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TREM2 modifies microglial phenotype and provides neuroprotection in P301S tau transgenic mice

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ABSTRACT

As a novel risk gene for Alzheimer's disease (AD), triggering receptor expressed on myeloid cells 2 (*TREM2*) gene encodes a type I transmembrane receptor that is uniquely expressed by the microglia in the brain. Emerging evidence indicates a strong association between TREM2 and tau pathology in the cerebral spinal fluid or brain tissue of AD patients. In line with these clinical findings, we found that TREM2 was upregulated in the brain of P301S mice, an animal model of tau pathology, during disease progression. However, despite this information, the precise role of TREM2 in tau pathology remains largely unknown. In our recent studies, we revealed that silencing microglial TREM2 expression in P301S mice exacerbated spatial cognitive deficits and tau pathology. Based on this evidence, we hypothesized that TREM2 might exert a protective effect in tau-related neurodegenerative diseases. In the present study, to test this hypothesis, a lentiviral-mediated strategy was employed to selectively overexpress TREM2 on microglia in the brain of P301S mice. For the first time, we showed that TREM2 overexpression rescued spatial cognitive impairments and ameliorated neuropathologies including neuronal and synaptic loss as well as tau hyperphosphorylation. Meanwhile, this protective effect was likely attributed to the suppression of neuroinflammation and subsequent attenuation of tau kinase activity, since the expression of pro-inflammatory cytokines including *Tnf*, *Il1b* and *Il6* as well as the activity of tau kinase including glycogen synthase kinase 3β and cyclin-dependent kinase 5 was significantly reduced following TREM2 overexpression. Additionally, the suppressed neuroinflammation might be ascribed to the M2 activation of microglia induced by TREM2, as the expression of M2 phenotype makers including *Arg1*, *Retnla*, *Il4* and *Il10* was markedly increased. Taken together, these findings support the concept of TREM2 as a valuable target against AD as well as other tau-related neurodegenerative diseases.

1. Introduction

Recently, two genetic studies have identified triggering receptor expressed on myeloid cells 2 (*TREM2*) gene as a novel risk gene for Alzheimer's disease (AD), as a rare coding variant (R47H) within exon 2 of this gene considerably increases the disease susceptibility in Caucasians with an odds ratio similar to that of an *APOE* ε4 allele (Guerreiro et al., 2013; Jonsson et al., 2013). Further functional studies indicate that the additional risk may be attributed to dysfunctions of TREM2 protein caused by this variant (Kleinberger et al., 2014; Wang et al., 2015), implying a crucial role of TREM2 in the pathogenesis of AD.

TREM2 is a type I transmembrane receptor that belongs to the immunoglobulin superfamily (Colonna, 2003). As its name indicates, TREM2 is mainly expressed by a subset of myeloid cells,

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such as dendritic cells, monocytes, osteoclasts, and tissue macrophages in peripheral tissues, coupling with DAP12 for its signaling and functions (Jiang et al., 2013; Ma et al., 2015). In the brain, TREM2 was uniquely expressed by the microglia, the main immune cell within the central nervous system (Frank et al., 2008; Takahashi et al., 2005). Although the natural ligands of TREM2 remain unknown, activation of TREM2 was revealed to regulate the functions of microglia including phagocytosis and cytokines production (Kawabori et al., 2015; Takahashi et al., 2007; Zhong et al., 2015). In addition, recent evidence also emphasized a crucial role for TREM2 in maintaining expansion and survival of microglia (Poliani et al., 2015; Wang et al., 2015).

Previously, by employing AD animal models, we and others showed that TREM2 was upregulated in plaque-associated microglia, and might exert a protective effect by enhancement of amyloid-β (Aβ) clearance (Jiang et al., 2014a; Wang et al., 2015). This partly uncovered the role of TREM2 in the pathogenesis of this disease. In addition to amyloid plaques, tau pathology is considered as another pathological hallmark for AD, which correlates better with the decline of cognitive functions in patients as well as animal models of this disease (Giannakopoulos et al., 2003; Xu et al., 2014). Interestingly, a close association between TREM2 and tau pathology has been revealed by recent clinical studies. Cruchaga and colleagues showed that *TREM2* R47H variant was related to higher levels of hyperphosphorylated tau protein in the cerebral spinal fluid (CSF) of AD patients (Cruchaga et al., 2013). Meanwhile, this finding has been confirmed by Lill et al. in a larger cohort of AD patients (Lill et al., 2015). Furthermore, Lue et al. found that TREM2 expression was positively correlated with phosphorylated tau protein in postmortem brain samples from patients with AD (Lue et al., 2015). In line with these clinical findings, we found that TREM2 was upregulated during disease progression in the brain of P301S mice, an animal model of tau pathology. Intriguingly, silencing of microglial TREM2 in this animal model markedly exacerbated spatial cognitive deficits and tau pathology (Jiang et al., 2015). Based on this evidence, we hypothesized that TREM2 might also exert a protective effect in tau-related neurodegenerative disease.

To test our hypothesis, in this study, we selectively overexpress TREM2 on microglia in the brain of P301S mice. For the first time, we show that TREM2 overexpression rescues spatial cognitive impairments and ameliorates neuropathologies including neuronal and synaptic loss as well as tau hyperphosphorylation. Meanwhile, this protective effect is likely attributed to the suppression of neuroinflammation and subsequent attenuation of tau kinase activity. Additionally, the suppressed neuroinflammation may be ascribed to the M2 activation of microglia induced by TREM2. Taken together, our findings support the concept of TREM2 as a valuable target against AD as well as other tau-related neurodegenerative diseases.

2. Materials and methods

2.1. Animals

P301S-tau transgenic mice (expressing P301S mutant human microtubule-binding protein tau on a C3H×C57BL/6 hybrid genetic background) and their age-matched wide-type (WT) mice were purchased from The Jackson Laboratory. To avoid the interference of estrogen on cognitive functions, only male mice were used in this study. Animal Care and Management Committee of Nanjing First Hospital approved the whole study protocol. All animal experiments were conducted in accordance with Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, and were reported in accordance with the ARRIVE guidelines (Hooijmans et al., 2011). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Lentiviral particles preparation

To get better transduction specificity for microglia *in vivo*, a lentiviral vector encoding the mouse WT *Trem2* cDNA (NM_031254.3) under the myeloid-specific CD11b promoters and a control lentiviral vector were constructed as previously described (Jiang et al., 2014a). They were purified and then co-transfected together with packaging vectors (Invitrogen) into 293FT cells (Invitrogen). Supernatant was collected after 48 h, and lentiviral particles in the supernatant were concentrated and recovered by suspension in PBS. Viral titers were determined by a commercial titration ELISA kit (titer for lentiviral particles containing TREM2 vector: 5.1×10^7 TU/μl; titer for control lentiviral particles: 5.5×10^7 TU/μl).

2.3. Intracerebral lentiviral particles injection

Stereotactic intracerebral injection of lentiviral particles was conducted by technicians who were blinded to the experimental groups as previously described (Jiang et al., 2014a). Briefly, five-month-old P301S mice and their age-matched WT mice were anesthetized and fixed on a stereotactic frame (David Kopf Instruments). Lentiviral preparation (2 μl) was injected into the cerebral cortex (two deposits) and hippocampus (one deposit) of each hemisphere using a 30-gauge blunt micropipette attached to a 10 μl Hamilton syringe (Hamilton). Stereotactic coordinates of injection sites from bregma were (1) anteroposterior: −0.3, mediolateral: 2, dorsoventral: −1.5 mm and anteroposterior: −2, mediolateral: 1.2, dorsoventral: −1.2 mm for cerebral cortex; and (2) anteroposterior: −2; mediolateral: 1.2; dorsoventral: −2 mm for hippocampus. The efficiency of lentiviral-mediated TREM2 overexpression was determined by quantitative real-time polymerase chain reaction (qRT-PCR) and immunofluorescence staining at 2 months after injection. According to one of our preliminary experiments, the TREM2 levels in brain of P301S mice reached a relatively steady level at 1 month after lentiviral particle injection (data not shown). Meanwhile, as reported by Dodart and colleagues, obvious neuronal loss was observed in the brain at 3 months after lentiviral vector-mediated gene delivery, possibly due to the long-term neurotoxicity of lentivirus (Dodart et al., 2005). On consideration of these factors, '2-month' was selected as the time point for investigation.

