebellar granule neurons**. *Neurosci Lett* 2010, **485**(3):212-216. DOI: 10.1016/j.neulet.2010.09.014

41. Zhang J, Yang Z, Liang Z, Wang M, Hu C, Chang C, Shi L, Ji Q, Liu L: **Anti-Interleukin- 16 Neutralizing Antibody Treatment Alleviates Sepsis-Induced Cardiac Injury and Dysfunction via the Nuclear Factor Erythroid-2 Related Factor 2 Pathway in Mice**. *Oxid Med Cell Longev* 2021, **2021**:6616422. DOI: 10.1155/2021/6616422
42. Koutsodendris N, Nelson MR, Rao A, Huang Y: **Apolipoprotein E and Alzheimer's Disease: Findings, Hypotheses, and Potential Mechanisms**. *Annu Rev Pathol* 2021. DOI: 10.1146/annurev-pathmechdis-030421-112756

Figures

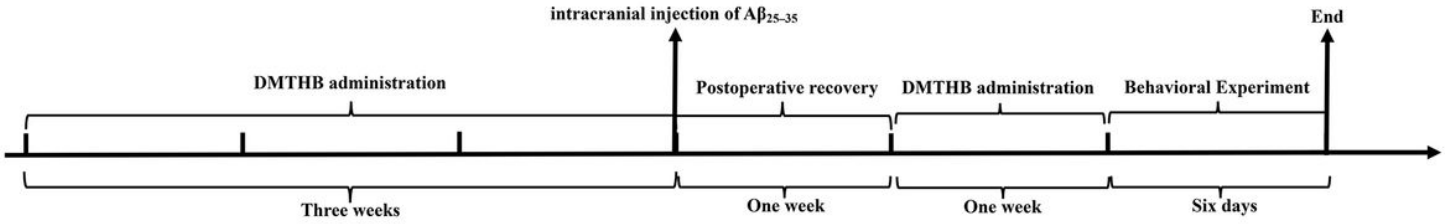

Figure 1

Experiments of AD mouse model induced by Aβ25–35 and and DMTHB treamtment

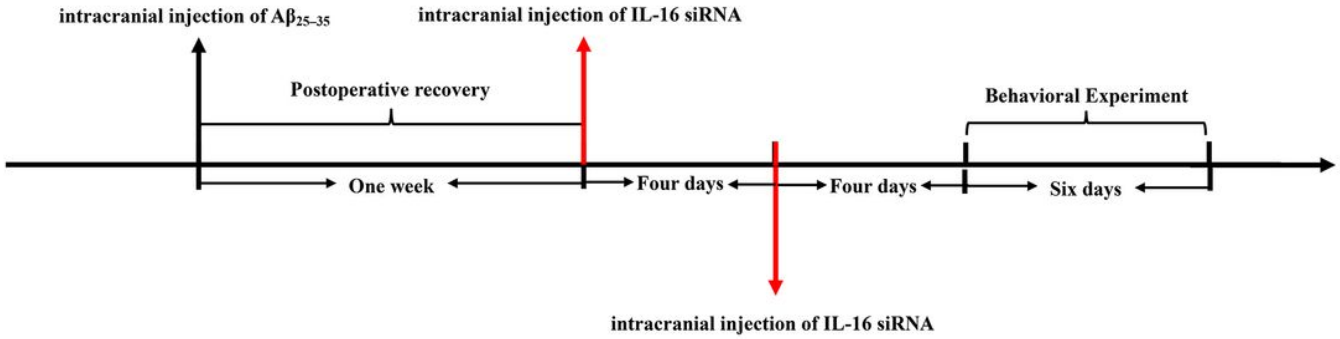

