Received: 19 September 2022 | Revised: 21 December 2022 | Accepted: 6 January 2023

DOI: 10.1096/fj.202201512R

THE FASEB JOURNAL

RESEARCH ARTICLE

Deficiency of IKKβ in neurons ameliorates Alzheimer's disease pathology in APP- and tau-transgenic mice

Laura Schnöder[1] | Wenqiang Quan[1,2] | Ye Yu[1,3] | Inge Tomic[1] | Qinghua Luo[1] | Wenlin Hao[1] | Guoping Peng[4] | Dong Li[2] | Klaus Fassbender[1] | Yang Liu[1,2]

[1]Department of Neurology, Saarland University, Homburg, Germany

[2]Department of Clinical Laboratory, Tongji Hospital, Tongji University Medical School, Shanghai, China

[3]Department of Neurology, The Second Affiliated Hospital of Nanchang University, Nanchang, China

[4]Department of Neurology, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China

Correspondence
Yang Liu, Department of Neurology, Saarland University, Kirrberger Straße, 66421 Homburg/Saar, Germany.
Email: a.liu@mx.uni-saarland.de

Funding information
National Natural Science Foundation of China (NSFC), Grant/Award Number: 81771371; Alzheimer Forschung Initiative (AFI), Grant/Award Number: #18009; Youth Science Foundation of Jiangxi Province (Youth Science Foundation of Jiangxi), Grant/Award Number: 20181BAB215016

Abstract

In Alzheimer's disease (AD) brain, inflammatory activation regulates protein levels of amyloid-β-peptide (Aβ) and phosphorylated tau (p-tau), as well as neurodegeneration; however, the regulatory mechanisms remain unclear. We constructed APP- and tau-transgenic AD mice with deletion of IKKβ specifically in neurons, and observed that IKKβ deficiency reduced cerebral Aβ and p-tau, and modified inflammatory activation in both AD mice. However, neuronal deficiency of IKKβ decreased apoptosis and maintained synaptic proteins (e.g., PSD-95 and Munc18-1) in the brain and improved cognitive function only in APP-transgenic mice, but not in tau-transgenic mice. Additionally, IKKβ deficiency decreased BACE1 protein and activity in APP-transgenic mouse brain and cultured SH-SY5Y cells. IKKβ deficiency increased expression of PP2A catalytic subunit isoform A, an enzyme dephosphorylating cerebral p-tau, in the brain of tau-transgenic mice. Interestingly, deficiency of IKKβ in neurons enhanced autophagy as indicated by the increased ratio of LC3B-II/I in brains of both APP- and tau-transgenic mice. Thus, IKKβ deficiency in neurons ameliorates AD-associated pathology in APP- and tau-transgenic mice, perhaps by decreasing Aβ production, increasing p-tau dephosphorylation, and promoting autophagy-mediated degradation of BACE1 and p-tau aggregates in the brain. However, IKKβ deficiency differently protects neurons in APP- and tau-transgenic mice. Further studies are needed, particularly in the context of interaction between Aβ and p-tau, before IKKβ/NF-κB can be targeted for AD therapies.

KEYWORDS
Alzheimer's disease, amyloid-beta (Aβ), IKKβ, neurodegeneration, tau

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β peptide; APP, amyloid precursor protein; CCL-2, chemokine (C–C motif) ligand 2; CHI3L3, chitinase-like 3; CREB, cAMP response element-binding protein; CX3CL1, chemokine (C-X3-C motif) ligand 1; GSK-3β, glycogen synthase kinase 3β; Iba-1, ionized calcium-binding adapter molecule −1; IκB, inhibitor of NF-κB; IKKβ, IκB kinase-β; IL-1β, interleukin-1β; MRC1, mannose receptor C type 1; NF-κB, nuclear factor κB; PP2A, protein phosphatase 2A; p-tau, phosphorylated tau; RIPA buffer, radioimmunoprecipitation assay buffer; TNF-α, tumor necrosis factor α; t-tau, total tau.

Laura Schnöder, Wenqiang Quan, and Ye Yu contributed equally to this project.

1 | INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease pathologically characterized by extracellular deposits of amyloid β peptide (Aβ), intracellular neurofibrillary tangles (NFT; primarily consisting of hyper-phosphorylated tau protein [p-tau]), and microglia-dominated inflammatory activation.[1] Extracellular Aβ and intracellular p-tau injure neurons directly, and indirectly through triggering neurotoxic inflammatory activation.[2–4] Conversely, chronic inflammatory activation in the brain upregulates Aβ production and induces tau phosphorylation and spreading, creating a positive feedback cycle in AD pathogenesis.[5–8] To date, the signaling pathways that mediate neuronal responses to inflammatory stimuli and regulate AD pathogenesis remain unclear.