2.4. Open-field exploratory test

At the last day before mice were killed, an open-field exploratory test was carried out by technicians who were blinded to the experimental groups. It was conducted in a 40 cm × 40 cm clear arena. Each mouse was placed in this arena for 10 min free exploration. The activity of mice was recorded by a camera connecting with the EthoVisionXT tracking system (Noldus Information Technology), and the total distance moved and movement speed during the test were analyzed.

2.5. Y-maze test

At the last day before mice were killed, Y-maze test described by Laurent et al. was performed in blind manner to evaluate short-term spatial memory (Laurent et al., 2014). During the exposure phase, mice were placed at the end of the 'start' arm and were allowed to explore the first arm (defined as 'start' arm) and the second arm (defined as 'other' arm) of the maze for 5 min. At this

stage, the access to the third arm of the maze (defined as 'new' arm) was temporarily blocked. Afterwards, the mouse was removed from the maze and returned to its home cage for 2 min. During the test phase, the mouse was placed again in the 'start' arm. Meanwhile, the door of the 'new' arm was removed and the mouse was allowed to explore the whole maze for 1 min. The time that each mouse spent in each arm of the maze during these two phases was recorded using the EthovisionXT tracking system. For the exposure phase, we calculated the percentage of time spent in the 'other' arm versus the 'start' arm. For the test phase, a discrimination index [new arm/(new + other arm)] × 100 was calculated. It is worthy to note that testing was performed in the same room and at the same time to ensure environmental consistency, and sawdust was placed in the maze during the experiments and mixed between each phase to minimize odor cues.

2.6. Morris water maze test

Morris water maze test was performed in a blind manner as described (Tan et al., 2014). Mice were given four training trials per day for 5 consecutive days. The path length to the submerged platform was recorded, and the average time of four trials was calculated. Twenty-four hours after the last trial, mice were subjected to a probe test in which the platform was removed, and their swimming paths were recorded using the EthovisionXT tracking system.

2.7. Western blot analysis

Brain tissues were homogenized in a lysis buffer containing complete protease inhibitor cocktail. Different samples with an equal amount of protein were separated on SDS polyacrylamide gels, transferred to PVDF membranes, and then blocked with 5% bovine serum albumin (BSA). Membranes were incubated with the primary antibody against tau protein hyperphosphorylated at Ser^{202}/Thr^{205} (AT8 epitope, Product#: MN1020, 1:1000, Pierce Biotechnology), tau protein hyperphosphorylated at Thr^{212}/Ser^{214} (AT100 epitope, Product#: MN1060, 1:1000, Pierce Biotechnology), tau protein hyperphosphorylated at Thr^{231} (AT180 epitope, Product#: MN1040, 1:1000, Pierce Biotechnology), total tau protein (Product#: ab80579, 1:800, Abcam), p35/p25 subunit of cyclin-dependent kinase 5 (CDK5) (Product#: 2680, 1:500, Cell Signaling Technology), total CDK5 (Product#: 2506, 1:1000, Cell Signaling Technology), neuron-specific nuclear protein (NeuN) (Product#: ab104224, 1:800, Abcam) or synaptophysin (Product#: ab32127, 1:1000, Abcam), then washed again and incubated with appropriate HRP-coupled secondary antibody. After washing, protein bands were detected with a chemiluminescent HRP substrate (Thermo Scientific). The signal intensity of primary antibody binding was analyzed using Quantity One software 4.6.2 (Bio-Rad Laboratories).

2.8. ELISA for the measurement of sarkosyl-insoluble tau levels

The sarkosyl-insoluble fraction of tau was extracted as described by Chu and colleagues (Chu et al., 2015). The sarkosyl-insoluble tau levels were measured by a commercial available human total tau ELISA kit.

2.9. qRT-PCR

Total RNA in brain tissues was extracted by Trizol reagent (Invitrogen). Equal amounts of total RNA were reverse transcribed under standard conditions using the PrimeScript RT Master Mix (Takara). After that, qRT-PCR reactions were performed with SYBR Premix Ex Taq (Takara) and specific mouse primers (see Supplementary Table 1 for detail) according to the manufacturer's instructions. *Gapdh* was adopted as an internal control.

2.10. Measurement of glycogen synthase kinase 3β (GSK3β) and protein phosphatase 2A (PP2A) activity

For the measurement of GSK3β and PP2A activity, brain tissues were lysed in an extraction buffer (Beyotime). The activity of GSK3β was assessed using a corresponding commercial available detection kit (Sigma-Aldrich Inc.) as previously described (Jiang et al., 2014c). Meanwhile, PP2A activity was measured according to a protocol described by Sun et al. (Sun et al., 2012): endogenous free phosphate and high concentrations of ATP in the supernatants were removed through Sephadex G25 columns. Total proteins (5 mg) were incubated with a chemically synthesized phosphopeptide, which is an optimal substrate for PP2A. The phosphate released from the substrate was detected by measuring the absorbance of a molybdate-malachite green-phosphate complex at 630 nm. The activity of PP2A was evaluated by the release of phosphate (pmol/μg/min). Final data were expressed as a fold change relative to non-treated animals.

2.11. Histological analysis

Brain section for histologic analysis was prepared as described (Jiang et al., 2014a). Nissl staining and immunofluorescence staining of hyperphosphorylated tau (AT8 and AT180 epitopes) were performed as described (Jiang et al., 2014a). Three coronal sections at different depths on the rostro-caudal axis (−0.8 mm, −1.8 mm, and −2.8 mm from bregma) were imaged for each mice, and six fields of cortex and hippocampus on each coronal section were then randomly selected for quantitative analysis. For Nissl staining, neurons with dark violet nucleus and intact morphology were identified as Nissl-positive neurons, and the numbers of Nissl-positive neurons were counted by observers who were unaware of the experimental groups. For immunofluorescence staining, the mean fluorescence signal for hyperphosphorylated tau was measured using Image J software 2.1 by observers who were blinded to the experimental groups.

2.12. Statistical analysis

One sample t-test, independent samples t-test or one-way ANOVA followed by Tukey's *post hoc* test were used to analyze differences among groups. For the hidden-platform training of the Morris water maze test, the path length was analyzed by repeated-measures ANOVA followed by Tukey's *post hoc* test. Data are expressed as mean ± SD. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Intracerebral injection of the lentivirus containing Trem2 cDNA effectively increases TREM2 expression in the brain of P301S mice

To investigate the role of TREM2 in spatial cognitive impairments and tau-related neuropathologies, a lentiviral-mediated strategy was employed to overexpress TREM2 in the brain of animals. At 2 months after injection of lentivirus, the mRNA levels of *Trem2* in the cerebral cortex and hippocampus of P301S mice were elevated by 3.2- ($n = 8$, $q = 22.34$, $P < 0.05$) and 2.9-fold ($n = 8$, $q = 22.53$, $P < 0.05$), respectively (Supplementary Fig. 1A and B). This increase was confirmed at protein levels using immunofluorescence staining (Supplementary Fig. 1C and D). It seemed that the

morphology of microglia was unaffected by lentiviral infection or TREM2 overexpression (See Supplementary Fig. 2 for the double immunofluorescence staining for TREM2 and IBA1, a microglial marker). Of note, As shown by Supplementary Fig. 1A and B, the mRNA levels of *Aif1* (encoding IBA1) stayed unchanged after lentiviral particle injection, suggesting that the upregulation of TREM2 level was not attributed to the proliferation of microglia. In addition, the mRNA levels of *Tyrobp* (encoding DAP12, the adapter protein for TREM2) were not altered following TREM2 overexpression either.