Figure 2

IL1-6 siRNA interference in mice induced by Aβ25–35

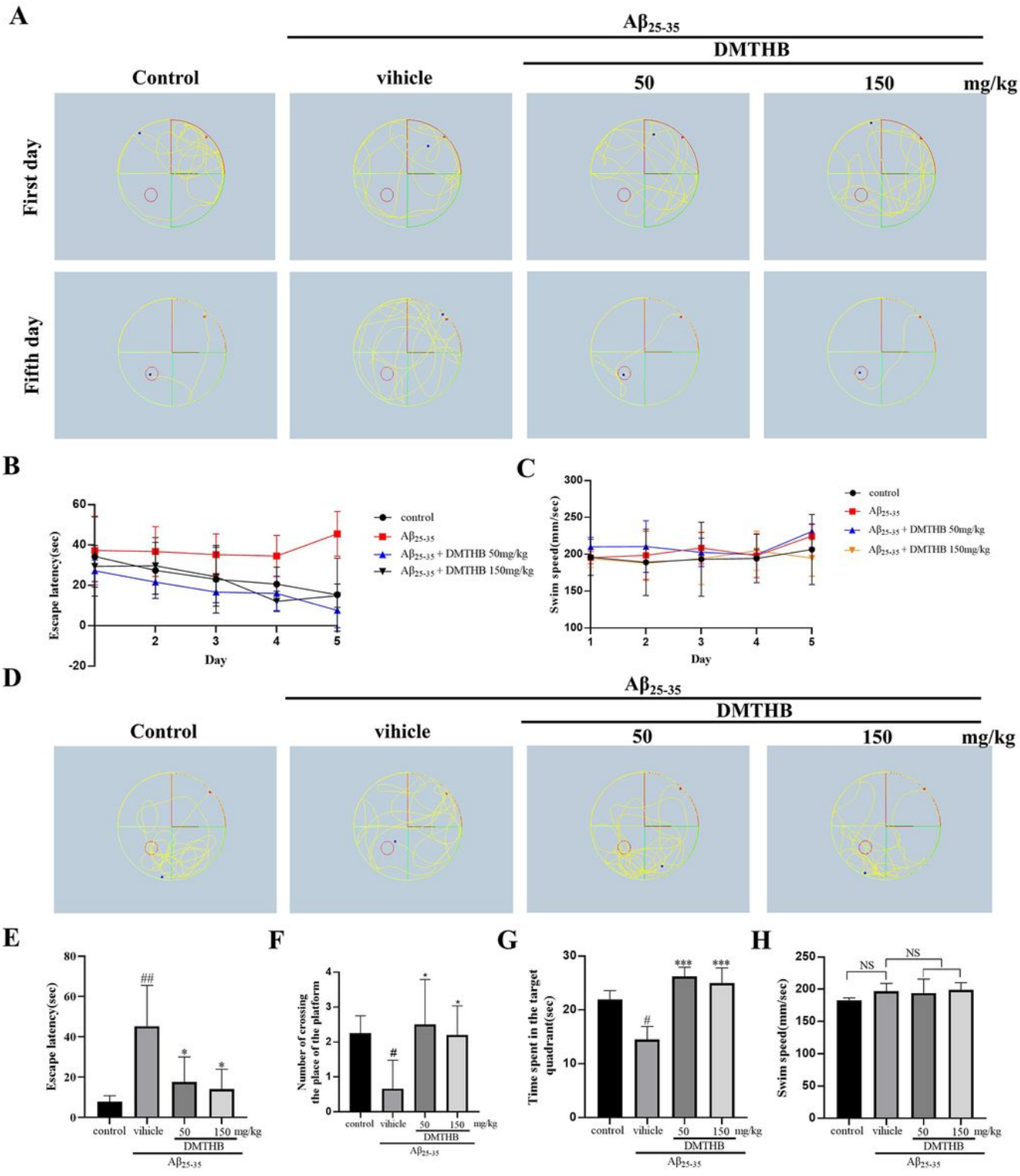

Figure 3

The beneficial effect of DMTHB on learning and memory in AD mice models induced by icv-Aβ25–35. The MWM was conducted for testing the learning and memory abilities of mice in different groups. n = 8. The representative swim paths (A), the escape latency (B), and average swimming speed (C) in training trials, and the representative swim paths (D), the escape latency (E), the number of crossing the place of the platform (F), time spent in the target quadrant (G), and average swimming speed (H) in spatial probe

test were shown. # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the control group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the Aβ25–35 group.