Nuclear factor κB (NF-κB), mediating inflammatory responses in various cells, is strongly activated in neurons in the vicinity of Aβ plaques in AD brain.[9,10] NF-κB is sequestered in the cytoplasm in the resting state by binding to its inhibitor, especially, inhibitor of NF-κB (IκB)-α. Once activated, for example, by tumor necrosis factor (TNF)-α and interleukin (IL)-1β, IκBα is phosphorylated by IκB kinase (IKK)-β, ubiquitinated and degraded in the proteasome. Thereafter, NF-κB can enter the nucleus to "turn on" the expression of specific genes.[11] Multiple NF-κB binding sites have been identified in promoters of *amyloid precursor protein* (*APP*), *β-secretase* (*BACE1*), and *a-secretase* (*ADAM10*) genes,[12–15] suggesting that IKKβ/NF-κB activation may increase Aβ generation in neurons. However, there are no in vivo studies directly addressing NF-κB in amyloid pathology in AD brains. The role of neuronal IKKβ/NF-κB in the development of tauopathy in AD is unknown. It is also unclear whether neuronal IKKβ/NK-κB affects neurodegeneration in AD, although overexpression of a constitutively active IKKβ in mouse forebrain neurons activates NF-κB and causes neuron loss.[16] In an ischemia stroke mouse model, inhibition of IKKβ/NF-κB signaling prevents neuronal apoptosis and reduces infarct size.[17]

It should be noted that NF-κB is constitutively active in glutamatergic neurons of cortex and hippocampus.[18] The activation of IKKβ/NF-κB has the potential to promote neuronal survival, neurite outgrowth, synaptogenesis, and neuronal plasticity.[11] Inhibition of NF-κB in forebrain excitatory neurons by overexpressing dominant-negative IKKβ promotes neuronal apoptosis in closed-head injury mice.[19] Knocking out IKKβ and inhibiting NF-κB in neurons increases the striatal neurodegeneration in the R6/1 mouse model of Huntington's disease.[20] In cultured neurons, pretreatment with Aβ or TNF-α at low concentrations activates NF-κB and protects neurons from the damage caused by Aβ treatments at high concentrations.[9,21,22] Thus, IKKβ/NF-κB activation may serve both toxic and protective effects on neurons, depending on distinct pathophysiological conditions.

Our previous study has shown that IKKβ deficiency in myeloid cells attenuates inflammatory activation and Aβ load in the brain, and improves cognitive function of APP-transgenic mice.[23] In this study, we continued to address the question of whether deletion of IKKβ in neurons also prevents AD pathogenesis in both APP- and tau-transgenic mice.

2 | MATERIALS AND METHODS

2.1 | Animal models and cross-breeding

TgCRND8 APP-transgenic mice (APP^{tg}) expressing a transgene incorporating both the Indiana mutation (V717F) and the Swedish mutations (K670N/M671L) in the human *APP* gene under the control of hamster prion protein (PrP) promoter were kindly provided by D. Westaway (University of Toronto). In this mouse strain, the Aβ load does not differ between male and female mice.[24] $IKK\beta^{fl/fl}$ mice carrying loxP site-flanked *Ikbkb* alleles were kindly provided by M. Pasparakis (University of Cologne[25]). Nex-Cre mice expressing Cre recombinase from the endogenous locus of the *Nex* gene that encodes a neuronal basic helix–loop–helix (bHLH) protein were kindly provided by K. Nave, Max-Planck Institute for Medicine, Göttingen. APP^{tg}, $IKK\beta^{fl/fl}$, and Nex-Cre mice, all on a C57BL6 genetic background, had been cross-bred to build AD animal models with ($APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$) and without ($APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$) deletion of IKKβ in neurons. In order to investigate physiological function of IKKβ in neurons, we also examined non-APP-transgenic (APP^{wt}) mice with ($APP^{wt}IKK\beta^{fl/fl}Cre^{+/-}$) and without ($APP^{wt}IKK\beta^{fl/fl}Cre^{-/-}$) deletion of neuronal IKKβ. To evaluate the effect of neuronal IKKβ on p-tau-induced phenotype, we cross-bred $IKK\beta^{fl/fl}$ and Nex-Cre mice, with P301S tau-transgenic (tau^{tg}) mice (imported from the Jackson Laboratory, Bar Harbor, MA, USA; Stock number: 008169), which over-express the human tau mutant (P301S) under the direction of mouse prion protein promoter.[3] For this study, APP-transgenic mice and tau-transgenic mice were analyzed by 6 and 9 months of age, respectively, as both AD mice clearly displayed cognitive dysfunction and AD-associated pathologies in the brain at these ages. All animal experiments were performed in accordance with relevant national rules and authorized by the local research ethical committee (permission numbers: 13/2018).