It should be noted that lentivirus infection or *in vivo* TREM2 overexpression was well tolerated by animals, as no sign of neurotoxicity including hind-limb paralysis, vocalization, food intake or neuroanatomical damage was observed during the whole study. Meanwhile, lentivirus infection or *in vivo* TREM2 overexpression did not significantly influence animal body weight or cause abnormal behaviors in animals (data not shown).

3.2. TREM2 overexpression rescues spatial cognitive impairments in P301S mice

At 2 months following lentivirus injection (7 months of age), an open-field exploratory test was carried out to investigate the influence of TREM2 overexpression on general behavioral and motor performance of animals. Overexpression of TREM2 did not affect gross behavioral and motor functions in both WT and P301S mice, which enabled us to exclude the confounding effects of movement factors on cognitive performance (Fig. 1A and B).

We then evaluated the effects of TREM2 overexpression on short-term spatial memory using Y-maze test. During the exposure phase, all groups spent a similar amount of time in the two available arms (Fig. 1C; n = 16, $F = 1.282$, n.s.), suggesting that they explored the maze equally. During the test phase, we revealed that the discrimination index, reflecting the preference of animals for the “new arm” versus “other arm”, was significantly above 50% chance for WT mice (Fig. 1D; n = 16; $t = 7.174$, $P < 0.05$). When compared with WT mice, P301S mice exhibited an impaired preference for the “new arm” (n = 16; Compared to 50% chance: $t = -7.284$, $P < 0.05$; Compared to WT mice: $t = 10.44$, $P < 0.05$). Importantly, this impairment was completely reversed by TREM2 overexpression (n = 16; Compared to 50% chance: $t = 5.487$, $P < 0.05$; Compared to P301S mice receiving control lentivirus: $q = 12.27$, $P < 0.05$). It should be noted that lentivirus injection did not affect the performance in both strains during the test phase. Meanwhile, in WT mice, TREM2 overexpression had no effect on the test performance (n = 16, $q = 0.4281$, n.s.).

Next, Morris water maze test was conducted in animals during the last 5 days before execution. First, we compared the swimming speed between these two strains, but no difference was observed (Fig. 1E; n = 16, $t = 0.8192$, n.s.). Injection of lentivirus or overexpression of TREM2 did not markedly affect swimming speed in both WT and P301S mice. These results enabled us to exclude the confounding effects of motivational and sensorimotor factors on test performance. We then employed a hidden platform test to assess the spatial learning functions in animals. As shown in Fig. 1F, WT mice swam less distance than P301S mice to find the hidden platform from day 2 to day 5 (day 2: 7.88 ± 0.69 vs. 9.31 ± 1.06 m; day 3: 6.24 ± 0.81 vs. 7.97 ± 0.84 m; day 4: 5.13 ± 0.78 vs. 7.45 ± 0.65 m; day 5: 4.41 ± 0.69 vs. 6.95 ± 0.91 m; $P < 0.05$). Two-way repeated-measures ANOVA showed that WT mice performed better during the whole task ($F_{genotype}$ (1150) = 177.6, $P < 0.05$; F_{days} (4150) = 227.6, $P < 0.05$; $F_{genotype \times days}$ (4150) = 9, $P < 0.05$), suggesting that P301S mice displayed evident spatial learning impairments at 7 months of age. Importantly, TREM2 overexpression rescued this impairment in P301S mice ($F_{treatment}$ (1150) = 62.8, $P < 0.05$; F_{days} (4150) = 172.1, $P < 0.05$; $F_{genotype \times days}$ (4150) = 6.6, $P < 0.05$). It is worthy to note that lentivirus injection did not affect the performance in both strains during the hidden platform test. Meanwhile, TREM2 overexpression had no influence on the test performance in WT mice ($F_{treatment}$ (1150) = 0.3, n.s.; F_{days} (4150) = 324.4, $P < 0.05$; $F_{genotype \times days}$ (4150) = 1.4, n.s.). To determine the effect of TREM2 overexpression on spatial memory, the platform was removed from the water and probe trials were conducted at 24 h after the last training trial. In contrast to WT mice, P301S mice spent almost the same length of time in the target quadrant and in any other quadrant (Fig. 1G, n = 16; 24.58 ± 4.52% vs. 25.14 ± 1.51%; n.s.), implying an evident spatial memory impairments. Importantly, P301S mice receiving *Trem2* lentivirus spent significantly more time in the target quadrant than in any other quadrant (n = 16; 35.1 ± 6.34% vs. 21.63 ± 2.11%; $P < 0.05$), indicating an improvement of spatial memory functions. Of note, lentivirus injection did not affect the performance in both strains during probe trials. Meanwhile, TREM2 overexpression had no influence on the test performance in WT mice.

3.3. TREM2 overexpression prevents neuronal and synaptic loss in the brain of P301S mice

Loss of neurons and synaptic in the hippocampus and parietal cortex closely correlates with the decline of spatial cognitive functions (Arendt, 2009; Giannakopoulos et al., 2003). In line with this notion, when compared with WT mice, seven-month-old P301S mice exhibited apparent neuronal (showed by Nissl staining) and synaptic losses (indicated by synaptophysin levels, see Jiang et al., 2014a; Jiang et al., 2015) in the above-mentioned brain regions (Fig. 2A-D). TREM2 overexpression markedly attenuated neuronal loss in the brain of P301S mice, as the percentage of Nissl-positive neurons in the cortex and hippocampus was increased by 2.92- (n = 8, $q = 26.01$, $P < 0.05$) and 3.57-fold (n = 8, $q = 25.17$, $P < 0.05$), respectively (Fig. 2A–C). Meanwhile, overexpression of TREM2 also led to a 2.76-fold increase in synaptophysin levels (n = 8, $q = 14.69$, $P < 0.05$), implying a prevention of synaptic loss in P301S mice brain (Fig. 2D). It is noteworthy that lentivirus injection did not cause significant neuronal and synaptic loss in the brain of both strains. Meanwhile, in WT mice, overexpression of TREM2 had no influence on the density of neurons and synapses in the brain, as the protein levels of NeuN (representing the density of living neurons) and synaptophysin stayed unchanged after TREM2 overexpression (Supplementary Fig. 3).

3.4. TREM2 overexpression ameliorates tau hyperphosphorylation in the brain of P301S mice

As progression of tau pathology directly associated with injury and even loss of neurons and synapses (Frost et al., 2015), we then determined whether the prevention of neuronal and synaptic loss in the brain of P301S mice was attributed to any alterations in tau pathology. First, we determined the effects of TREM2 overexpression on tau hyperphosphorylation. Overexpression of TREM2 reduced the levels of tau hyperphosphorylated at Ser^{202}/Thr^{205} (AT8 epitope) and Thr^{231} (AT180 epitope) by 67.8% (n = 8, $q = 15.74$, $P < 0.05$) and 64.9% (n = 8, $q = 15.27$, $P < 0.05$), respectively, in the brain of P301S mice (Fig. 3A-C). This reduction was further confirmed by immunofluorescence staining. As demonstrated by Fig. 3F and G, the immunofluorescence signal of AT8 in the cerebral cortex and hippocampus of P301S mice was reduced by 60.5% (n = 8, $q = 13.28$, $P < 0.05$) and 64.2% (n = 8, $q = 17.71$, $P < 0.05$), respectively, following TREM2 overexpression. Overexpression of TREM2 also reduced the immunofluorescence signal of AT180 in the cerebral cortex and hippocampus of P301S mice by 66% (n = 8,