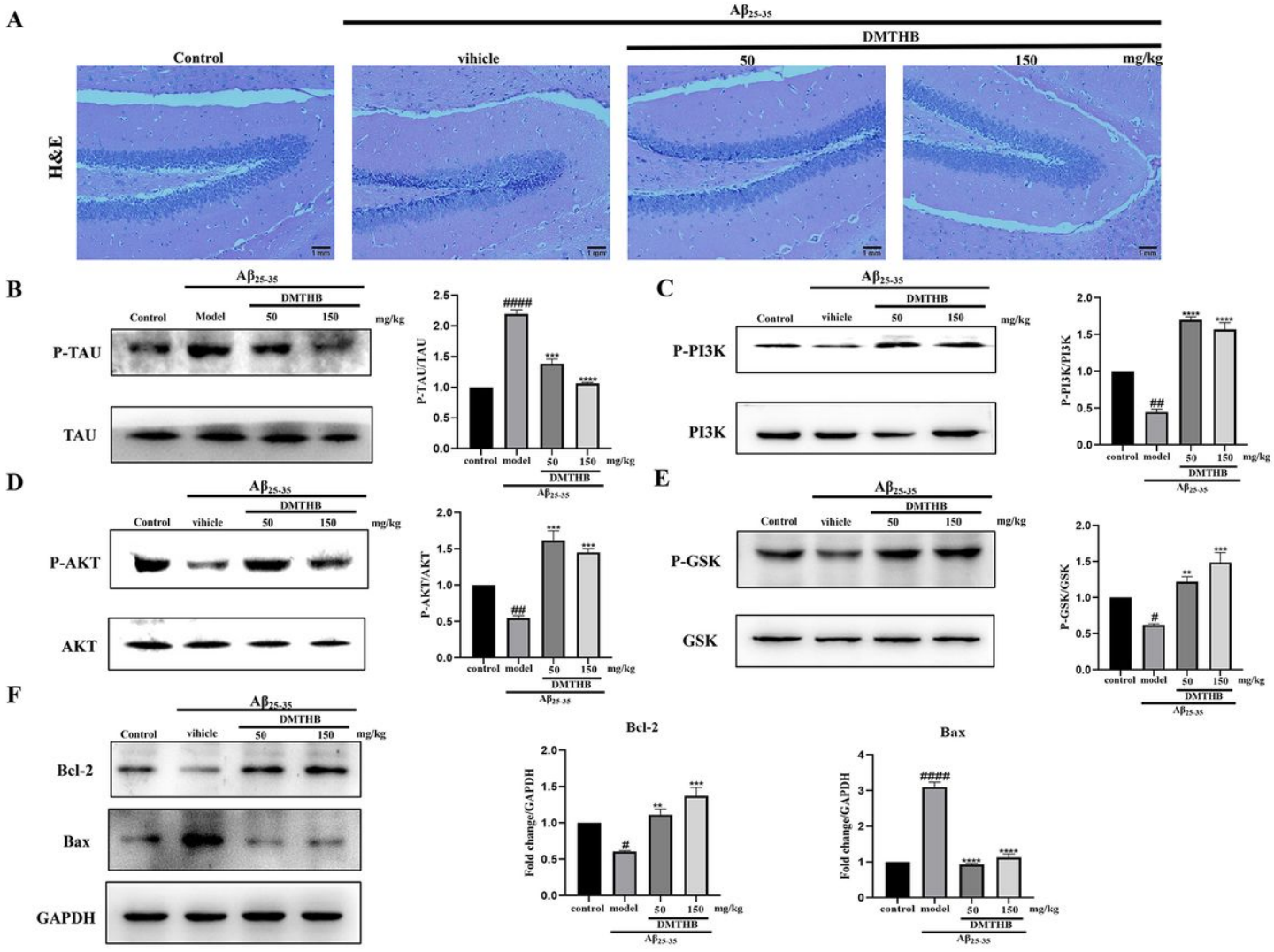

Figure 4

DMTHB protects neurons against Tau pathology and apoptosis in Aβ25–35-induced mice. (A) H&E staining of brain tissue of each group. (B) Representative immunoblots of p-Tau / Tau expression in brain. (C-E) Representative immunoblots of p-PI3K / PI3K, p-AKT / AKT and p-GSK / GSK expression in brain. (F) Representative immunoblots of Bcl-2 and Bax, GAPDH was used as loading control. n=8 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the control group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the Aβ25–35 group.