2.2 | Morris water maze

The Morris water maze test was used to assess the cognitive function of APP^{tg} or tau^{tg} mice and their APP^{wt} littermates, as previously described.[26] Mice were trained to find the hidden escape platform. There were four trials per training day; with a trial interval of 15 min. Latency time, path length, and velocity were recorded with Ethovision video tracking equipment and software (Noldus Ethovision, Wageningen, The Netherlands). After 6 training days, there were 1 day of rest, and a probe trial on the 8th day. During the probe trial, the platform was removed, and the swimming path was recorded during 5 min. The frequency of entries in the location of original platform were measured.

2.3 | Tissue collection for histological and biochemical analysis

Animals were euthanized by over-dose inhalation of isoflurane. The whole brain was collected and divided along the interhemispheric fissure. The left hemisphere was immediately fixed in 4% paraformaldehyde (Sigma-Aldrich GmbH, Taufkirchen, Germany) and embedded in paraffin. The right hemisphere was dissected to remove the cerebellum, brainstem, thalamus, hypothalamus, and olfactory bulb. A 0.5-mm-thick piece of sagittal tissue was cut from the medial side and homogenized in TRIzol (Thermo Fisher Scientific, Darmstadt, Germany) for RNA isolation. The rest of the right hemisphere was snap frozen in liquid nitrogen and stored at −80°C until biochemical analysis was performed.

2.4 | Immunohistological analysis

Serial 50-μm-thick sagittal sections were cut from the paraffin-embedded hemisphere. Human Aβ in APP^{tg} mouse brains was stained with rabbit anti-human Aβ antibody (1:1000; clone D12B2; Cell Signaling Technology, Frankfurt am Main, Germany), microglia and astrocytes were stained with rabbit anti-ionized calcium-binding adapter molecule (Iba)-1 antibody (1:500; Cat.-No: 019-19 741; Wako Chemicals, Neuss, Germany), and rabbit anti-glial fibrillary acidic protein (GFAP) antibody (1:500; Code-No: Z0334; Agilent Technologies Deutschland GmbH, Waldbronn, Germany) respectively. The VectaStain *Elite* ABC kit (Vector Laboratories, Burlingame, CA, USA) and diaminobenzidine tetrahydrochloride hydrate (Sigma-Aldrich GmbH) were used for visualization of immunoreactive cells. For each animal, we labeled four serial sections with an interval of 10 layers between each two adjacent sections. In the whole hippocampus and cortex, volumes of Aβ were estimated with the *Cavalieri* method, and Iba-1 or GFAP-positive cells were counted with Optical Fractionator as described previously[23] on a Zeiss AxioImager.Z2 microscope equipped with a Stereo Investigator system (MBF Bioscience, Williston, VT, USA). Immunohistochemistry with rabbit anti-CD8a antibody (1:100; Cat.-No: HS-361003; Synaptic Systems GmbH, Göttingen, Germany) was performed on four serial brain sections from each tau^{tg} mouse as described above. Because the immunoreactive cells were enriched in the dentate gyrus, we counted all cells only in this brain region and calculated the cell density by dividing with the area of interest.

To evaluate tau pathology in tau^{tg} mice, four serial 50-μm-thick sections were chosen as for Aβ analysis. Brain tissues were stained according to our established protocols[26] with a mouse monoclonal antibody against human phospho-tau (Ser202, Thr205) (5 μg/ml; clone: AT8; Thermo Fisher Scientific). Because of low numbers of p-tau-positive cells in the cortex and hippocampus, we did not use stereological analysis, but counted labeled cells in the whole brain region. Data were recorded as the number of labeled cells divided by the full area (in square millimeters) of interest.

To demonstrate the colocalization of Cre and neurons/astrocytes, we used 30-μm-thick sagittal sections that were cut from the dehydrated and cryoembedded left brain hemisphere of 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice. The guinea pig anti-Cre antibody (1:500; Cat.-No: 257004; Synaptic Systems GmbH) and Alexa Fluor 488-conjugated second antibodies were first incubated with brain sections. After thorough washing, additional antibodies against various cellular markers (mouse anti-NeuN, clone A60, Sigma-Aldrich; or rabbit anti-GFAP, Agilent Technologies Deutschland) were added and were visualized with relevant Alexa Fluor 546-conjugated second antibodies (all second antibodies were from Thermo Fisher Scientific).