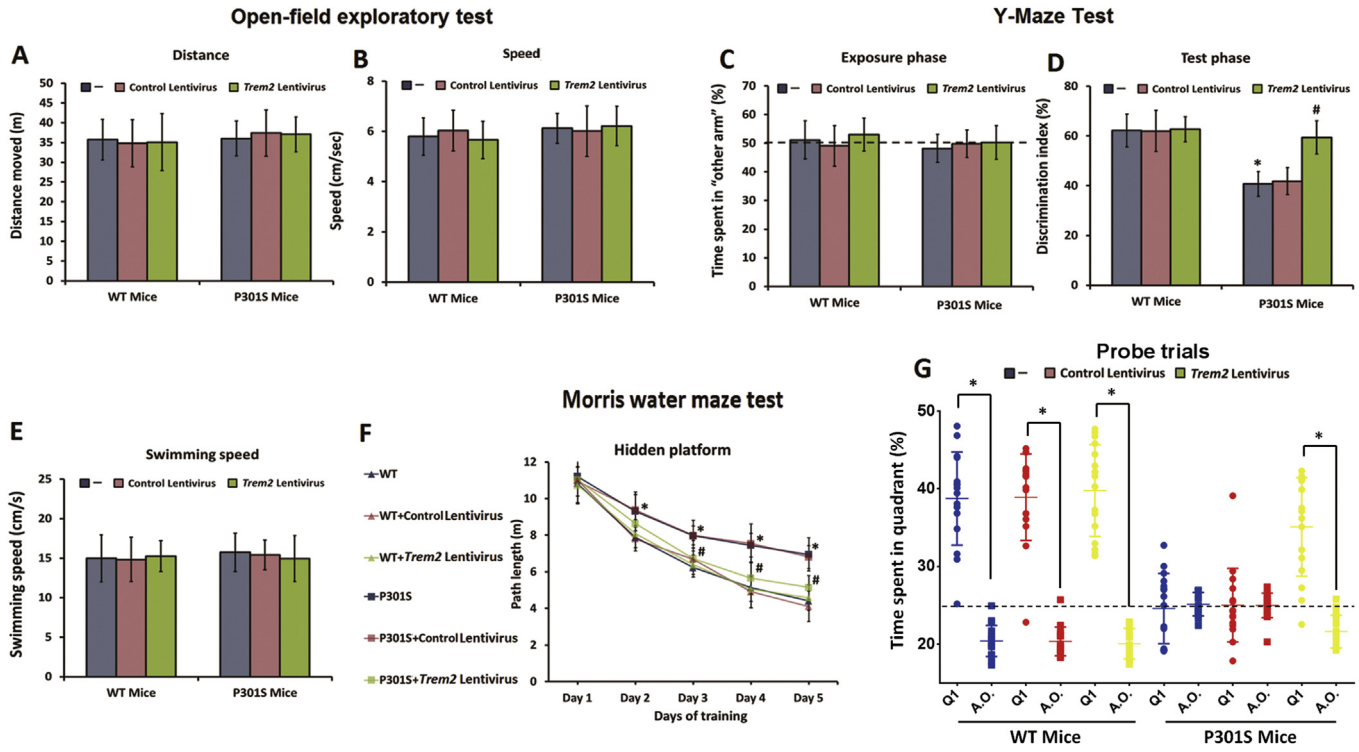

Fig. 1. TREM2 overexpression rescues spatial cognitive impairments in P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice and age-matched WT mice, and an open-field exploratory test was conducted at 2 months after lentivirus injection to investigate whether TREM2 overexpression affected general behavioral and motor performance of animals. During the test, the activity of mice was recorded by a camera connecting with a tracking system, and the distance moved (A) and movement speed (B) were analyzed. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Afterward, Y-Maze test was performed to evaluate short-term spatial memory. During the exposure phase, mice were placed at the end of the 'start' arm and were allowed to explore the first arm (defined as 'start' arm) and the second arm (defined as 'other' arm) of the maze for 5 min. At this stage, the access to the third arm of the maze (defined as 'new' arm) was temporarily blocked, and (C) the percentage of time spent in the 'other' arm versus the 'start' arm was calculated. Data were analyzed by one sample t-test (versus 50% chance) or one-way ANOVA followed by Tukey's post hoc test (between groups). Afterwards, the mouse was removed from the maze and returned to its home cage for 2 min. During the test phase, the mouse was placed again in the 'start' arm. Meanwhile, the door of the 'new' arm was removed and the mouse was allowed to explore the whole maze for 1 min. (D) For the test phase, a discrimination index [new arm/(new + other arm)] × 100 was calculated. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. *P < 0.05 versus non-treated WT mice. #P < 0.05 versus P301S mice receiving the control lentivirus. During the last 5 days before execution, the spatial cognitive functions were also assessed by Morris water maze test. (E) Swimming speed of each group during the 5-day task. Data were analyzed by one-way ANOVA followed by Tukey's post hoc test. (F) Path length of each group in the hidden platform task. Data were analyzed by two-way repeated-measures ANOVA followed by Tukey's post hoc test. *P < 0.05 versus non-treated WT mice. #P < 0.05 versus P301S mice receiving control lentivirus. (G) Data from probe trials are presented as the percentage of time spent in the target quadrant (Q1) vs the averaged time spent in other three quadrants (a.o.). Data were analyzed by independent samples t-test. *P < 0.05 vs averaged time spent in other three quadrants. Columns represent mean ± s.d. (n = 16 per group).

$q = 17.37$, $P < 0.05$) and 67.8% (n = 8, $q = 15.08$, $P < 0.05$), respectively (Fig. 3F and H). However, TREM2 overexpression led to a moderate reduction in tau hyperphosphorylated at Thr^{212}/Ser^{214} (AT100 epitope) in the brain of P301S mice, but this did not reach statistical significance (Fig. 3A and D, n = 8, $q = 3.519$, n.s.). It is worthy to note that no significant alteration was observed in total tau levels in the brain of P301S mice (Fig. 3A and E) and WT mice (Supplementary Fig. 4A and B) following TREM2 overexpression.

Next, to study the effects of TREM2 overexpression on tau aggregation, total tau levels in sarkosyl-soluble and insoluble fractions were analyzed. As shown by Fig. 3I and J, overexpression of TREM2 did not significantly alter the levels of both sarkosyl-soluble and sarkosyl-insoluble tau in the brain of P301S mice, suggesting that the process of tau aggregation was unaffected by TREM2 overexpression.

3.5. TREM2 overexpression attenuates the activity of tau kinases in the brain of P301S mice

Afterwards, we investigated the possible mechanisms by which TREM2 overexpression attenuated tau hyperphosphorylation. As CDK5 and GSK3β are major kinases responsible for tau hyperphosphorylation (Lopes and Agostinho, 2011; Takashima, 2006), we then determined their activity in the brain of P301S mice. First, we detected the ratio of p25/p35 subunit of CDK5, which represented an activation marker of this kinase (Shah and Lahiri, 2014). The ratio of p25/p35 was decreased by 79.5% after TREM2 overexpression (n = 8, $q = 16.71$, $P < 0.05$), implying that the CDK5 activity was markedly attenuated (Fig. 4A). We also noted a slight reduction in CDK5 levels in the brain of P301S mice after TREM2 overexpression, but this did not reach statistical significance (Fig. 4A, n = 8, $q = 1.34$, n.s.). Meanwhile, a 69.3% decrease in the activity of GSK3β was noted in P301S mice brain following TREM2 overexpression (Fig. 4B, n = 8, $q = 16.27$, $P < 0.05$). Since phosphatase is involved in dephosphorylation of tau protein, and upregulation of phosphatase such as PP2A directly attenuates the hyperphosphorylation of tau (Martin et al., 2013), we then determined the PP2A activity in brain of P301S mice following TREM2 overexpression. Overexpression of TREM2 did not significantly affect brain PP2A activity (Fig. 4C, n = 8, $q = 1.03$, $P < 0.05$). Additionally, in the brain of WT mice, TREM2 overexpression had no influence on the activity of CDK5, GSK3β and PP2A (Supplementary Fig. 4A and C-E).