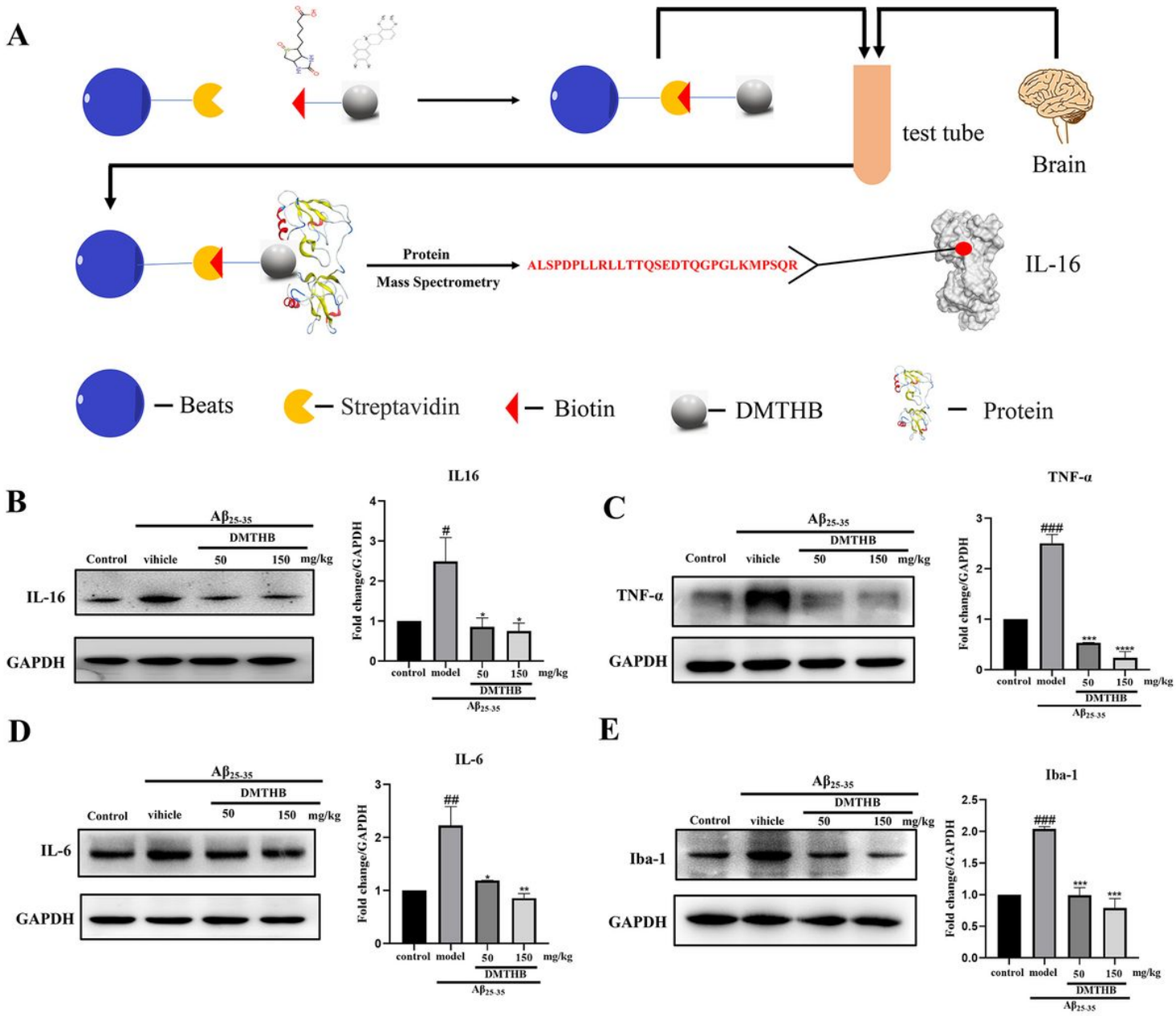

Figure 5

DMTHB targets IL-16 to ameliorates neuroinflammation in AD model mice. (A) Schematic diagram for screening of DMTHB target. (B-E) Representative immunoblots of IL-16, TNF-α, IL-6 and Iba-1, GAPDH was used as loading control. n=8 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the control group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the Aβ25–35 group.

