Impaired 26S proteasome causes leaning and memory deficiency and induces neuroinflammation mediated by NF-κB in mice

Running title: Impaired proteasome and neuroinflammation

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Abstract

Background

Aging and many neurodegenerative disorders, including Alzheimer's disease (AD), show reduced proteasome activity, loss of synapses and increased neuroinflammation in the brain. However, whether proteasome dysfunction causes neuroinflammation remains less understood.

Methods

Here, we studied the effect of impaired 26S proteasome on neuroinflammation in the *Psmc1* knockout (KO) mice deficient of a 19S proteasome subunit selectively in the forebrain region. We initially assessed the proteasome activity in the KO and control mouse brains and performed cognitive behavioral test to the animals. The synaptosomes were isolated from the forebrain and subjected to mass spectrometric analysis of total synaptic proteins. The identified synaptic proteins were then assessed using bioinformatic approaches. To validate identified proteins, Western blot analysis and immunofluorescent staining of specific protein were utilized. To validate NF-κB mediating neuroinflammation, we administered the KO animals at 5 weeks of age with a selective NF-κB inhibitor for three weeks and then, animal behaviors and neuroinflammation were assessed when they reached to eight weeks of age.

Results

Our results revealed that impaired 26S proteasome led to AD-like cognitive deficiency and overt neuroinflammation in the synapses of the *Psmc1* KO brain at eight weeks of age. Pronounced neuroinflammation also occurred in other regions and glial cells, which was confirmed by increased levels of several key immune response-related proteins, including Stat1, Trem2 and NF-κB, and by activation of astrocytes and microglia in the KO brain. Following treating the KO

mice with a selective NF-κB inhibitor, the KO mice exhibited improved behaviors and reduced neuroinflammation compared to the control animals.

Conclusions

These data indicate that impaired 26S proteasome causes AD-like cognitive deficiency and induces neuroinflammation mediated largely by NF-κB, which will help develop effective therapeutics and aid to better understand the pathogenesis of AD and many other neurodegenerative disorders where impaired proteasome is consistently coupled with neuroinflammation.

Key Words: proteasome; aging; neuroinflammation; neurodegenerative disease; Alzheimer's disease; mice; NF-kB; inhibitor

Introduction

Misfolded, aberrant, or unneeded intracellular proteins can be degraded by the ubiquitin-proteasome system (UPS), a major protein degradation system of the cell that functions to maintain intracellular proteostasis. However, a number of previous studies have shown that proteasome activity decreases in the brain affected by Alzheimer's disease (AD) and many other neurodegenerative diseases [1-5]. Associated with reduced proteasome function is neuroinflammation in these diseases [6], an inflammatory response that is considered to be neuroprotective initially in the brain by eliminating diverse pathogens following microbial infections [7], boosting tissue repair, and removing debris. However, increasing evidence has suggested that sustained neuroinflammation is detrimental to neuronal function and survival [8]. Many endogenous stimuli, such as genetic mutation and protein accumulation and aggregation,

can persist to activate microglia and astrocytes to transition into proinflammatory phenotypes and cause neurodegeneration [9, 10]. However, how neuroinflammation occurs in AD and many other neurodegenerative diseases where proteasome function is impaired remains less understood.

For a protein to be degraded by the UPS, it will need to be tagged by a polyubiquitin chain through a series of reaction known as polyubiquitination that requires a series of biochemical reactions catalyzed by the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the ubiquitin ligase (E3). Additional ubiquitins are added onto the first one, leading to formation of a polyubiquitin chain on the substrate protein. The substrate protein now can be recognized and degraded by the proteasome. The proteasome is an ATP-dependent complex consisting of the 20S core particle attached to one or two 19S regulatory particles to constitute the 26S or 30S proteasome, respectively. The 20S core particle is arranged in repeating $\alpha_{1\text{-}7}$-$\beta_{1\text{-}7}$- $\beta_{1\text{-}7}$-$\alpha_{1\text{-}7}$. Three β subunits have enzymatic activity: β1 subunits have caspase-like activity; β2 subunits have trypsin-like activity; and β5 subunits have chymotrypsin-like activity. On one or either side of the 20S core particle sits the 19S regulatory particle to make 26S or 30S proteasome. The 19S regulatory particle consists of the lid and base, with the lid subunits responsible for deubiqutylating the substrate protein and the base responsible for ubiquitin recognition and binding. Rpn1 and Rpn2, and Rpt1-6 are required for the assembly of the 19S regulatory particle. Rpt2, also known as *Psmc1*, is responsible for opening the entry pore gated by the α-subunits to allow a substrate protein for translocation into the 20S core particle for degradation [11-13]. The UPS plays a crucial role in various activities in a brain cell, including proteostasis, cognitive function, synaptic plasticity, signal transduction, and immune responses [12, 14, 15].

Given the fact that reduced proteasome activity and increased neuroinflammation are two common pathological features of aging, AD and many other neurodegenerative disorders, we hypothesize that 26S proteasome deficiency is a causative mechanism to induce neuroinflammation in the brain. To test this hypothesis, we genetically ablated *Psmc1*, one 19S proteasomal subunit of the 26S proteasome, in neurons of the forebrain region. This genetic ablation made the 19S regulatory particle unable to bind to the 20S core particle, rendering the deficiency of the 26S proteasome in this brain region. Utilizing this model, we discovered that 26S proteasome deficiency drives neuroinflammation and AD-like cognitive behaviors mediated by the NF-κB signaling pathway, while inhibition of the NF-κB pathway attenuates neuroinflammation and cognitive impairment.

Materials and Methods

Animals

The procedures and handling of mice were approved by the Institutional Animal Care and Use Committee at the University of South Dakota and were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Animals were housed in up to four animals per cage on a 14/10 hour light/dark cycle. Animals were allowed to access to water and food ad libitum. Floxed *Psmc1* animals were previously described [16]. CamKIIα-Cre (T29-Cre) transgenic animals were obtained from the Jackson Laboratory (stock number: 005359). *Psmc1*(fl/wt);T29-Cre(Tg/wt) females were crossed with *Psmc1*(fl/fl) males, according to the breeding recommendation of Jackson Laboratory, to generate *Psmc1* KO (fl/fl);T29-Cre(Tg/wt) animals in the forebrain. The experimenter was blinded to the genotypes of the animals throughout the experiments. Mice were two months of age when used for various experiments.

Proteasome activity assay

Proteasome activity assay was performed according previous described methods [17].

Animal behavior tests

The learning and memory capability of mice was assessed using the radial arm water maze (RAWM) as previously described [18, 19].

Synaptosome isolation

Synaptosome isolation was carried out following previously described protocols [20]. One hemisphere of the cortex of each mouse brain was homogenized on ice in 7 mL of 0.32 M sucrose containing protease inhibitor cocktail, 10 mM DDT, and deubiquitinase inhibitors (5 mM *N*-ethylmaleimide and 50 mM iodoacetamide) in a 2-mL dounce homogenizer for ten strokes. Homogenates were centrifuged at 4°C for 10 minutes at 1,000 × *g*. The supernatant was then transferred on top of an equal volume of 1.2 M sucrose in ultracentrifuge tubes and ultracentrifuged at 4°C for 35 minutes at 160,000 × *g* in an SW41 rotor in the Optima XPN-100 Beckman Coulter Ultracentrifuge. The synaptosomes from the cloudy interface between 0.32 M and 1.2 M sucrose was collected.

Mass spectrometric analysis of synaptic proteins

The sample analysis was performed based on previously described methods [21, 22]. Briefly, the proteins were separated using 12% SDS-PAGE and the gel was stained with colloidal Coomassie G250 (BioRad) and destained overnight. Each lane of gel corresponding to each replicate/group was cut into 14 equal gel slices. Each gel slice was destained, followed by reduction, alkylated, and subjected to in-gel digestion before analyzed in an Easy nLC 1200 using a trapping and desalting online trap column (300 µm x 20 mm Acclaim PepMap C18 100Å (Thermo Scientific), and separated by an Easy-Spray PepMap RSLC 2 µm, 75 µm x 15 cm, nanoViper (Thermo Scientific) coupled to nanoESI orbitrap Exploris 240 HR/MA mass spectrometer. The chromatography conditions were as follows: 0–1 min, 1 % B isocratic; 2–60 min, 1–25%, 61-90 min 25-50%, and 92-101 min 50-100% B linear. Mobile Phase A (Water/Formic Acid, 99.9:0.1%

v/v), and Phase B (Water/Acetonitrile/Formic Acid, 20/80/0.1% v/v). The solvent flow rate was 300 nL per min. The orbitrap Exploris 240 instrument was operated under dependent mode (DDS) top 12 modes to automatically switch between full scan MS and MS/MS acquisition. The ions were analyzed in positive MS ion mode (m/z 375 −1500) with 120,000 resolution (m/z 200) after accumulation with target ions to 1 × 106 value based on predictive AGC. The MS/MS ions selection was set at m/z 65-1800 to 1 × 105 counts. The spectrum was deconvoluted and analyzed using the Mascot Distiller v2.6 (www.matrixscience.com) and Proteome Discoverer v2.5 (Thermo Scientific). A mascot generic format list (MGF format) was generated to identify +1 or multiple charged precursor ions from the MS data file.