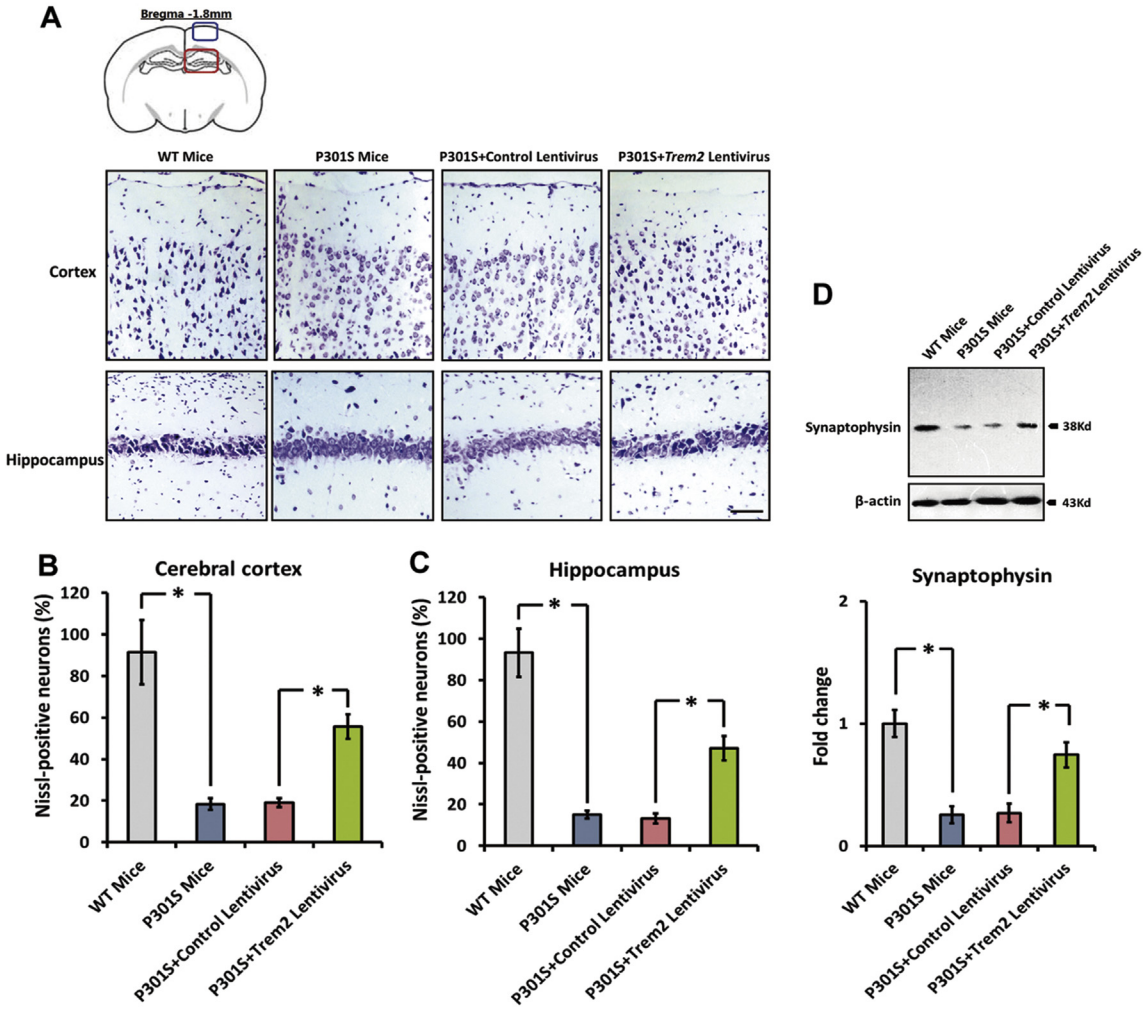

Fig. 2. TREM2 overexpression prevents neuronal and synaptic loss in the brain of P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice. Two months later, mice were killed for subsequent analysis. (A) Nissl staining for detection of neuronal loss in the cortex and hippocampus of 7-month-old P301S and WT mice. Neurons with dark violet nucleus and intact morphology were identified as Nissl-positive neurons. Scale bar = 100 μm. Blue box on the top atlas shows the region where the photo of the cortex was taken, and red box on the left atlas shows the region where the photo of the hippocampus was taken. (B, C) P301S mice at 7 months of age showed an obvious reduction in the percentage of Nissl-positive neurons in the hippocampus and cortex in comparison with age-matched WT mice, whereas this reduction was rescued by TREM2 overexpression. (D) Protein levels of synaptophysin in the whole brain of 7-month-old P301S mice and WT mice were detected by Western blot. Data were normalized to β-actin. All data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Columns represent mean ± s.d. (n = 8 per group). *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.6. TREM2 overexpression suppresses neuroinflammation in the brain of P301S mice

Neuroinflammation was directly associated with the hyperactivation of tau kinases (Gorlovoy et al., 2009; Lee et al., 2010), subsequently leading to the hyperphosphorylation of tau. Coincidently, TREM2 was reported to mitigate inflammatory responses in the brain (Hickman and El Khoury, 2014). Therefore, we investigated whether the attenuation of tau kinases activity in the brain of P301S mice was attributed to suppressed inflammatory responses after TREM2 overexpression. At 7 months of age, P301S mice exhibited apparent inflammatory responses in the brain, as mRNA levels of proinflammatory cytokines including *tnf* (by 1.94-fold, n = 8, $t = 7.49$, $P < 0.05$), *Il1b* (by 1.73-fold, n = 8, $t = 6.26$, $P < 0.05$), *Il6* (by 2.53-fold, n = 8, $t = 9.78$, $P < 0.05$) and *Nos2* (by 1.59-fold, n = 8, $t = 6.07$, $P < 0.05$) were significantly higher than those in the brain of age-matched WT mice (Fig. 5A-C). Overexpression of TREM2 led to the significant reduction in mRNA levels of *tnf* (Fig. 5A, by 33.4%, n = 8, $q = 6.05$, $P < 0.05$), *Il1b* (Fig. 5B, by 38.1%, n = 8, $q = 7.51$, $P < 0.05$) and *Il6* (Fig. 5C, by 45.2%, n = 8, $q = 7.9$, $P < 0.05$). Meanwhile, corresponding increments in protein levels of TNF-α, IL-1β and IL-6 were observed following TREM2 overexpression (Fig. 5E–G). It is worthy to note that overexpression of TREM2 did not affect the mRNA expression of proinflammatory cytokines including *tnf*, *Il1b*, *Il6* and *Nos2* in the brain of WT mice (Supplementary Fig. 5).

3.7. TREM2 overexpression elevated microglial M2 phenotype makers in the brain of P301S mice

As reduced inflammatory response represents a major characteristic of M2 phenotype of microglia, we then hypothesized that TREM2 overexpression suppressed neuroinflammation through switching microglia toward the M2 phenotype. To test this hypothesis, the mRNA levels of M2 phenotype makers including *Arg1, Retnla*, *Il4* and *Il10* in the whole brain of P301S mice were measured by qRT-PCR. As shown by Fig. 6, overexpression of TREM2 significantly increased the mRNA levels of *Arg1* (Fig. 6A, by 1.67-fold,