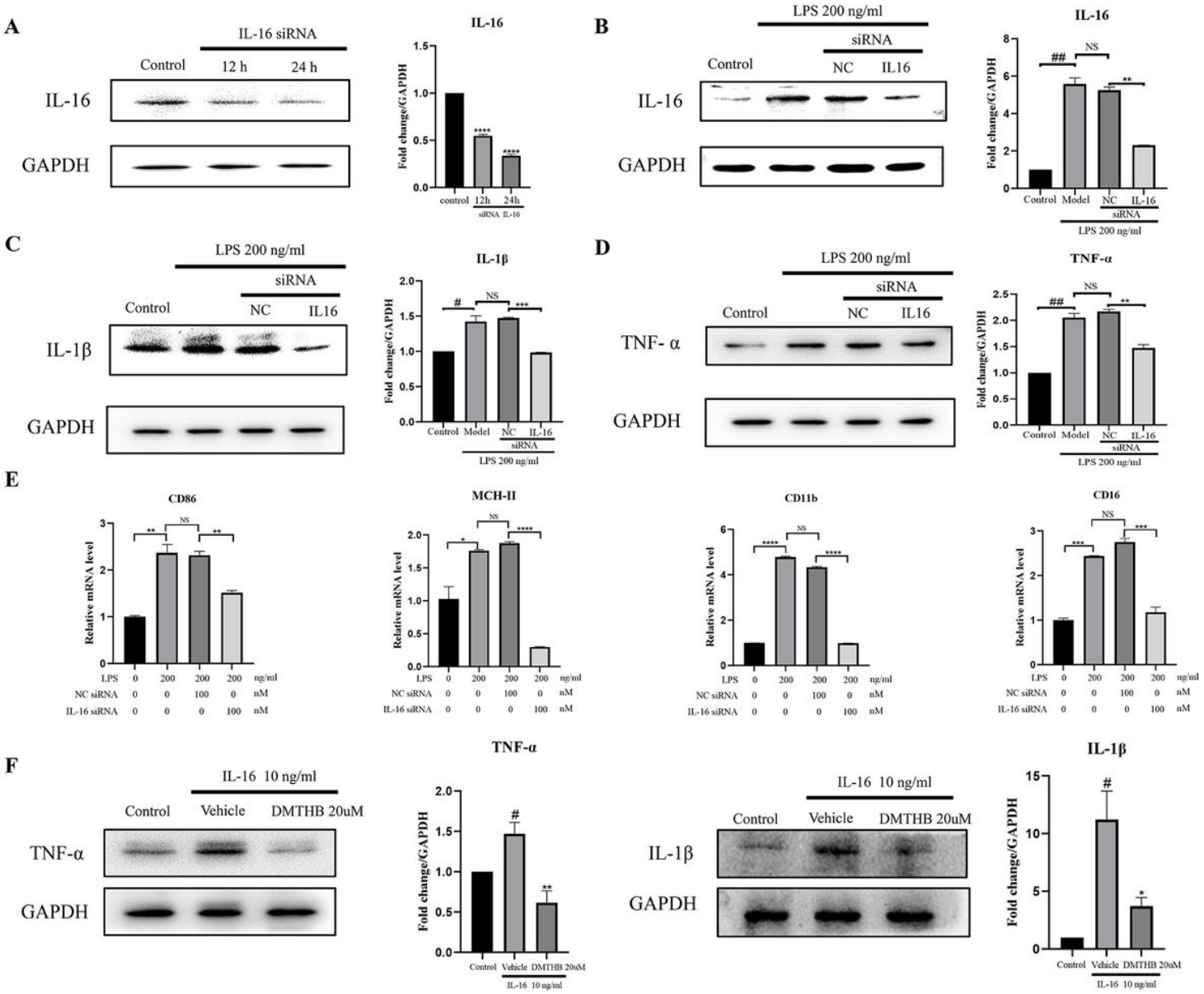

Figure 6

IL-16 is important factor for neuroinflammation in cell model. (A) Representative immunoblot of IL-16 in BV-2 cells treated with IL-16 siRNA. n=3 per group, **** P < 0.0001 compared with the control group. (B-D) Representative immunoblot of IL-16, IL-1β, and TNF-α in BV-2 cells induced by LPS. (E) Relative mRNA levels of CD86, MHC II, CD11b and CD16 in BV-2 cells induced by LPS. n=3 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the control group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the NC siRNA group. (F) Representative immunoblot of IL-1β and TNF-α in BV-2 cells induced by IL-16. n=3 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the control group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the Vehicle group.