Mascot server v2.8.1 (www.matrix-science.com, UK) in MS/MS ion search mode (local licenses) was applied to conduct peptide matches (peptide masses and sequence tags) and protein searches against SwissProt Mus musculus 092022 (17562 sequences; 9,985,721 residues) database. The following parameters were set for the search: carbamidomethyl (C) on cysteine was fixed; variable modifications included asparagine and glutamine deamidation, and methionine oxidation. Two missed cleavages were allowed; monoisotopic masses were counted; the precursor peptide and fragment mass tolerance were set at 15 ppm and 0.02 Da, respectively; the ion score or expected cut-off was set at 5. The MS/MS spectra were searched with MASCOT using a 95% confidence interval (% C.I.) threshold ($p<0.05$), with which a minimum score of 27 was used for peptide identification. The protein redundancy at the database under different accession numbers was limited to Mus musculus. All the proteins identified in the current study were found in these domains. The comparative study was performed using ProteoIQ v2.8 (local license) [22, 23].

Gene Ontology (GO) enrichment analysis of the identified proteins

GO analysis of the proteins was carried out by using DAVID functional annotation tool (https://david.ncifcrf.gov/summary.jsp) as previously described [21]. The UniProt IDs for each protein were converted to their official gene IDs, with Mus musculus selected as the species. The default settings for thresholds of 2 genes per term and an EASE threshold of 0.1 were utilized for the analysis. The p-value was converted to the -log(p). The Venny 2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/) was used to determine GO terms and pathways differences between control and *Psmc1* KO samples.

Western blot analysis

Western blot analysis was followed previously described methods [24, 25]. Briefly, the isolated cellular fractions or cortical lysates were diluted with a cell lysis buffer[26]. An equal amount of protein was resolved on 12% SDS-PAGE gels and the separated proteins were then transferred onto nitrocellulose membranes under standard conditions. The membrane was blocked in 5% nonfat milk in Tris-buffered saline supplemented with 0.5% Tween-20 and probed with the following primary antibodies: anti-Stat1 (1:1000, Cell Signaling), anti-Trem2 (1:1000, Cell Signaling), anti-Actin (1:1000, Developmental Studies Hybridoma Bank), anti-PSD95 (1:1000, Cell Signaling), anti-NF-κB (1:1000, Cell Signaling), and anti-GFAP (1:1000, Millipore). Following overnight incubation of the primary antibody at 4ºC, an appropriate secondary antibody was incubated at room temperature for one hour. The secondary antibodies used were conjugated with infrared dyes (1:3000, LI-COR, #926-32211; #92668072, #926-33214). Protein band intensities were quantified using ImageJ.

Histopathological analysis

Brain fixation, section and immunohistochemical procedure was based on previously described protocols [24]. Briefly, the brain sections were blocked in a blocking buffer (1% normal donkey

serum, 0.1% Triton, and 0.05% Tween). Then, the samples were incubated with the following primary antibodies: anti-GFAP (1:1000, EMD Millipore) or anti-Iba1 (1:200, FUJIFILM Wako) overnight at 4ºC. Next, the sections were incubated with a Cy3-conjugated goat anti-rabbit secondary antibody (1:200, Jackson ImmunoResearch). The nuclei were counterstained with the DNA-binding dye Hoechst 33342 (1:1000, Thermo Fisher). Images were collected using the Zeiss Axiovert Inverted Epifluorescent Microscope. Three sections per animal were analyzed. The analysis of GFAP and Iba1 consisted of counting the GFAP- or Iba1-positive cells before dividing them by the total (neuronal and glial) number of cells (Hoechst 33342-positive cells).

Treating mice with NF-κB inhibitor

Psmc1 KO mice were treated with pyrrolidinedithiocarbamate ammonium (PDTC), a selective NF-κB inhibitor[27], daily by intraperitoneal injections at a dose of 50 mg/kg for three weeks once mice reached five weeks of age. At eight weeks of age, mice received radial arm water maze test as described above. On the day of behavioral tests, injections were performed early in the morning, and behavioral tests began at midday. Biweekly body weights of the mice were taken to monitor for any side effects of the drug.

Statistical analysis

GraphPad Prism Statistical Software version 9.4.1 was utilized for statistical analysis and graphical displays. All numerical data are presented as mean ± SEM. A student's t-test was utilized to compare two groups. A Mann-Whitney Test was used for the comparison between control and KO animals in the hindlimb clasping. For the analysis of the RAWM, average errors to find the platform were calculated on a three-trial block. For comparisons between control and *Psmc1* KO animals in the RAWM, repeated measures, two-way ANOVA, and Sidak's multiple comparisons test were utilized. Statistical significance was accepted when $p < 0.05$.

Results

Genetically disrupting 26S proteasome reduces proteasome activity and impairs animal learning and memory

To understand the effect of 26S proteasome deficiency on brain function, we genetically ablated *Psmc1*, one 19S proteasomal subunit of the 26S proteasome, in the forebrain region. This causes the19S regulatory particle to be unable to bind to the 20S core particle, rendering the deficiency of the 26S proteasome in this brain region. Utilizing this model, we initially examined the three proteasome activities, chymotrypsin-, caspase- and trypsin -like proteasome activities, in the forebrain. As demonstrated in **Fig. 1A**, **1B,** and **1C**, all of the three proteasome activities were reduced in the KO brain tissues compared to the control animal tissues at two months of age. We then performed the radial arm water maze test to assess their learning and memory capabilities. *Psmc1* KO mice exhibited a significant increase in the number of incorrect arm entries (errors) to find the platform compared to control animals at blocks 4, 5, 6, 7, 8, and 10, suggesting an impairment in learning and memory (**Fig. 1D**). A significant increase in the time for the mice to find the platform was also observed in the *Psmc1* KO animals compared to the control animals at blocks 1, 2, 4, 5, and 10 (**Fig. 1E**). Thus, 26S proteasome deficiency impairs animal learning and memory.

26S proteasome deficiency causes synapse loss and neuroinflammation in the brain

Next, we decided to assess the synapses at two months of age, as synapse loss shows the strongest correlation for cognitive decline in AD [28-31]. We therefore isolated synaptosomes from the cortex of the *Psmc1* KO and the control animals to explore the possibility of synapse alterations. Synapse fractionation was confirmed by western blot (WB) analysis of a postsynaptic and a presynaptic marker protein. As shown in **Fig. 2A, 2B,** the postsynaptic marker, PSD95, in the *Psmc1* KO synaptosomes, exhibited a significant reduction in expression compared to control

synaptosomes, suggesting the possibility of synaptic loss occurring at this age. However, SNAP-25, a presynaptic marker, showed no differences between control and *Psmc1* KO animals in the synaptosome isolates at this stage (**Fig. 2A, 2C**).

With the isolated synaptosomes, we then performed proteomic analysis using the mass spectrometry (MS)-based approach, an unbiased method commonly used for proteomics. MS analysis of synaptosomal proteins in the control samples revealed 176 unique diverse proteins, Surprisingly, only 130 proteins were found exclusively in *Psmc1* KO synapses (**Fig. 1D**). Gene Ontology (GO) analysis of control and *Psmc1* KO synaptosomal proteins identified differential molecular functions between the two groups. The biological processes identified, from control synaptosomes, the proteins essential for mitochondria function and nervous system development (**Fig. 1E**). However, the *Psmc1* KO synaptosomes exhibited biological processes associated with the immune system response and inflammatory response (**Fig. 1F**). The molecular functions identified in control synaptosomes consisted of the structural constituent of the ribosome and RNA binding as the top two pathways (data not shown). In contrast, *Psmc1* KO synaptosomes consisted of molecular functions associated with inflammation and transmembrane signaling receptor activity (data not shown), providing further support for an inflammatory response occurring at the synapse. This data suggests that *Psmc1* KO synaptosomes contain proteins associated with inflammation that are absent from control synaptosomes.