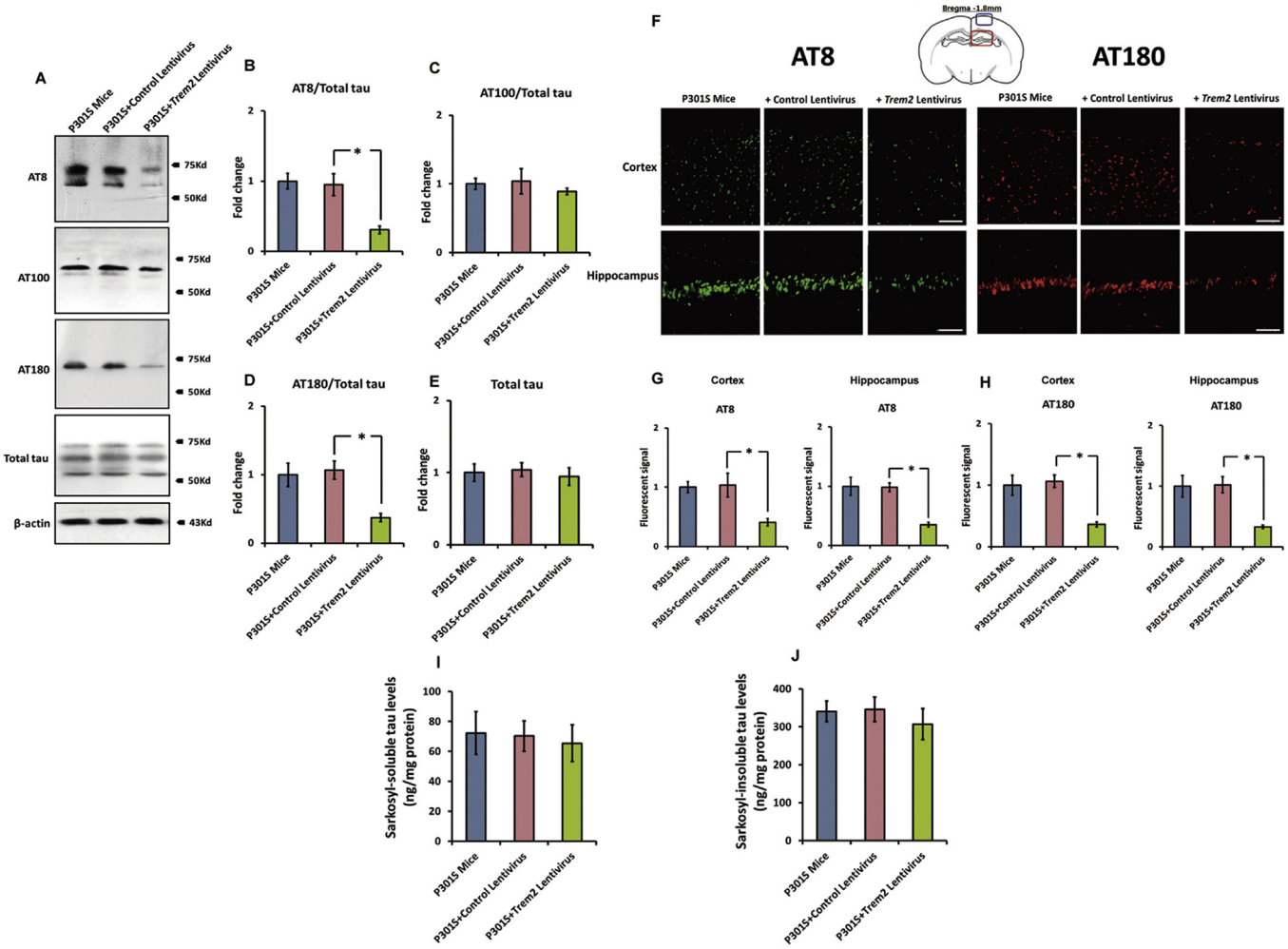

Fig. 3. TREM2 overexpression ameliorates tau hyperphosphorylation in the brain of P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice. Two months later, animals were killed for subsequent analysis. (A) The levels of AT8 (Recognize tau hyperphosphorylated at Ser^{202}/Thr^{205}), AT100 (Recognize tau hyperphosphorylated at Thr^{212}/Ser^{214}), AT180 (Recognize tau hyperphosphorylated at Thr^{231}) and total tau levels in the whole brain of P301S mice were detected by Western blot. (B–D) Quantification of AT8, AT100 and AT180 epitopes in the brain of P301S mice. Data were normalized to total tau levels. (E) Quantification of total tau levels in the brain of P301S mice. Data were normalized to β-actin. (F) Alterations of AT8 and AT180 in the brain of P301S mice were further confirmed by immunofluorescence staining at 2 months after injection. Scale bar in the cortex = 100 μm, Scale bar in the cortex = 50 μm. Blue box on the top atlas shows the region where the photo of the cortex was taken, and red box on the top atlas shows the region where the photo of the hippocampus was taken. (G, H) Quantification of AT8 and AT180 fluorescent signal in the brain of P301S mice. (I, J) The levels of total tau in sarkosyl-soluble and insoluble fraction in the brain of P301S mice were detected by ELISA. All data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Columns represent mean ± s.d. (n = 8 per group). *P < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

n = 8, $q = 11.62$, $P < 0.05$), *Retnla* (Fig. 6B, by 1.71-fold, n = 8, $q = 9.22$, $P < 0.05$), *Il4* (Fig. 6C, by 2.01-fold, n = 8, $q = 13.94$, $P < 0.05$) and *Il10* (Fig. 6D, by 2.34-fold, n = 8, $q = 12.44$, $P < 0.05$).

4. Discussion

Progressive spatial cognitive impairment is a characteristic symptom in several tau-related neurodegenerative diseases (Giannakopoulos et al., 2003; Yoshiyama et al., 2013). Here, using Y-Maze and Morris water maze tests, we showed that TREM2 overexpression rescued spatial cognitive impairments in P301S mice. This was compatible with our recent findings that overexpression of TREM2 ameliorates spatial cognitive deficits in *APP*/*PS1* mice, a transgenic model of AD (Jiang et al., 2014a). It should be noted that our results did not support a direct involvement of TREM2 in spatial cognitive functions, as overexpression of TREM2 in the brain of WT mice was not associated with an improved performance in Y-Maze and Morris water maze tests.

Loss of neuron and synapse in brain regions including the hippocampus and parietal cortex correlates well with the decline of spatial cognitive functions (Arendt, 2009; Giannakopoulos et al., 2003). In accordance with this notion, we revealed that P301S mice at 7 months of age exhibited apparent neuronal and synaptic loss in the above-mentioned brain regions. Excitingly, this phenomenon was prevented by overexpression of TREM2, as the percentage of Nissl-positive neurons and levels of synaptophysin were markedly increased in the brain of P301S mice. This was further supported by our recent observation that TREM2 overexpression rescued Aβ-induced injury of neurons and synapses in the brain of *APP*/*PS1* mice (Jiang et al., 2014a). In view of the above information, it seemed that the prevention of neuronal and synaptic loss may contribute to the improvement of spatial cognitive functions after TREM2 overexpression.

As progression of tau pathology directly led to neurodegenerative changes including dysfunction and even loss of neurons and synapses (Frost et al., 2015), we then determined whether the