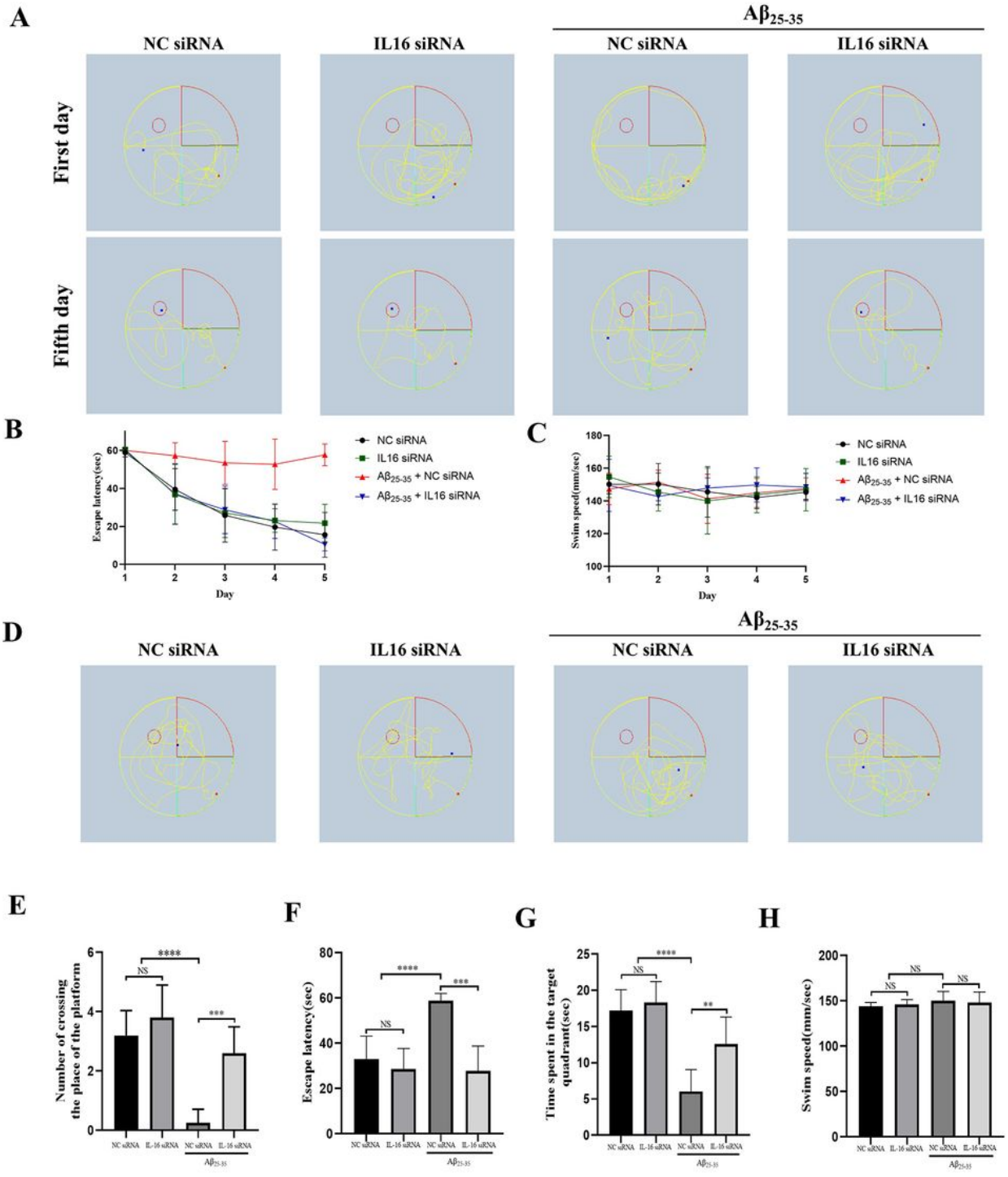

Figure 7

Blockade of IL-16 ameliorates the learning and memory of AD mice models induced by icv-Aβ25–35. The MWM was conducted for testing the learning and memory abilities of mice in different groups. n = 8. The representative swim paths (A), the escape latency (B), and average swimming speed (C) in training trials, and the representative swim paths (D), the number of crossing the place of the platform (E), the escape

latency (F), time spent in the target quadrant (G), and average swimming speed (H) in spatial probe test were shown. * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the NC siRNA + Aβ25–35 group.