26S proteasome deficiency causes accumulation of inflammation-associated proteins

As inflammation is a biological response that is not limited to synapses, we next examined some of the identified inflammation-related proteins, including Stat1, Trem2, and NF-κB in the brain cortex lysates by western blot. Stat1and NF-κB are transcription factors and activation of the two pathways are the most commonly inflammatory signaling pathways following immune responses[32], whereas TREM2 is an innate immune cell surface receptor regulating microglial

function and is involved in the pathogenesis of many neurodegenerative diseases[33, 34]. Recent genome-wide association studies have identified rare variants in the gene triggering receptor expressed on myeloid cells 2 (*TREM2*) that are associated with a high risk for the development of late-onset AD [35-37]. Stat1 expression significantly increased in *Psmc1* KO brain samples compared to control samples (**Fig. 3A, 3B**). Similarly, we observed a significant increase in TREM2 and NF-κB in *Psmc1* KO brain samples compared to control samples (**Fig. 3A-3D**). Therefore, 26S proteasome deficiency upregulates the key inflammatory regulatory proteins, including NF-κB, a master regulator of innate immune response [38].

26S proteasome deficiency activates astrocytes and microglia

As activation of microglia and astrocytes is a common feature of neuroinflammation and involved in a number of neurodegenerative diseases[8], we next assessed whether the two types of glia were activated. The glial fibrillary acidic protein (GFAP), a protein indicative of astrocytes, represents a biomarker of neuroinflammation, as an increase in its expression indicates more of a reactive phenotype of astrocytes [39]. WB analysis of GFAP protein level in the brains of controls and *Psmc1* KO samples indicated a significant increase in GFAP in *Psmc1* KO samples compared to controls, providing further support for overt neuroinflammation occurring in the brain following 26S proteasome disruption (**Fig. 3E, 3F**).

Immunohistochemistry analysis of GFAP revealed significantly more GFAP staining in the cortex of *Psmc1* KO brains compared to control samples, suggesting reactive gliosis occurring following 26S proteasome disruption (**Fig. 3G-3I**). Ionized calcium-binding adaptor molecule 1 (Iba1) is a marker for microglia, the resident macrophage cells in the brain [40]. An increase in Iba1 expression is indicative of microglial activation [41]. There was a significant increase in Iba1-positive cells in the brains of *Psmc1* KO animals compared to control samples,

providing further support for an inflammatory response occurring in the brains of *Psmc1* KO animals (**Fig. 3J-3L**).

Inhibition of NF-κB improves learning and memory in *Psmc1* KO animals

Aforementioned results consistently support that disrupting the 26S proteasome causes neuroinflammation and activation of the NF-κB pathway. As NF-κB is a master regulator of innate immune responses and plays crucial role in modulating inflammatory responses [38, 42], we assessed whether treatment of Psmc1 KO mice with the PDTC, a selective and blood-brain barrier-permeable NF-κB inhibitor [43], confers any beneficial effect on the mice. We therefore treated the KO mice at five weeks of age and then continued the treatment for three weeks by daily intraperitoneal injections of 50 mg/kg PDTC prior to behavioral testes at seven weeks and sacrifice at eight weeks for examination of inflammation (**Fig. 4A**). Behavioral tests for PDTC-treated animals began when they reached seven weeks of age (**Fig. 4A**). The learning and memory capability of animals was assessed using the RAWM following NF-κB inhibition. *Psmc1* KO + PDTC-treated animals demonstrated better learning and memory capability compared to the KO control animals at blocks 7 – 10 trials for Day 2 of testing (**Fig. 4B**). Furthermore, *Psmc1* KO + PDTC-treated animals spent less time to find the platform in the RAWM compared to the KO control mice (**Fig. 4C**). This data suggests that NF-κB inhibition can reverse the learning and memory impairments caused by 26S proteasome disruption.

Inhibition of NF-κB improves 26S proteasome deficiency-caused neuroinflammation

As we observed increased inflammation-associated proteins in the brains of *Psmc1* KO animals, we wondered what the effect NF-κB inhibition would have on Stat1 and Trem2. To that end, we performed Western blot analysis of the proteins following the drug treatment. Stat1 expression significantly decreased when *Psmc1* KO animals were treated with the NF-κB inhibitor compared to the KO control samples (**Fig. 5A, 5B**). We found a significant decrease in Trem2 in

Psmc1 KO + PDTC treated samples compared to the KO control samples (**Fig. 5A, 5C**). These results indicate that NF-κB inhibition suppresses 26S proteasome deficiency caused upregulation of Stat1 and Trem2 in *Psmc1* KO animals.

Immunohistochemistry analysis of GFAP revealed significantly reduced GFAP in the cortex of *Psmc1* KO + PDTC treated mice compared to the KO control animals, suggesting blockage of reactive astrocytes following the inhibition of NF- κB (**Fig. 5D-5F**). Similarly, there was a significant decrease in Iba1-positive cells in the brains of *Psmc1* KO + PDTC treated mice compared to the KO control animals (**Fig. 5G-5I**), providing further support for the attenuation of the inflammatory response in *Psmc1* KO animals following NF-κB inhibition.

Discussion

Impaired proteasome and neuroinflammation have been implicated in various neurodegenerative disorders, including Alzheimer's disease. However, whether impaired proteasome is a causative mechanism to neuroinflammation remains to be defined. Here, we took advantage of a genetic mouse model to ablate the function of the 26S proteasome that is primarily responsible for ubiquitin-dependent protein degradation to determine the effect of disrupting 26S proteasome on neuroinflammation and neuronal function. Our results consistently indicated that 26S proteasome deficiency caused synapse loss, neuroinflammation, and learning and memory dysfunction. Importantly, we identified that 26S proteasome deficiency-caused neuroinflammation was mediated largely by the NF-κB signaling pathway. Hence, our data provide direct evidence that impaired 26S proteasome causes neuroinflammation in the brain.

In addition to neuroinflammation, loss of synapses is another key pathological hallmark of AD. In the isolated synaptosomes, we observed reduced total synaptic proteins, and

diminished level of PSD95, a postsynaptic marker, in the in the *Psmc1* KO mouse brain, indicative of synapse loss occurred in the KO brain. Previous data has indicated that reduced PSD95 level can alter neuronal functions, consequently leading to the synaptic loss and neurodegeneration [44]. Interestingly, SNAP25, a presynaptic marker, did not decrease in the isolated synaptosomes from KO brain at this stage. Neuroinflammation observed in the KO mouse brain may account for the reduced PSD95, because some inflammation- related transcription factors, such as Stat1, are responsible for impairing the structure and function of synapses [45-48].

Our data consistently support a significant role of NF-κB, a master regulator of innate immune responses [38, 42, 49, 50], in 26S proteasome deficiency-induced neuroinflammation. First, our proteomics and WB results showed that NF-κB levels were increased in *Psmc1* KO brain. Second, our transcriptome analysis identified NF-κB signaling pathway as one of the top listed pathways among the upregulated genes following proteasome disruption. Third, inhibition of NF-κB was able to attenuate 26S proteasome deficiency-caused neuroinflammation in Psmc1 KO mouse brain and reduce learning and memory impairment. These data suggest that 26S proteasome deficiency-caused neuroinflammation is mediated, at least partially, through NF-κB signaling pathway (**Fig. 6**). Activated NF-kB then induces neuroinflammation via Stat1, TREM2 and other downstream factors, leading to synapse loss and impaired cognitive function (**Fig. 6**).

As NF-κB can be degraded by the 26S proteasome [51], impaired proteasome would result in increased NF-κB. As a master regulator of immune responses, NF-κB induces the expression of diverse proinflammatory genes, including those encoding cytokines and chemokinesy [42]. A high NF-κB level is correlated with a high level of β-site amyloid precursor protein (APP) cleaving enzyme-1 (BACE) and the p65 subunit of NF-κB can bind to the κB elements on the promoter of BACE to facilitate the expression of β-secretase [52]. As a result,

the cleavage of APP into Aβ increases, leading to the formation of Aβ fibrils. Additionally, Aβ oligomers can also activate NF-κB [52], astrocytes, and microglia [53]. Therefore, 26S proteasome disruption may induce a chain reaction, involving multiple mechanisms and causing NF-κB levels even higher. All of these are responsible for the marked neuroinflammation observed here. Moreover, we found that other immune response related proteins, Stat1 and Trem2, also increased in the KO mouse brain. Since NF-κB regulates Trem2 to shape innate immune and phagocytic responses by microglia that contribute to neuroinflammation [54]. Although Stat1 is also upregulated in the KO mouse brain, it may not be a targeted gene of NF-κB, as the two transcriptional factors are activated by distinct extracellular stimuli and cross talk to confer a synergy in regulating the expression of inflammatory genes at the transcriptional level [55]. Conversely, inhibition of NF-κB reduced Stat1 level, which should be neuroprotective in the KO mouse brain [47]. These different mechanisms work together contributing to neuroinflammation observed in mice.