2.5 | Western blot analysis

Frozen brain tissues were homogenized in 5× volumes of ice-cold radioimmunoprecipitation assay buffer (RIPA buffer; 50 mM Tris [pH 8.0], 150 mM NaCl, 0.1% SDS, 0.5% sodiumdeoxy-cholate, 1% NP-40, and 5 mM EDTA) supplemented with protease inhibitor cocktail (Roche Applied Science, Mannheim, Germany) and phosphatase inhibitors (50 nM okadaic acid, 5 mM sodium pyrophosphate, and 50 mM NaF; Sigma-Aldrich), followed by centrifugation at 16 000*g* for 30 min at 4°C. After determination of protein concentrations with Bio-Rad

Protein Assay (Bio-Rad Laboratories GmbH, München, Germany), the protein samples were separated through 10% or 12% SDS-PAGE gels. Proteins were then transferred onto polyvinylidene difluoride (PVDF) or nitrocellulose membranes and incubated overnight at 4°C with the following antibodies: mouse monoclonal antibody against Aβ (clone W0-2; Sigma-Aldrich); rabbit monoclonal antibodies against beclin1 (clone D40C5), phosphor-glycogen synthase kinase (GSK)-3β (clone D3A4), GSK-3β (clone 27C10), phospho-CREB (Ser133) (clone 87G3), CREB (clone 48H2), cleaved Caspase-3 (clone 5A1E), PSD-95 (clone D27E11), synaptophysin (clone D8F6H), SNAP25 (clone D110), and Munc18-1 (clone D4O6V), and rabbit polyclonal antibodies against LC3B (Cat.-No: NB100-2220; Novus Biologicals, Centennial, USA), SQSTM1/p62 (Cat.-No: 5114), phospho-p38-MAPK (Thr180/Tyr182) (Cat.-No: 9211), p38-MAPK (Cat.-No: 9212), and protein phosphatase type 2A (PP2A) catalytic subunit (Cat.-No: 2038) (all antibodies except anti-LC3B were bought from Cell Signaling Technology, Danvers, USA). To evaluate the efficiency of Nex-Cre-mediated deletion of IKKβ, and the activity of NF-κB, the brain lysate was detected with rabbit monoclonal antibody against IKKβ (clone D30C6) and mouse monoclonal antibody against IκBa (clone l35A5; both from Cell Signaling Technology). After thoroughly washing, HRP-conjugated relevant second antibodies were used. The detected proteins were visualized via Plus-ECL method (PerkinElmer, Waltham, USA). To quantify proteins of interest, rabbit monoclonal antibody against β-actin (clone 13E5), vinculin (clone E1E9V), or GAPDH (clone 14C10) from Cell Signaling Technology, or α-tubulin (clone DM1A) from Abcam (Cambridge, United Kingdom) was used as a protein loading control. Densitometric analysis of band densities was performed with Image-Pro PLUS software version 6.0.0.260 (Media Cybernetics, Inc., Rockville, USA).

To quantify p-tau and total tau (t-tau) proteins, the brain tissue was sequentially homogenized in ice-cold high-salt reassembly buffer (RAB; 0.1 M MES, 1 mM EGTA, 0.5 mM MgSO4, 0.75 M NaCl, 20 mM NaF, and 1 mM PMSF), RIPA buffer and 70% formic acid (FA) as we did in previous studies.[26,27] For the Western blot detection, mouse monoclonal antibodies against p-tau (clone AT8) and t-tau (clone HT7) from Thermo Fisher Scientific were used.

2.6 | Quantitative PCR for analysis of gene transcripts

Total RNA was isolated from mouse brains and reverse transcribed. Gene transcripts of pro-and anti-inflammatory markers were quantified with our established protocol[23] using Taqman gene expression assays of mouse *Tnf-α*, *Il-1β*, *Chemokine* (*C–C motif*) *ligand 2* (*Ccl-2*), *Il-10*, *Mannose receptor C type 1* (*Mrc1*), *Chitinase-like 3* (*Chi3l3*), *Glyceraldehyde 3-phosphate dehydrogenase* (*Gapdh*), and *18 s RNA* (Thermo Fisher Scientific). *Gapdh* and 18 s RNA were detected as internal controls. Their threshold cycle (Ct) values in real-time PCRs were significantly correlated (see Figure S1). For the detection of *Ikbkb* transcript, Taqman gene expression assay (Mm01222249_m1) was used with the amplified PCR product overlapping 6–7 exon boundary of *Ikbkb* as we did in a previous study.[23] The transcription of following target genes: *APP*, *TAU*, *Cd200*, and *Chemokine* (*C-X3-C motif*) *ligand 1*(*Cx3cl1*), as well as *Ppp2ca* and *Ppp2cb* encoding PP2A catalytic subunit isoform α and β, and *Ppp3ca* and *Ppp3cb* encoding PP2B catalytic subunit isoform α and β, was determined using SYBR green binding technique with primers shown in Table 1.