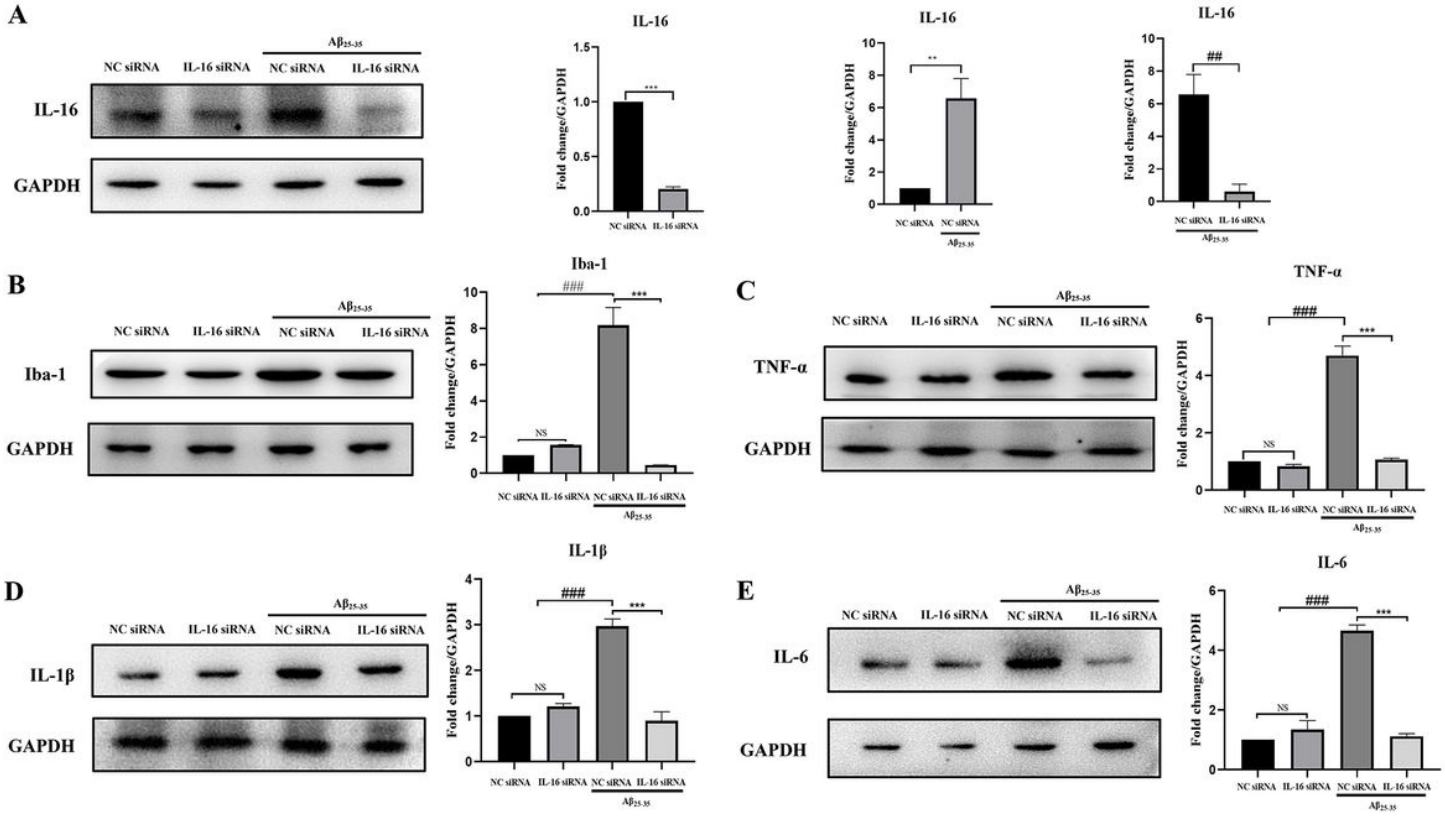

Figure 8

Blockade of IL-16 ameliorates neuroinflammation in AD model mice. (A) Representative immunoblot of IL-16 expression in brain, GAPDH was used as loading control. n=8 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the NC siRNA + Aβ25–35 group, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the NC siRNA group. (B-E) Representative immunoblots of Iba-1, TNF-α, IL-1β and IL-6 expression in brain, GAPDH was used as loading control. n=8 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the NC siRNA + Aβ25–35, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the NC siRNA+ Aβ25–35 group.