In humans, variance of *Psmc1* gene is associated with an autosomal recessive syndrome of severe developmental delay and intellectual disability, hearing loss, and other problems [56]. Impaired learning and memory observed from *Psmc1* KO mice are consistent with this clinical data, indicating that impaired proteasome could be a driver for the development of an AD-like phenotype. This study demonstrates that proteasome function and neuroinflammation may go "hand-in-hand," as neuroinflammation was exacerbated in 26S proteasome deficiency mice. Indeed, this possibility is strengthened by our data that inhibiting one inflammation signaling pathway, NF-κB, can reverse the phenotype-induced by 26S proteasome disruption.

Conclusion

In this study, we discovered that 26S proteasome deficiency impairs learning and memory and causes neuroinflammation. We also identified a significant mediator, NF-κB, in modulating the 26S impairment-caused neuroinflammation and neurodysfunction. Our results will help better understand the pathogenesis of AD and many other neurodegenerative disorders where impaired proteasome is consistently coupled with neuroinflammation.

Availability of data and materials
Data and materials supporting the findings of this manuscript are available from the corresponding author upon reasonable request.

Author contributions
Hongmin Wang conceived the presented idea and model. Hongmin Wang, Christa Huber, and Eduardo Callegari designed the experiments, and Christa Huber, Eduardo Callegari, Maria Paez, and Xiaoping Li performed experiments and analyzed results. Christa Huber and Hongmin Wang interpreted the data and wrote the manuscript.

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Conflict of interest statement
The authors declare no conflicts of interest.

Ethics approval and consent to participate statement
Not applicable.

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References

1. Keller JN, Hanni KB, Markesbery WR: **Impaired proteasome function in Alzheimer's disease**. *J Neurochem* 2000, **75**(1):436-439.

2. Liu Y, Hettinger CL, Zhang D, Rezvani K, Wang X, Wang H: **The proteasome function reporter GFPu accumulates in young brains of the APPswe/PS1dE9 Alzheimer's disease mouse model**. *Cell Mol Neurobiol* 2014, **34**(3):315-322.
3. Wang X, Wang H: **Priming the Proteasome to Protect against Proteotoxicity**. *Trends Mol Med* 2020, **26**(7):639-648.
4. Li H, Guo M: **Protein degradation in Parkinson disease revisited: it's complex**. *J Clin Invest* 2009, **119**(3):442-445.
5. Medina DX, Caccamo A, Oddo S: **Methylene blue reduces abeta levels and rescues early cognitive deficit by increasing proteasome activity**. *Brain Pathol* 2011, **21**(2):140-149.
6. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N: **Neuroinflammation as a Common Feature of Neurodegenerative Disorders**. *Front Pharmacol* 2019, **10**:1008.
7. Tran VTA, Lee LP, Cho H: **Neuroinflammation in neurodegeneration via microbial infections**. *Front Immunol* 2022, **13**:907804.
8. Kwon HS, Koh SH: **Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes**. *Transl Neurodegener* 2020, **9**(1):42.
9. Zhao R, Hu W, Tsai J, Li W, Gan WB: **Microglia limit the expansion of beta-amyloid plaques in a mouse model of Alzheimer's disease**. *Mol Neurodegener* 2017, **12**(1):47.
10. Wei Y, Li X: **Different phenotypes of microglia in animal models of Alzheimer disease**. *Immun Ageing* 2022, **19**(1):44.
11. Budenholzer L, Cheng CL, Li Y, Hochstrasser M: **Proteasome Structure and Assembly**. *J Mol Biol* 2017, **429**(22):3500-3524.
12. Ding M, Shen K: **The role of the ubiquitin proteasome system in synapse remodeling and neurodegenerative diseases**. *Bioessays* 2008, **30**(11-12):1075-1083.
13. Fernandez-Cruz I, Reynaud E: **Proteasome Subunits Involved in Neurodegenerative Diseases**. *Arch Med Res* 2021, **52**(1):1-14.
14. Gupta I, Singh K, Varshney NK, Khan S: **Delineating Crosstalk Mechanisms of the Ubiquitin Proteasome System That Regulate Apoptosis**. *Front Cell Dev Biol* 2018, **6**:11.
15. Collins GA, Goldberg AL: **The Logic of the 26S Proteasome**. *Cell* 2017, **169**(5):792-806.
16. Bedford L, Hay D, Devoy A, Paine S, Powe DG, Seth R, Gray T, Topham I, Fone K, Rezvani N *et al*: **Depletion of 26S proteasomes in mouse brain neurons causes neurodegeneration and Lewy-like inclusions resembling human pale bodies**. *J Neurosci* 2008, **28**(33):8189-8198.
17. Liu Y, Qiao F, Leiferman PC, Ross A, Schlenker EH, Wang H: **FOXOs modulate proteasome activity in human-induced pluripotent stem cells of Huntington's disease and their derived neural cells**. *Hum Mol Genet* 2017, **26**(22):4416-4428.
18. Adegoke OO, Qiao F, Liu Y, Longley K, Feng S, Wang H: **Overexpression of Ubiquilin-1 Alleviates Alzheimer's Disease-Caused Cognitive and Motor Deficits and Reduces Amyloid-beta Accumulation in Mice**. *J Alzheimers Dis* 2017, **59**(2):575-590.
19. Alamed J, Wilcock DM, Diamond DM, Gordon MN, Morgan D: **Two-day radial-arm water maze learning and memory task; robust resolution of amyloid-related memory deficits in transgenic mice**. *Nat Protoc* 2006, **1**(4):1671-1679.
20. Hajos F: **An improved method for the preparation of synaptosomal fractions in high purity**. *Brain Res* 1975, **93**(3):485-489.
21. Huber CC, Callegari EA, Paez MD, Romanova S, Wang H: **Heat Shock-Induced Extracellular Vesicles Derived from Neural Stem Cells Confer Marked Neuroprotection Against Oxidative Stress and Amyloid-β-Caused Neurotoxicity**. *Molecular Neurobiology* 2022.
22. Paez MD, Callegari EA: **Proteomics Analysis of the Estrogen Effects in the Rat Uterus Using Gel-LC and Tandem Mass Spectrometry Approaches**. *Methods Mol Biol* 2022, **2418**:289-311.