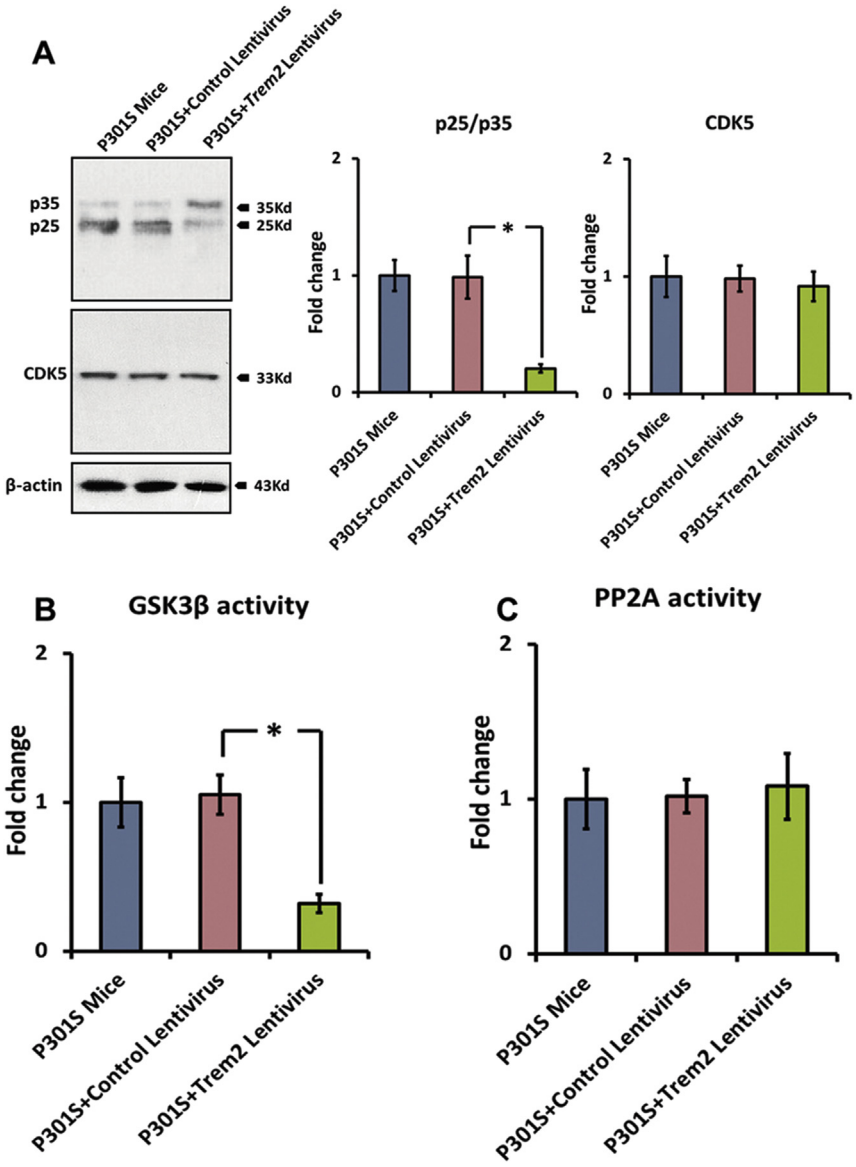

Fig. 4. TREM2 overexpression attenuates the activity of tau kinases in the brain of P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice. Two months later, mice were killed for subsequent analysis. (A) The protein levels of CDK5 and its subunits p25 and p35 in the whole brain of P301S mice were determined by Western blot. (B, C) The activity of GSK3β and PP2A in the whole brain of P301S mice was detected by commercial detection kits. Data are expressed as a fold change relative to non-treated P301S mice. All data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Columns represent mean ± s.d. (n = 8 per group). *P < 0.05.

prevention of neuronal and synaptic loss in the brain of P301S mice was attributed to any alterations in tau pathology. For the first time, we showed that overexpression of TREM2 significantly ameliorated the hyperphosphorylation of tau at multiple pathological sites including AT8 and AT180. In combination with our previous observation that TREM2 silencing enhanced tau hyperphosphorylation at these pathological sites in P301S mice, these results strongly suggested a protective effect of TREM2 against tau pathology. However, TREM2 overexpression might not affect the aggregation of tau in this study, as the immunoreactivity of AT100 epitope and levels of sarkosyl-insoluble tau remained unchanged in the brain of P301S mice. This suggested that the prevention of neuronal and synaptic loss following TREM2 overexpression was likely attributed to alterations in tau hyperphosphorylation status rather than tau aggregation.

Tau hyperphosphorylation usually results from the hyperactivation of tau kinase and/or inactivation of tau phosphatases in the brain. Here, we showed that the activity of GSK3β, the major tau kinase implicating in tau hyperphosphorylation (Takashima, 2006), was significantly reduced in the brain of P301S mice after TREM2 overexpression. In addition, the activity of CDK5, another kinase responsible for abnormal tau phosphorylation (Lopes and Agostinho, 2011), was also dramatically decreased following overexpression of TREM2. It is worthy to note that TREM2 overexpression did not alter the activity of PP2A, the main phosphatase that participated in tau dephosphorylation (Martin et al., 2013). These findings implied that the protective effects of TREM2 against tau pathology were achieved, at least in part, by attenuating the activity of tau kinases.

Moreover, in this study, we found that TREM2 overexpression suppressed neuroinflammation, as the expression of proinflammatory cytokines including TNF-α, IL-1β and IL-6 was reduced

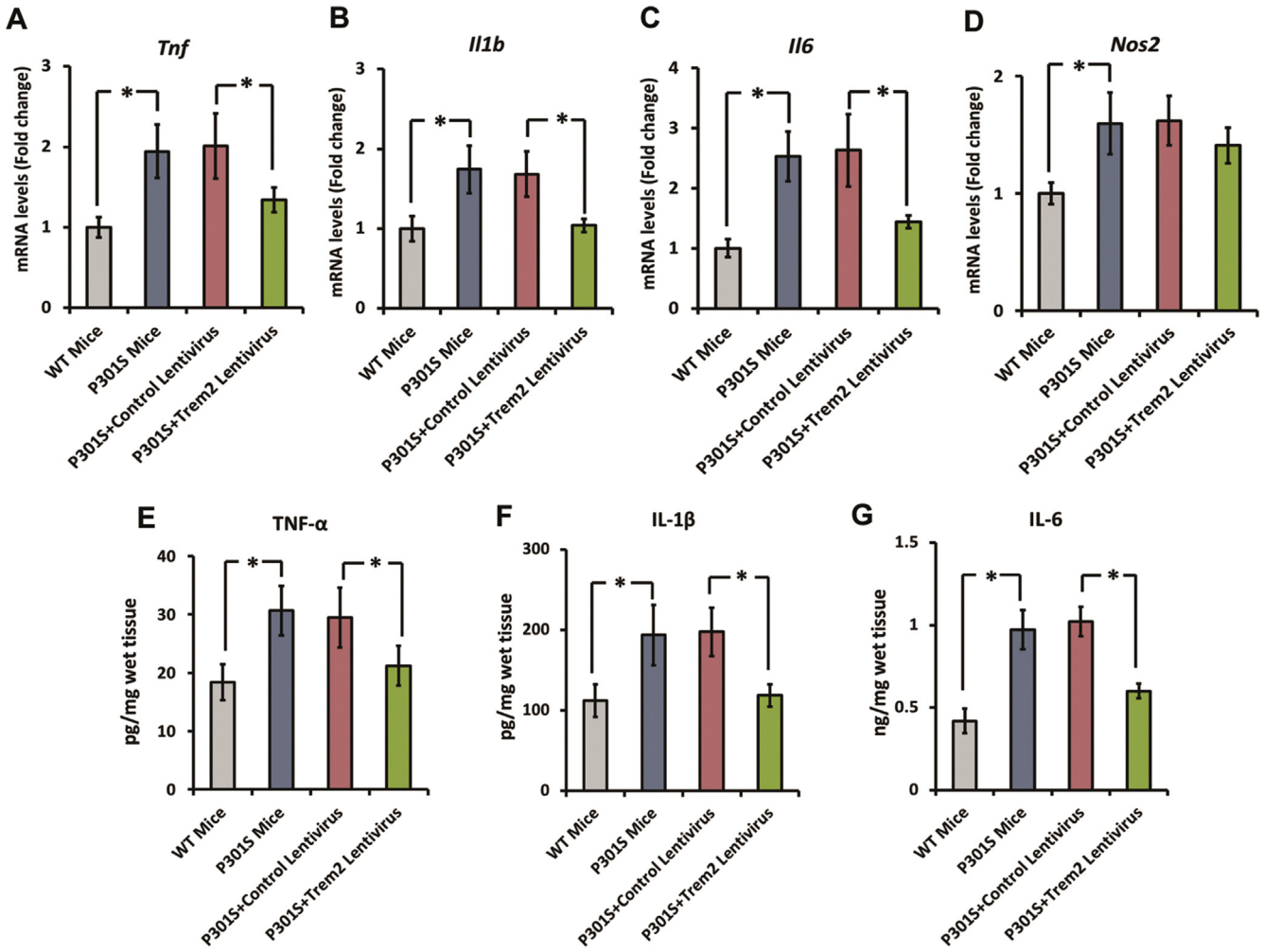

Fig. 5. TREM2 overexpression suppresses neuroinflammation in the brain of P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice. Two months later, mice were killed for subsequent analysis. (A–D) mRNA levels of Tnf, Il1b, Il6 and Nos2 in the whole brain of P301S mice were measured by qRT-PCR. (E–G) The protein levels of TNF-a, IL-1β and IL-6 in the whole brain of P301S mice were assessed by ELISA. All data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Columns represent mean ± s.d. (n = 8 per group). *P < 0.05.