2.7 | Construction of knockdown vectors

Two pcDNA6.2-GW/EmGFP-miR vectors (Thermo Fisher Scientific) (kd456 and kd1425) were engineered to contain different select hairpins targeting human IKKβ-encoding gene, *IKBKB* (Sequence ID: NM_001190720.3) using the protocol we established in the previous study.[28] Sequences of the DNA oligomers are listed in Table 2. pcDNA6.2-GW/EmGFP-miR-neg control plasmid (Thermo Fisher

TABLE 1 List of primer sequences used for SYBR green-based quantitative RT-PCR

Gene	Sense (5′-3′)	Antisense (5′-3′)
Cd200	CGGCGAATAGTAGTGTCCCT	TACCAGACTGCCCATCCTTG
Cx3cl1	TGCTGACCCGAAGGAGAAAT	CGGATTCAGGCTTTGTCAGG
Ppp2ca	TCTTCCTCTCACTGCCTTGG	ATCGAGTGCTCGGATGTGAT
Ppp2cb	TGTGCGAGAAGGCTAAGGAA	GGAATTGGCCATGCACATCT
Ppp3ca	AAAGTCGTGGTGGGTTTGTG	TGGGAGGTGTCCCATACAAC
Ppp3cb	TAGTGGAGTGTTGGCTGGAG	CATCTTGCTGCACAGCATCT
APP	TGCATGACTACGGCATGTTG	GTGGGCAACACACAAACTCT
TAU (1N4R)	GCGCCAGGAATTTGAAGTGA	GGTATAGCCGCCCTGATCTT

TABLE 2 Sequences of DNA oligomers inserted into pcDNA6.2-GW/EmGFP-miR to construct knockdown vectors

Oligonucleotides		Sequences (from 5′ to 3′)
Kd456	Top	TGCTGTCATGAAGGTATCTAAGCGCAGTTTTGGCCACTGAC TGACTGCGCTTATACCTTCATGA
	Bottom	CCTGTCATGAAGGTATAAGCGCAGTCAGTCAGTGGCCA AAACTGCGCTTAGATACCTTCATGAC
Kd1425	Top	TGCTGTTTCGGAGGAGATTCATCATGGTTTTGGCCACTGAC TGACCATGATGACTCCTCCGAAA
	Bottom	CCTGTTTCGGAGGAGTCATCATGGTCAGTCAGTGGCCA AAACCATGATGAATCTCCTCCGAAAC

Scientific) (kd-ct) containing scrambled sequence was used as a control knockdown vector. Thereafter, the sequence between Sac I and Xho I of each plasmid was subcloned into pCEP4 mammalian expression vector (Thermo Fisher Scientific) between Kpn I and Xho I. The terminals from cleavages of Sac I and Kpn I were both blunted with Klenow fragment (New England Biolabs GmbH, Frankfurt am Main, Germany). After transfection, the vector transcribed artificial miRNA, which have 100% homology to the gene sequence of interest, resulting in target RNA cleavage.

2.8 | Cell culture and establishment of cell lines

SH-SY5Y neuroblastoma cells were obtained from LGC Standards GmbH (Wesel, Germany) and maintained in DMEM supplemented with 10% fetal calf serum (FCS; PAN Biotech, Aidenbach, Germany). IKKβ-knockdown cell lines were established by transfecting cells with kd-ct, kd456, and kd1425 vectors, and selected with hygromycin until the stable transfection. All genetic modifications were confirmed by Western blot detection of IKKβ proteins using rabbit polyclonal antibody against IKKβ as described above.

2.9 | Preparation of membrane components from brain tissues and cultured cells

To measure β- and γ-secretase activity, membrane components were purified according to the published protocol.[29] Briefly, brain tissues or SH-SY5Y cell pellets were transferred into sucrose buffer (10 mM Tris/HCl, pH 7.4, including 1 mm EDTA and 200 mM sucrose) and homogenized on ice. The homogenate was centrifuged at 1000*g* for 10 min at 4°C to delete nuclei. The resulting post-nuclear supernatant was transferred to a new tube and centrifuged again at 10 000*g* at 4°C for 10 min. Finally, the resulting supernatant was centrifuged at 187 000*g* in an Optima MAX Ultracentrifuge (Beckman Coulter GmbH, Krefeld, Germany) for 75 min at 4°C. The resulting supernatant was discarded, and pellets were re-suspended using cannulas of decreasing diameter in sucrose buffer.