23. Bonilla JO, Callegari EA, Paez MD, Gil RA, Villegas LB: **Bivalent copper ions presence triggers removal and homeostatic mechanisms in the metal-resistant microorganism Apiotrichum loubieri M12**. *Res Microbiol* 2023, **174**(3):104013.
24. Liu Y, Subedi K, Baride A, Romanova S, Callegari E, Huber CC, Wang X, Wang H: **Peripherally misfolded proteins exacerbate ischemic stroke-induced neuroinflammation and brain injury**. *J Neuroinflammation* 2021, **18**(1):29.
25. Liu Y, Min JW, Feng S, Subedi K, Qiao F, Mammenga E, Callegari E, Wang H: **Therapeutic Role of a Cysteine Precursor, OTC, in Ischemic Stroke Is Mediated by Improved Proteostasis in Mice**. *Transl Stroke Res* 2020, **11**(1):147-160.
26. Dong G, Callegari EA, Gloeckner CJ, Ueffing M, Wang H: **Prothymosin-alpha interacts with mutant huntingtin and suppresses its cytotoxicity in cell culture**. *J Biol Chem* 2012, **287**(2):1279-1289.
27. Chu W, Song X, Yang X, Ma L, Zhu J, He M, Wang Z, Wu Y: **Neuropilin-1 promotes epithelial-to-mesenchymal transition by stimulating nuclear factor-kappa B and is associated with poor prognosis in human oral squamous cell carcinoma**. *PLoS One* 2014, **9**(7):e101931.
28. DeKosky ST, Scheff SW, Styren SD: **Structural correlates of cognition in dementia: quantification and assessment of synapse change**. *Neurodegeneration* 1996, **5**(4):417-421.
29. Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R: **Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment**. *Ann Neurol* 1991, **30**(4):572-580.
30. Scheff SW, Price DA: **Alzheimer's disease-related alterations in synaptic density: neocortex and hippocampus**. *J Alzheimers Dis* 2006, **9**(3 Suppl):101-115.
31. de Wilde MC, Overk CR, Sijben JW, Masliah E: **Meta-analysis of synaptic pathology in Alzheimer's disease reveals selective molecular vesicular machinery vulnerability**. *Alzheimers Dement* 2016, **12**(6):633-644.
32. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L: **Inflammatory responses and inflammation-associated diseases in organs**. *Oncotarget* 2018, **9**(6):7204-7218.
33. Diaz-Lucena D, Kruse N, Thune K, Schmitz M, Villar-Pique A, da Cunha JEG, Hermann P, Lopez-Perez O, Andres-Benito P, Ladogana A *et al*: **TREM2 expression in the brain and biological fluids in prion diseases**. *Acta Neuropathol* 2021, **141**(6):841-859.
34. Yang J, Fu Z, Zhang X, Xiong M, Meng L, Zhang Z: **TREM2 ectodomain and its soluble form in Alzheimer's disease**. *J Neuroinflammation* 2020, **17**(1):204.
35. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E, Cruchaga C, Sassi C, Kauwe JS, Younkin S *et al*: **TREM2 variants in Alzheimer's disease**. *N Engl J Med* 2013, **368**(2):117-127.
36. Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, Bjornsson S, Huttenlocher J, Levey AI, Lah JJ *et al*: **Variant of TREM2 associated with the risk of Alzheimer's disease**. *N Engl J Med* 2013, **368**(2):107-116.
37. Neumann H, Daly MJ: **Variant TREM2 as risk factor for Alzheimer's disease**. *N Engl J Med* 2013, **368**(2):182-184.
38. Dorrington MG, Fraser IDC: **NF-kappaB Signaling in Macrophages: Dynamics, Crosstalk, and Signal Integration**. *Front Immunol* 2019, **10**:705.
39. Wilhelmsson U, Bushong EA, Price DL, Smarr BL, Phung V, Terada M, Ellisman MH, Pekny M: **Redefining the concept of reactive astrocytes as cells that remain within their unique domains upon reaction to injury**. *Proc Natl Acad Sci U S A* 2006, **103**(46):17513-17518.
40. Guan YH, Zhang LJ, Wang SY, Deng YD, Zhou HS, Chen DQ, Zhang LC: **The role of microglia in Alzheimer's disease and progress of treatment**. *Ibrain* 2022, **8**(1):37-47.
41. Hovens I, Nyakas C, Schoemaker R: **A novel method for evaluating microglial activation using ionized calcium-binding adaptor protein-1 staining: cell body to cell size ratio**. *Neuroimmunology and Neuroinflammation* 2014, **1**(2).

42. Sun E, Motolani A, Campos L, Lu T: **The Pivotal Role of NF-kB in the Pathogenesis and Therapeutics of Alzheimer's Disease**. *Int J Mol Sci* 2022, **23**(16).
43. Kan MH, Yang T, Fu HQ, Fan L, Wu Y, Terrando N, Wang TL: **Pyrrolidine Dithiocarbamate Prevents Neuroinflammation and Cognitive Dysfunction after Endotoxemia in Rats**. *Front Aging Neurosci* 2016, **8**:175.
44. Sultana R, Banks WA, Butterfield DA: **Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease**. *J Neurosci Res* 2010, **88**(3):469-477.
45. Ma D, Huang R, Guo K, Zhao Z, Wei W, Gu L, Li L, Zhang L: **Cornel Iridoid Glycoside Protects Against STAT1-Dependent Synapse and Memory Deficits by Increasing N-Methyl-D-aspartate Receptor Expression in a Tau Transgenic Mice**. *Front Aging Neurosci* 2021, **13**:671206.
46. Li XG, Hong XY, Wang YL, Zhang SJ, Zhang JF, Li XC, Liu YC, Sun DS, Feng Q, Ye JW *et al*: **Tau accumulation triggers STAT1-dependent memory deficits by suppressing NMDA receptor expression**. *EMBO Rep* 2019, **20**(6).
47. Han W, Pu H, Li S, Liu Y, Zhao Y, Xu M, Chen C, Wu Y, Yang T, Ye Q *et al*: **Targeted ablation of signal transducer and activator of transduction 1 alleviates inflammation by microglia/macrophages and promotes long-term recovery after ischemic stroke**. *J Neuroinflammation* 2023, **20**(1):178.
48. Hsu WL, Ma YL, Hsieh DY, Liu YC, Lee EH: **STAT1 negatively regulates spatial memory formation and mediates the memory-impairing effect of Abeta**. *Neuropsychopharmacology* 2014, **39**(3):746-758.
49. Gu SM, Park MH, Hwang CJ, Song HS, Lee US, Han SB, Oh KW, Ham YW, Song MJ, Son DJ *et al*: **Bee venom ameliorates lipopolysaccharide-induced memory loss by preventing NF-kappaB pathway**. *J Neuroinflammation* 2015, **12**:124.
50. Khalil MNA, Choucry MA, El Senousy AS, Hassan A, El-Marasy SA, El Awdan SA, Omar FA: **Ambrosin, a potent NF-kappabeta inhibitor, ameliorates lipopolysaccharide induced memory impairment, comparison to curcumin**. *PLoS One* 2019, **14**(7):e0219378.
51. Liu S, Chen ZJ: **Expanding role of ubiquitination in NF-kappaB signaling**. *Cell Res* 2011, **21**(1):6-21.
52. Chen CH, Zhou W, Liu S, Deng Y, Cai F, Tone M, Tone Y, Tong Y, Song W: **Increased NF-kappaB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease**. *Int J Neuropsychopharmacol* 2012, **15**(1):77-90.
53. Di Benedetto G, Burgaletto C, Bellanca CM, Munafo A, Bernardini R, Cantarella G: **Role of Microglia and Astrocytes in Alzheimer's Disease: From Neuroinflammation to Ca(2+) Homeostasis Dysregulation**. *Cells* 2022, **11**(17).
54. Zhao Y, Bhattacharjee S, Jones BM, Dua P, Alexandrov PN, Hill JM, Lukiw WJ: **Regulation of TREM2 expression by an NF-small ka, CyrillicB-sensitive miRNA-34a**. *Neuroreport* 2013, **24**(6):318-323.
55. Barbosa Lima LE, Muxel SM, Kinker GS, Carvalho-Sousa CE, da Silveira Cruz-Machado S, Markus RP, Fernandes P: **STAT1-NFkappaB crosstalk triggered by interferon gamma regulates noradrenaline-induced pineal hormonal production**. *J Pineal Res* 2019, **67**(3):e12599.
56. Aharoni S, Proskorovski-Ohayon R, Krishnan RK, Yogev Y, Wormser O, Hadar N, Bakhrat A, Alshafee I, Gombosh M, Agam N *et al*: **PSMC1 variant causes a novel neurological syndrome**. *Clin Genet* 2022, **102**(4):324-332.

Figure Legends

Fig. 1. Genetically disrupting 26S proteasome reduces proteasome activity and impairs animal learning and memory. Chymotrypsin- (**A**), caspase- (**B**) and trypsin-like (**C**) proteasome activities were reduced in the forebrain of the KO mice (n = 3). The RAWM was used to assess animals' spatial learning and memory by counting the number of incorrect arm entries (errors) (**D**) and the time it takes each mouse to find the platform (**E**). Data are presented as mean ± SEM; n = 11 - 34 per genotype, * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (RAWM data analyzed by repeated measures, two-way ANOVA, and Sidak's multiple comparisons test. All other data was analyzed by a student's t-test).

Fig. 2. Mass spectrometry combined with bioinformatic analyses of synaptic proteins identifies inflammation and immune responses as the top listed pathways in *Psmc1* KO brains at two months of age. Synaptosomes were isolated from the cortex of control and *Psmc1* KO animals. *Psmc1* KO animals showed a significant decrease in PSD95, a postsynaptic marker, compared to control animals (**A, B**). SNAP-25, a presynaptic marker, exhibited no statistical difference between control and *Psmc1* KO animals (**A, C**). Data are presented as mean ± SEM; n = 3 per genotype, ** $p < 0.01$ (Data was analyzed by a student's t-test). Actin was used as a loading control. Mass spectrometric analysis of synaptic proteins revealed 176 proteins unique to control synaptosomes and 130 proteins unique to *Psmc1* KO synaptosomes (**D**). GO enrichment analysis for the top biological processes unique to control (**E**) and *Psmc1* KO synaptosomes (**F**) (n = 3 per genotype).