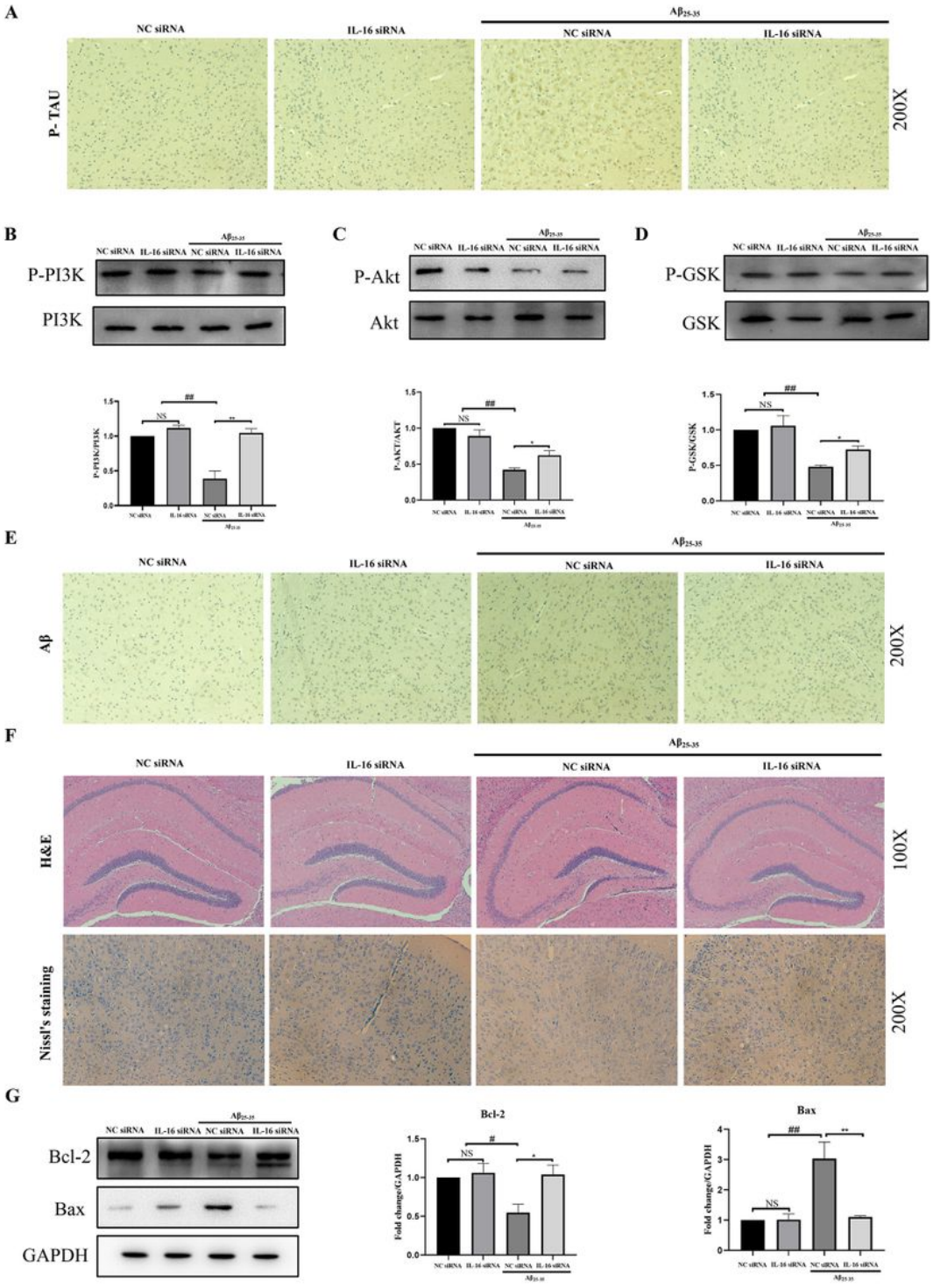

Figure 9

Blockade of IL-16 protects neuron against tau tangles and apoptosis in Aβ25–35-induced mice. (A) immunohistochemistry staining for P-TAU of brain tissue of each group, original magnification, 200×. (B-D) Representative immunoblots of p-PI3K / PI3K, p-AKT / AKT and p-GSK / GSK expression in brain. (E) immunohistochemistry staining for Aβ of brain tissue of each group, original magnification, 200×. (F) H&E staining of brain tissue of each group. (G) Representative immunoblots of Bcl-2 and Bax, GAPDH

was used as loading control. n=8 per group, # P < 0.05, ## P < 0.01 and ### P < 0.001 compared with the NC siRNA + Aβ25–35, * P < 0.05, ** P < 0.01 and *** P < 0.001 compared with the NC siRNA+ Aβ25–35 group.

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