2.10 | β- and γ-secretase activity assays

β- and γ-secretase activities were measured by incubating the crude membrane fraction with secretase-specific FRET substrates according to our established methods.[29] For measurement of β-secretase activity, the crude membrane fraction was resuspended in 500 µl β-secretase assay buffer (0.1 M sodium acetate, pH 4.5). The final concentrations for the β-secretase assay were: 0.1 mg/ml membrane protein (12.5 µg protein per well in 96-well plates), 10% dimethyl sulfoxide, and 8 µM β-secretase substrate IV (Calbiochem, Darmstadt, Germany). For measurement of γ-secretase activity, the crude membrane fraction was resuspended in 500 µl γ-secretase assay buffer (50 mM Tris/HCl, pH 6.8, 2 mM EDTA). Final concentrations for the γ-secretase assay were: 1 mg/ml membrane protein (125 µg protein per well in 96-well plates) and 8 µM γ-secretase substrate (Calbiochem). For both secretase assays, kinetics was performed at 37°C and fluorescence intensity in each well was measured for 8 h with intervals of 5 min with Synergy Mx Monochromator-Based Multi-Mode Microplate Reader (BioTek, Winooski, USA). Fluorescence intensity of the first cycle was considered as background and subtracted for each well.

To further analyze the β-secretase, SH-SY5Y neuroblastoma cells were lysed in RIPA buffer and detected with Western blot using rabbit monoclonal antibody against BACE1 (clone D10E5; Cell Signaling Technology) as described above.

2.11 | Statistics

Data were presented as mean ± SEM. For multiple comparisons, one-way or two-way ANOVA followed by

Bonferroni, Tukey, or Dunnett T3 *post-hoc* test (dependent on the result of Levene's test to determine the equality of variances) was used. Two independent-samples Students *t* test was used to compare means for two groups of cases. All statistical analyses were performed with SPSS version 19.0 for Windows (IBM, New York, NY, USA). Statistical significance was set at $p < .05$.

3 | RESULTS

3.1 | Establishment of APP-transgenic mice with deletion of IKKβ in neurons

To delete IKKβ specifically in neurons, we cross-bred TgCRND8 APP-transgenic mice (APP^{tg}) to *Ikbkb*-floxed mice ($IKK\beta^{fl/fl}$) and Nex-Cre knock-in mice ($Nex\text{-}Cre^{+/-}$) as we did in previous studies.[23,26] The cellular specificity of Nex-Cre-mediated gene recombination had been described in detail by our and other groups.[26,30] In this study, we co-stained Cre recombinase with various cell markers in brains of $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice. As shown in Figure 1A,B, Cre recombinase was expressed only in NeuN-positive cells (neurons) but not in GFAP-immunoreactive cells (astrocytes), confirming previous observations. As a control, there were no Cre-immunoreactive cells in the brains of $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 1C). Using real-time RT-PCR, we observed that transcription of IKKβ-encoding gene, *Ikbkb*, was down-regulated in the brains of $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared to $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (Figure 1D; *t* test, $p = .002$). Quantitative Western blot further showed that the protein level of IKKβ in homogenates of cortex and hippocampus from $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice was significantly lower than that in $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 1E,F; IKKβ/Vinculin: 0.218 ± 0.058 vs 1.056 ± 0.259; *t* test, $p = .004$). To investigate whether IKKβ deficiency regulated NF-κB activation, we detected IκB in brain homogenates and observed that the protein level of IκB was significantly increased in the brains of 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ controls (Figure 1G,H; IκB/β-actin: 0.717 ± 0.098 vs 1.271 ± 0.136; *t* test, $p = .004$), suggesting that IKKβ deficiency in neurons inhibits NF-κB activation in the brain.

3.2 | Neuronal deficiency of IKKβ reduces cerebral Aβ load in APP-transgenic mice