Fig. 3. 26S proteasome deficiency causes neuroinflammation at two months of age. Western blot analysis was conducted to define the expression of Stat1, Trem2 and NF-κB in *Psmc1* KO

and control brain samples (**A**). The representative ratio of inflammatory-associated proteins compared to Actin (loading control) for Stat1 (**B**), Trem2 (**C**), and NF-κB (**D**). Data are presented as mean ± SEM; n = 6 per genotype, * $p < 0.05$; ** $p < 0.01$ (Data analyzed by a student's t-test). Western blot analysis of GFAP (**E, F**) in the cortex of *Psmc1 KO* and control samples. Immunohistochemical analysis of the cortex of GFAP (**G – I**) and Iba1 (**J – L**) suggests proteasome disruption causes an overt inflammatory response. Scale bar, 50 µm. Data are presented as mean ± SEM; n = 3 – 6 per genotype, * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Data analyzed by a student's t-test; blue, Hoechst; green, GFAP; red, Iba1).

Fig. 4. Inhibition of NF-κB improves learning and memory in *Psmc1* KO mice. Mice were treated with the NF-κB inhibitor, PDTC, at five weeks of age for three weeks (**A**). Learning and memory tests were carried out after the mice received the final PDTC treatment (**A**). The RAWM was used to assess animals' learning and memory capabilities (**B**) and (**C**). Data are presented as mean ± SEM; n = 5 – 14 animals per genotype, * $p < 0.05$; ** $p < 0.01$. RAWM data analyzed by repeated measures, one-way ANOVA and Tukey's post hoc test.

Fig. 5. Inhibition of NF-κB attenuates neuroinflammation in *Psmc1* KO mice. Western blot analysis of the expression of Stat1 and TREM2 in *Psmc1* KO and control brain samples revealed that NF-κB inhibition decreased the level of Stat1 (**B & C**) and Trem2 (**B & D**) in the brains of *Psmc1* KO animals. (**A**). The representative ratio of inflammatory-associated proteins compared to Actin (loading control) for Stat1 (**C**), and Trem2 (**D**). Data are presented as mean ± SEM; n = 3 – 4 per genotype, * $p < 0.05$; ** $p < 0.01$ (Data analyzed by a student's t-test). Immunohistochemical analysis of the cortex of GFAP (**E-G**) and Iba1 (**H-J**). Scale bar, 50 µm.

Data are presented as mean ± SEM; n = 3 per genotype, * $p < 0.05$ and **** $p < 0.0001$ (Data analyzed by a student's t-test; blue, Hoechst; green, GFAP; red, Iba1).

Fig. 6. Working model. Impaired 26S proteasome causes neuroinflammation, synaptic loss, and disrupted learning and memory via activation of NF-kB.

Figure 1

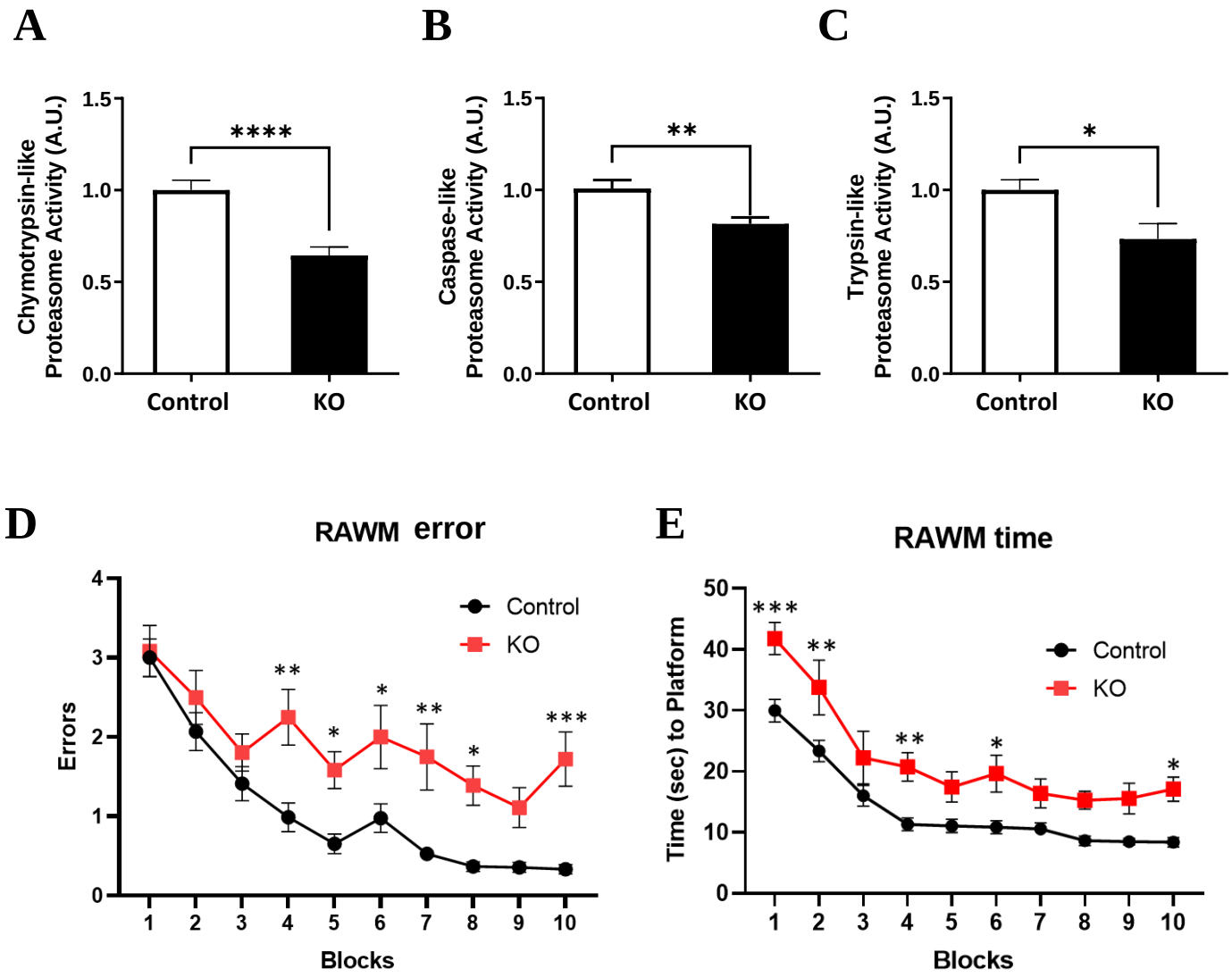

A
Chymotrypsin-like Proteasome Activity (A.U.)
1.5
1.0
0.5
0.0
****
Control
KO
B
Caspase-like Proteasome Activity (A.U.)
1.5
1.0
0.5
0.0
**
Control
KO
C
Trypsin-like Proteasome Activity (A.U.)
1.5
1.0
0.5
0.0
*
Control
KO
D
RAWM error
Errors
4
3
2
1
0
Control
KO
**
*
*
**
*
***
1 2 3 4 5 6 7 8 9 10
Blocks
E
RAWM time
Time (sec) to Platform
50
40
30
20
10
0
***
**
**
*
*
Control
KO
1 2 3 4 5 6 7 8 9 10
Blocks