in the brain of P301S mice following TREM2 overexpression. As a microglial immune receptor, TREM2 was revealed to regulate inflammatory response mediated by microglia (Hickman and El Khoury, 2014). Previously, we showed that knockdown of TREM2 on microglia exacerbated neuroinflammation in SAMP8 mice, an animal model of senescence (Jiang et al., 2014b). Meanwhile, Wang and colleagues revealed that TREM2 deficiency enhanced the microglial production of proinflammatory cytokines in 5 × FAD mouse model of AD (Wang et al., 2015). More importantly, a recent study by our group provided more direct evidence that selectively overexpressed TREM2 on microglia markedly ameliorated Aβ-induced neuroinflammation in *APP/PS1* mice (Jiang et al., 2014a). On consideration of this evidence, the attenuation in neuroinflammation might be due to a direct inhibition of TREM2 on microglia-mediated inflammatory response. Meanwhile, in view of the fact that neuroinflammation could directly elevate the activity of tau kinases and thus contributed to tau pathology (Ghosh et al., 2013; Gorlovoy et al., 2009; Lee et al., 2010), it is likely that the attenuated activity of tau kinases under this condition might be related to the suppressed neuroinflammation caused by TREM2 overexpression.

In brain, activation of microglia can be categorized into two opposite types: M1 phenotype and M2 phenotype. M2 phenotype of microglia is featured by an enhanced phagocytic activity as well as a reduced inflammatory response (Franco and Fernandez-Suarez, 2015). Interestingly, a decreased level of M2 phenotype markers including *Arg1, Retnla, Il4* and *Il10* in the brain of P301S mice after TREM2 overexpression was observed in this study, implying that TREM2 suppressed neuroinflammation through switching microglia toward the M2 phenotype. To our knowledge, this is the first study showing a modulatory effect of TREM2 on microglial phenotype. More importantly, this uncovered the potential mechanisms by which TREM2 inhibits microglial inflammatory response under the current condition.

In conclusion, this study provides the first evidence that TREM2 overexpression rescues spatial cognitive impairments and ameliorates neuropathologies including neuronal and synaptic loss as well as tau hyperphosphorylation in P301S mice, an animal model of tau pathology. Meanwhile, this protective effect is likely attributed to the suppression of neuroinflammation and subsequent attenuation of tau kinase activity. In addition, the suppressed neuroinflammation may be ascribed to the M2 phenotype of microglia induced by TREM2 (See Fig. 7 for a diagrammatic summary of this study). Taken together, these findings support the concept of TREM2 as a valuable target against AD as well as other tau-related neurodegenerative diseases.

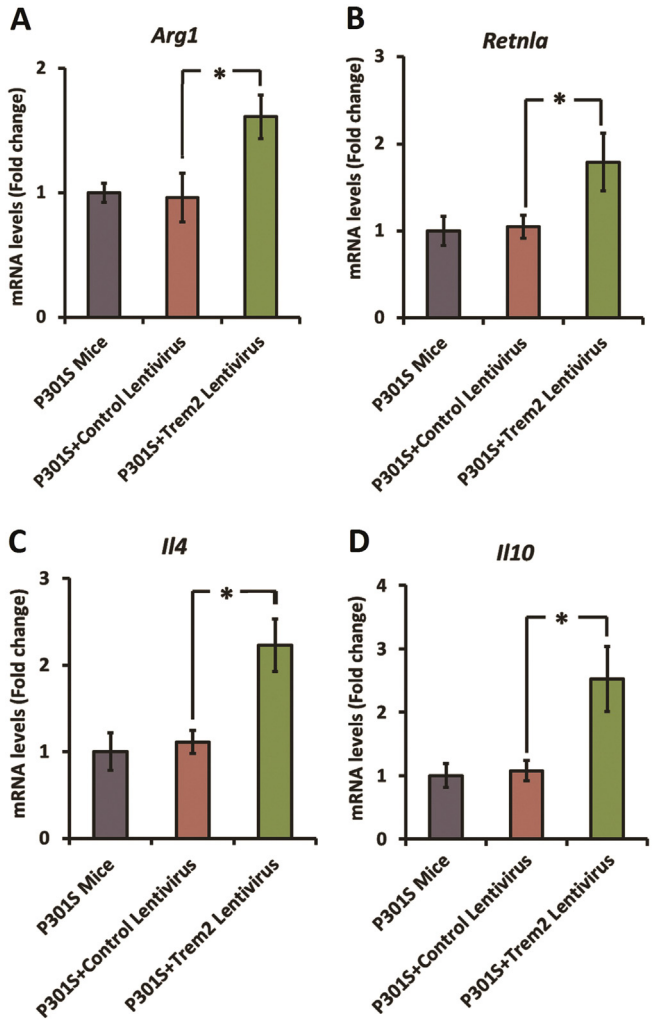

Fig. 6. TREM2 overexpression elevated microglial M2 phenotype makers in the brain of P301S mice. The lentivirus containing Trem2 cDNA or the control lentivirus was injected bilaterally into the cerebral cortex and the hippocampus of 5-month-old P301S mice. Two months later, mice were killed for subsequent analysis. (A–D) mRNA levels of microglial M2 phenotype makers including Arg1, Retnla, Il4 and Il10 in the whole brain of P301S mice were measured by qRT-PCR. All data were analyzed by one-way ANOVA followed by Tukey's post hoc test. Columns represent mean ± s.d. (n = 8 per group). *P < 0.05.

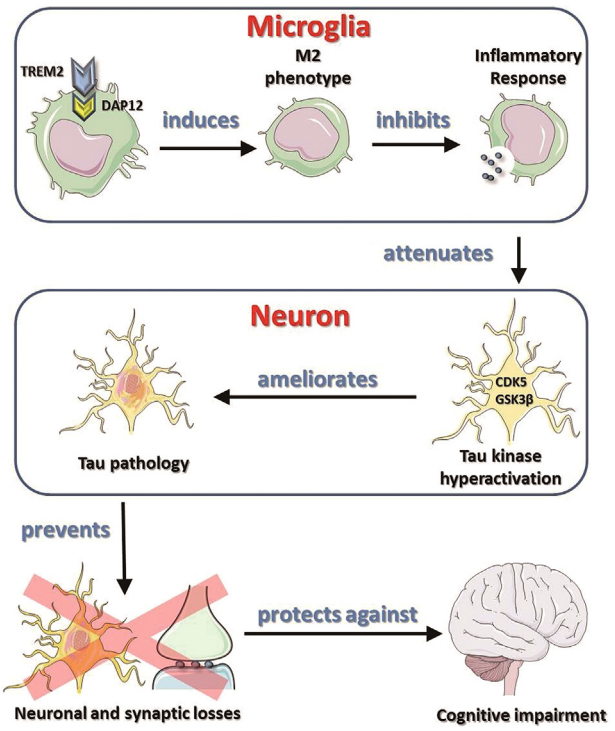

Fig. 7. A diagrammatic summary of this study. This study provides the first evidence that TREM2 overexpression rescues spatial cognitive impairments and ameliorates neuropathologies including neuronal and synaptic loss as well as tau hyperphosphorylation in P301S mice, an animal model of tau pathology. Meanwhile, this protective effect is likely attributed to the suppression of neuroinflammation and subsequent attenuation of tau kinase activity. In addition, the suppressed neuroinflammation may be ascribed to the M2 phenotype of microglia induced by TREM2.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.neuropharm.2016.01.028.

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