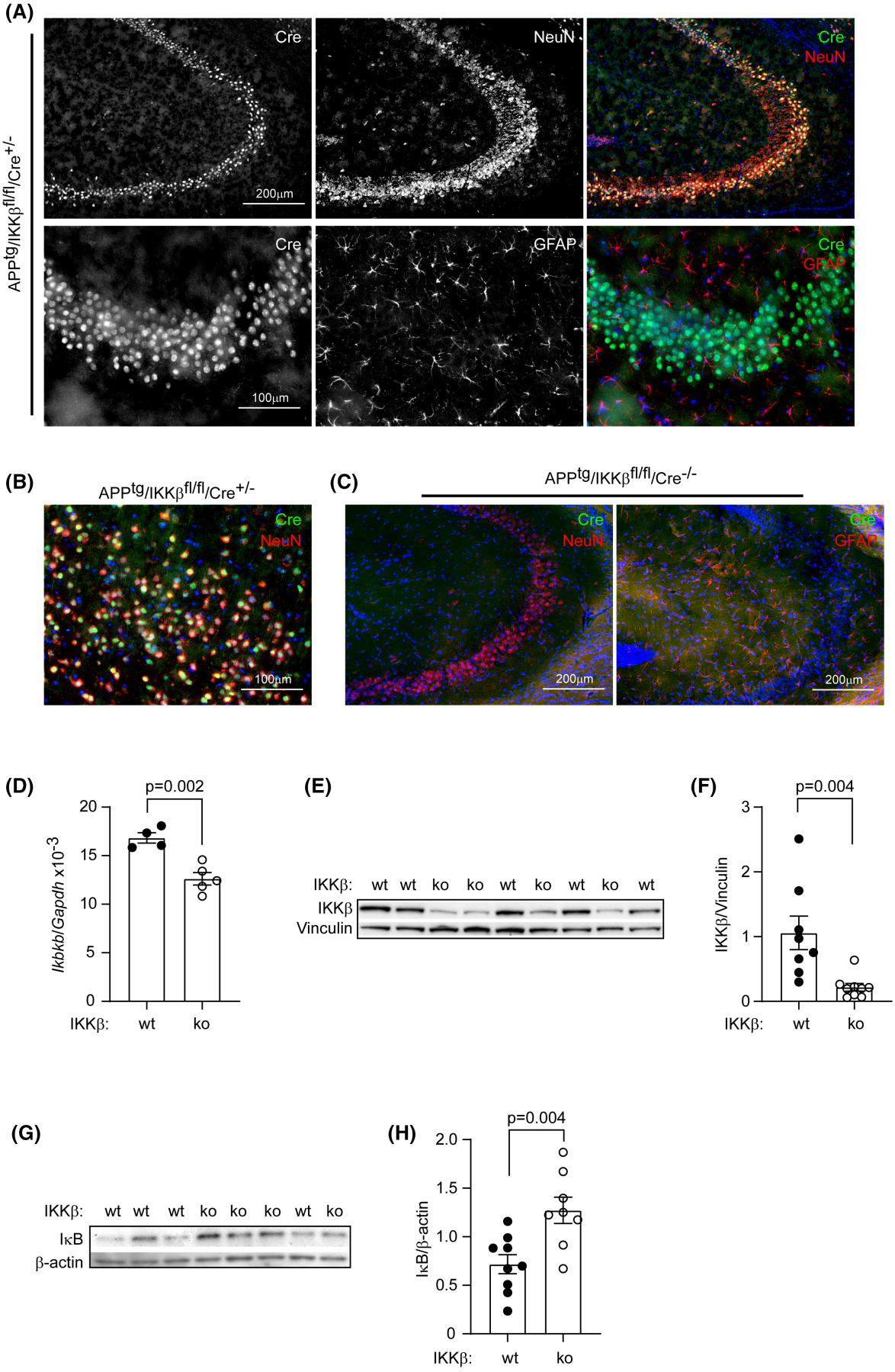
Aβ is a key molecule leading to neurodegeneration in AD.[31] We used the stereological *Cavalieri* method to measure Aβ volume, adjusted relative to the volume of analyzed tissues, in 6-month-old APP-transgenic mice. The volume of immunoreactive Aβ deposits in $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice ($1.094\% \pm 0.121\%$ in the hippocampus and $1.136\% \pm 0.124\%$ in the cortex) was significantly lower than that in $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice ($1.623\% \pm 0.109\%$ in the hippocampus and $1.582\% \pm 0.148\%$ in the cortex; Figure 2A–C; *t* test, $p = .006$ and .039 respectively).

Western blot analysis using human Aβ-specific antibody was performed to determine levels of Aβ monomers and dimers in the homogenate of cortex and hippocampus derived from 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermate mice. As shown in Figure 2D–F, deletion of IKKβ in neurons reduced dimeric Aβ by 35% (*t* test, $p = .019$). Thus, our study suggested that deletion of IKKβ in neurons reduces Aβ load in the brain of APP-transgenic mice.

In the following experiments, we examined how neuronal IKKβ regulates Aβ level in the brain. Six-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice did not differ in either gene transcription or protein expression of APP (Figure 3A–C; *t* test, $p > .05$). Interestingly, we observed that the activity of β-secretase but not γ-secretase, was significantly decreased in 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 3D,E; two-way ANOVA, $p = .046$).

To further ask whether IKKβ regulates β- and γ-secretase activity, we constructed two SH-SY5Y cell lines (kd456 and kd1425) with knock-down of *IKBKB* gene (Figure 3F,G; one-way ANOVA followed by *post-hoc* test, $p < .05$). Compared with the control cells (kd-ct), IKKβ deficiency significantly reduced both the protein expression of BACE1 (Figure 3H,I; one-way ANOVA followed by *post-hoc* test, $p < .05$), and β-secretase activity in kd456 and kd1425 cells (Figure 3J; two-way ANOVA followed by *post-hoc* test, $p < .05$). Deficiency of IKKβ did not change γ-secretase activity in kd456 and kd1425 cells compared with kd-ct cells (Figure 3K; two-way ANOVA, $p > .05$).