Figure 2

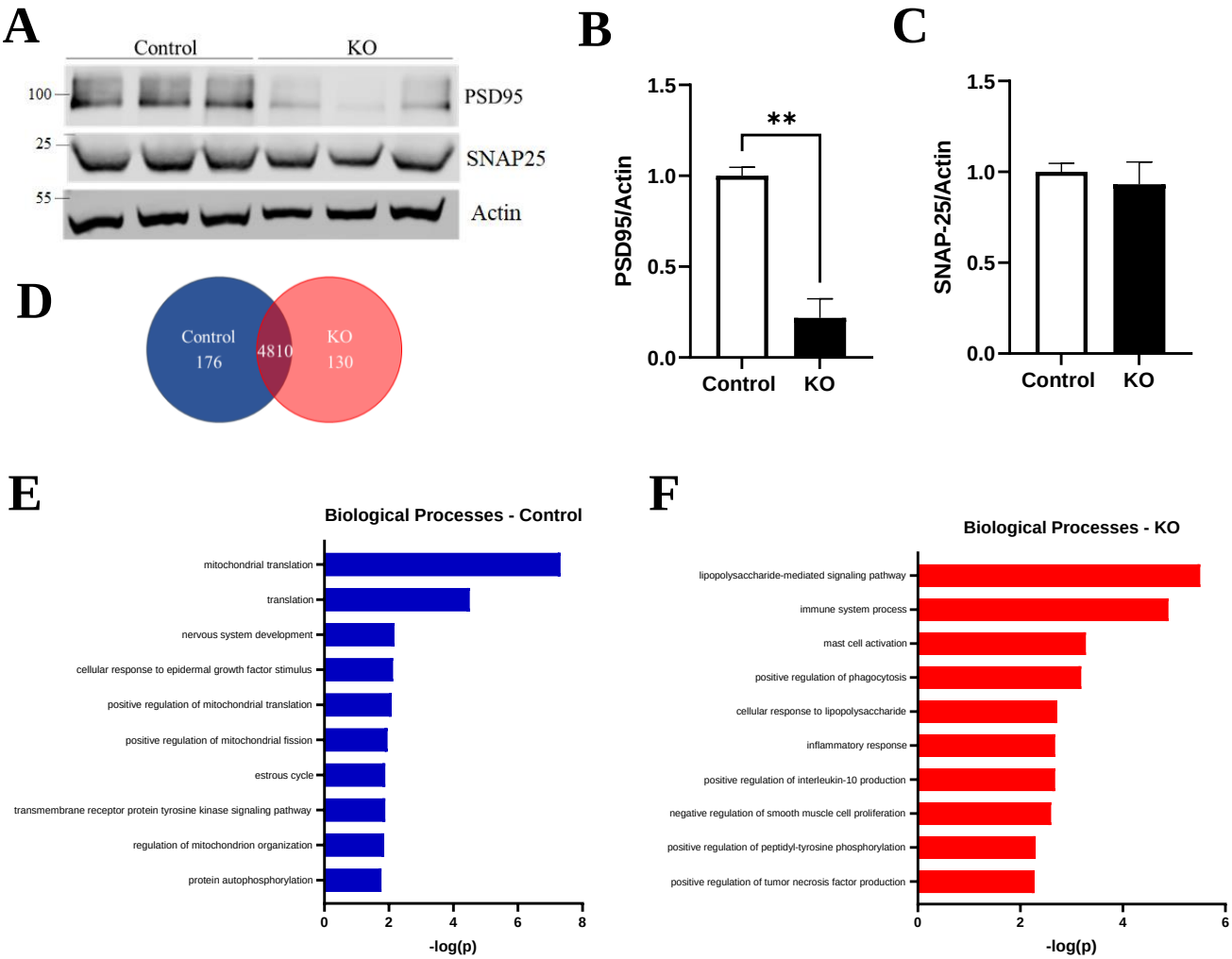

A
Control
KO
100
25
55
PSD95
SNAP25
Actin
B
PSD95/Actin
1.5
1.0
0.5
0.0
**
Control
KO
C
SNAP-25/Actin
1.5
1.0
0.5
0.0
Control
KO
D
Control
176
4810
KO
130
E
Biological Processes - Control
mitochondrial translation
translation
nervous system development
cellular response to epidermal growth factor stimulus
positive regulation of mitochondrial translation
positive regulation of mitochondrial fission
estrous cycle
transmembrane receptor protein tyrosine kinase signaling pathway
regulation of mitochondrion organization
protein autophosphorylation
0
2
4
6
8
-log(p)
F
Biological Processes - KO
lipopolysaccharide-mediated signaling pathway
immune system process
mast cell activation
positive regulation of phagocytosis
cellular response to lipopolysaccharide
inflammatory response
positive regulation of interleukin-10 production
negative regulation of smooth muscle cell proliferation
positive regulation of peptidyl-tyrosine phosphorylation
positive regulation of tumor necrosis factor production
0
2
4
6
-log(p)

Figure 3

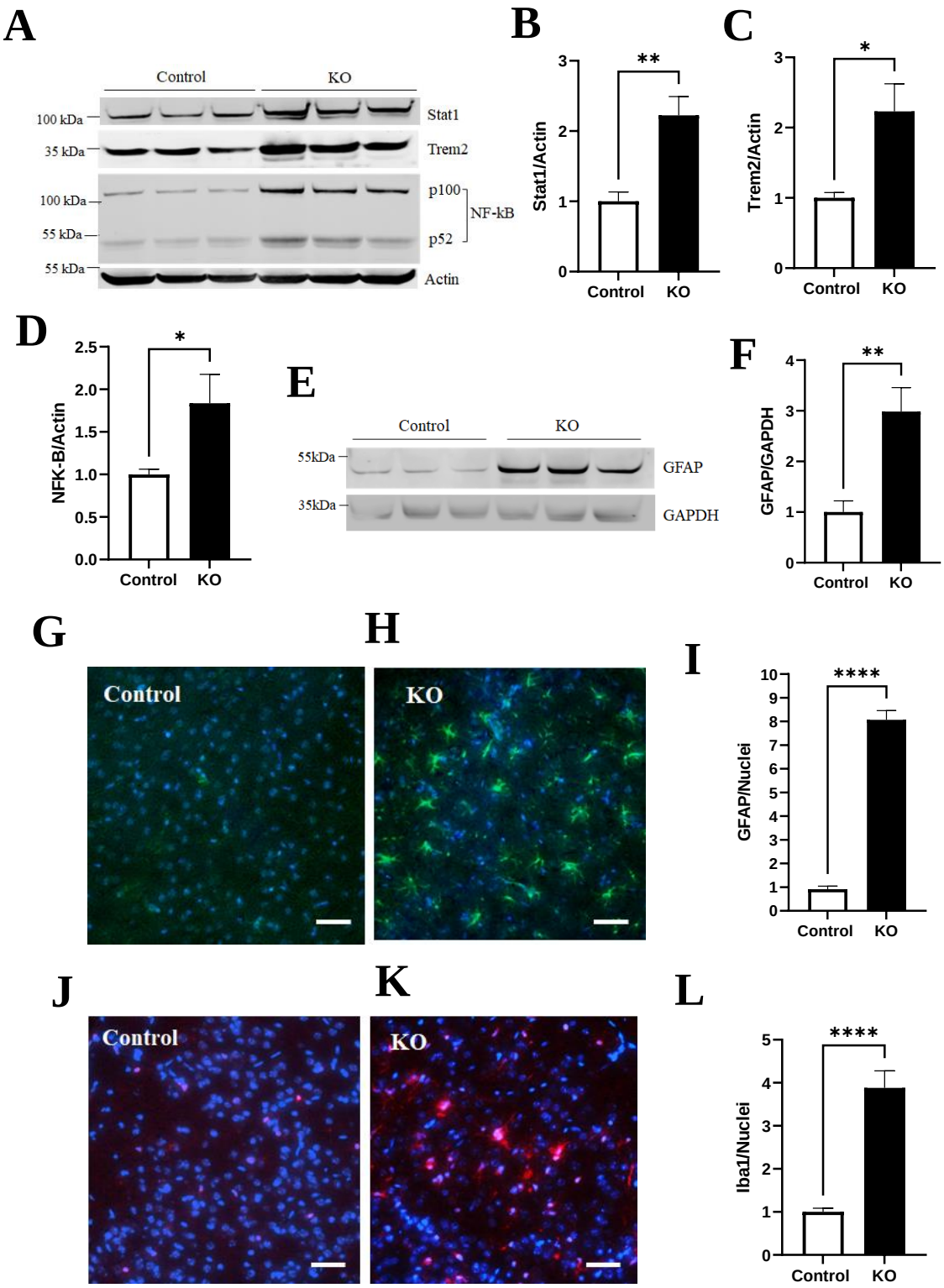
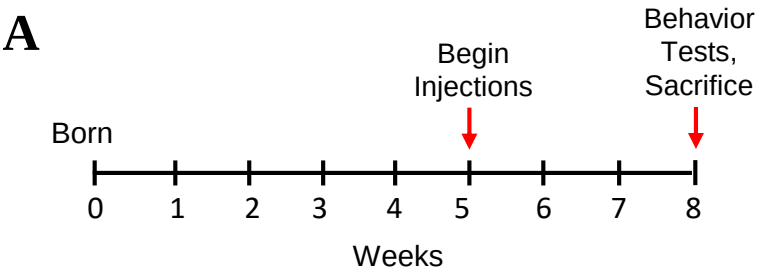
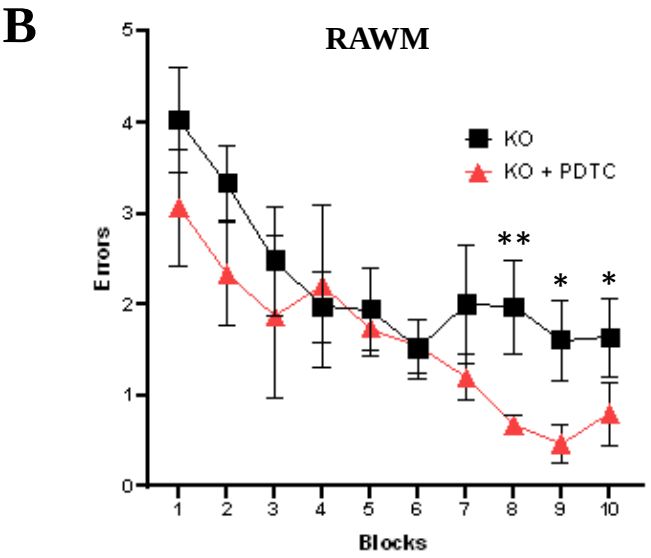

A
Control
KO
100 kDa
35 kDa
100 kDa
55 kDa
55 kDa
Stat1
Trem2
p100
p52
NF-kB
Actin
B
Stat1/Actin
**
Control
KO
C
Trem2/Actin
*
Control
KO
D
NFK-B/Actin
*
Control
KO
E
Control
KO
55kDa
35kDa
GFAP
GAPDH
F
GFAP/GAPDH
**
Control
KO
G
Control
H
KO
I
GFAP/Nuclei
****
Control
KO
J
Control
K
KO
L
Iba1/Nuclei
****
Control
KO

Figure 4


A
Begin
Injections
Behavior
Tests,
Sacrifice
Born
0
1
2
3
4
5
6
7
8
Weeks


B
RAWM
KO
KO + PDTC
Errors
Blocks
**
*
*

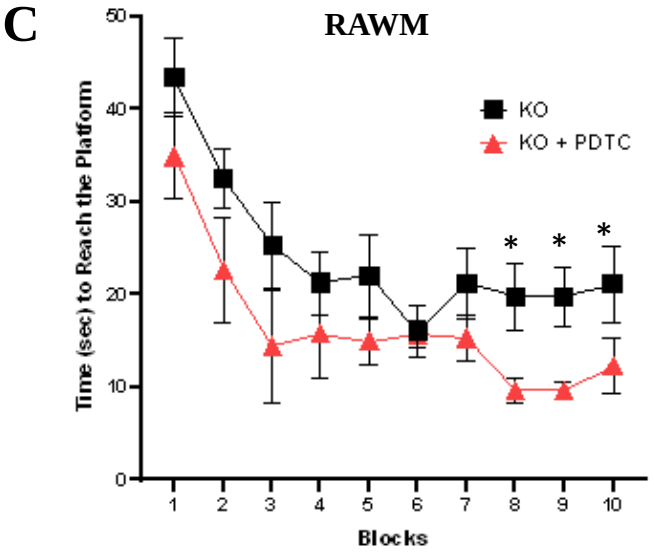

C
RAWM
KO
KO + PDTC
Time (sec) to Reach the Platform
Blocks
*
*
*

Figure 5

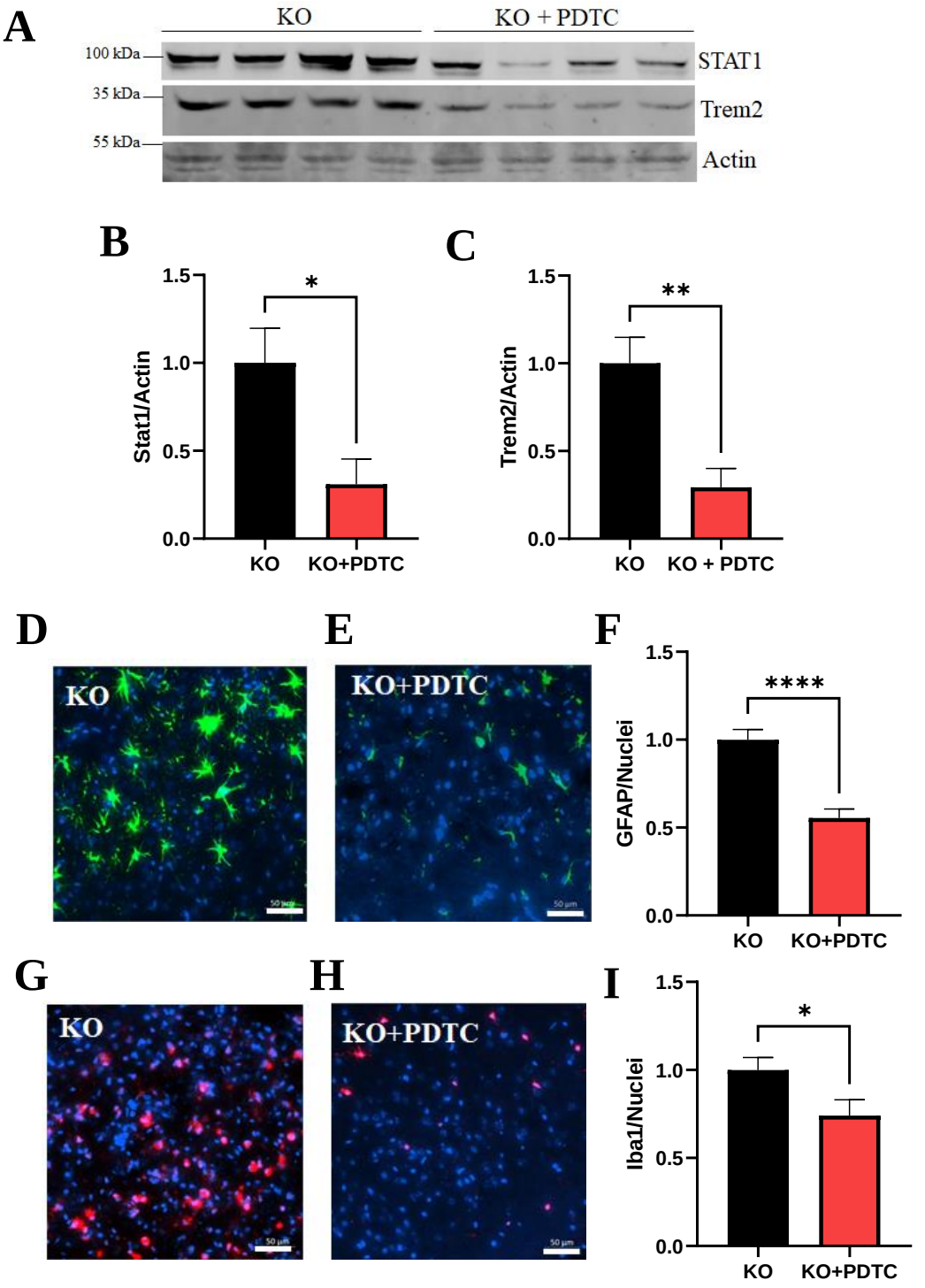
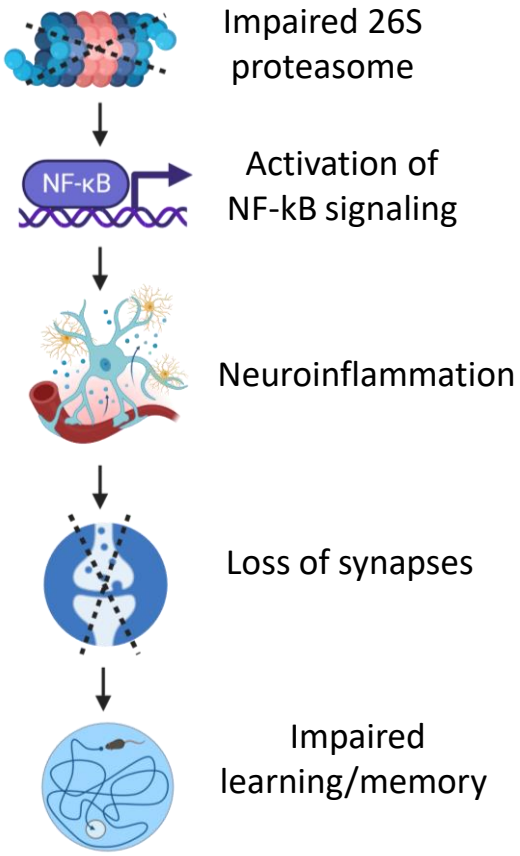

A
KO
KO + PDTC
100 kDa
35 kDa
55 kDa
STAT1
Trem2
Actin
B
Stat1/Actin
1.5
1.0
0.5
0.0
*
KO
KO+PDTC
C
Trem2/Actin
1.5
1.0
0.5
0.0
**
KO
KO + PDTC
D
KO
E
KO+PDTC
F
GFAP/Nuclei
1.5
1.0
0.5
0.0
****
KO
KO+PDTC
G
KO
H
KO+PDTC
I
Iba1/Nuclei
1.5
1.0
0.5
0.0
*
KO
KO+PDTC

Figure 6


Impaired 26S proteasome
NF-κB
Activation of NF-kB signaling
Neuroinflammation
Loss of synapses
Impaired learning/memory