FIGURE 1 IKKβ is efficiently deleted in neurons of $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice. Brain sections from 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were co-stained for Cre-recombinase in green and NeuN or GFAP in red (A–C). Cre is present in the nuclei of NeuN but not GFAP-positive cells in both the hippocampus (A) and cortex (B) of IKKβ ko mice. As a control, Cre is absent in the brain of IKKβ wt mice (C). Transcripts of *Ikbkb* gene in the brain of 6-month-old IKKβ ko and wt mice were determined with quantitative RT-PCR (D; *t* test, $n \geq 4$ per group). The protein levels of IKKβ and IκB in brain homogenates from these two groups of AD mice were further detected with quantitative Western blot. IKKβ significantly decreases IKKβ and increases IκB (E–H; *t* test, $n \geq 8$ per group).



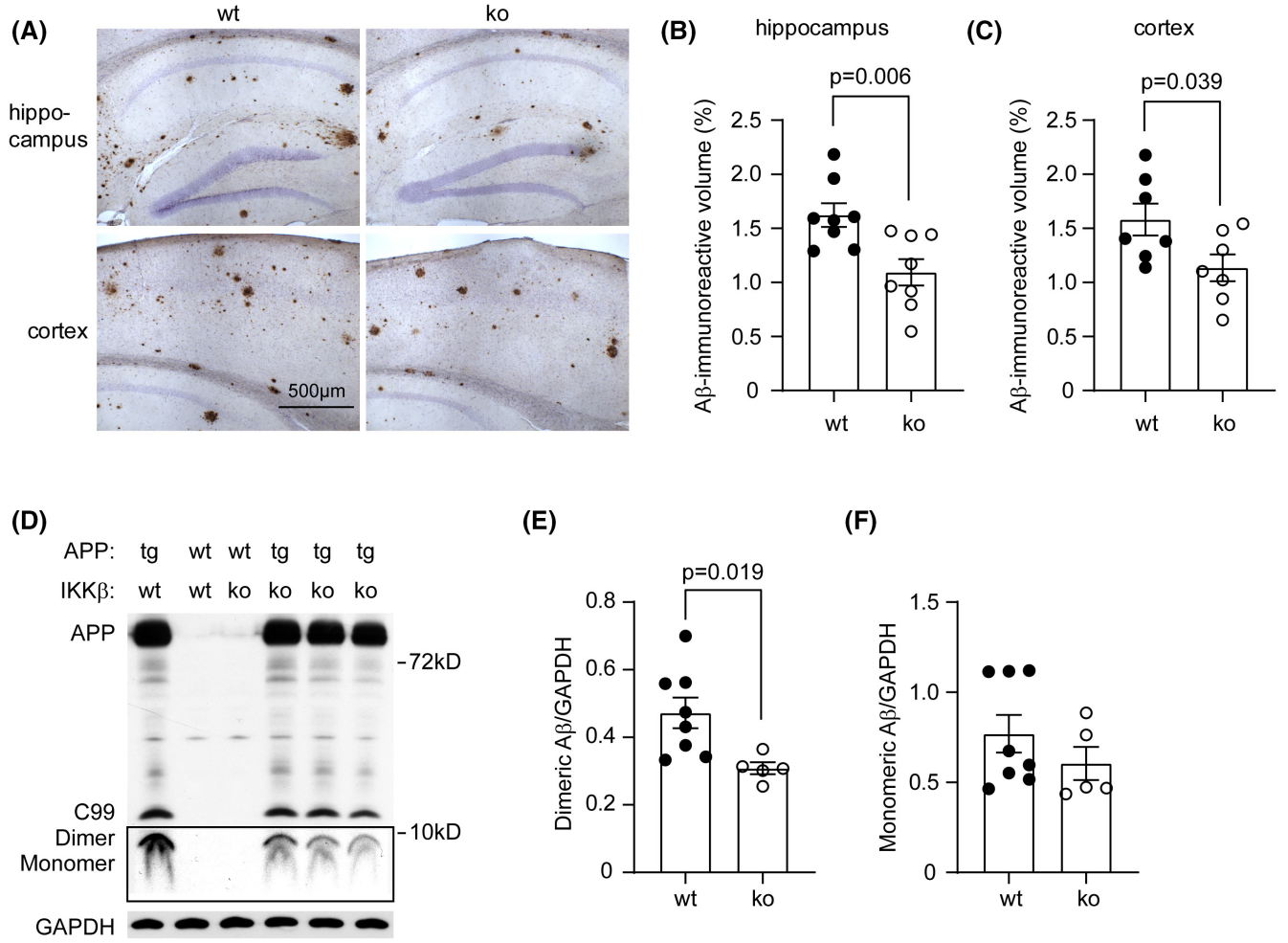


FIGURE 2 Deficiency of IKKβ in neurons reduces cerebral Aβ in APP-transgenic mice. Six-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were analyzed for cerebral Aβ load after immunohistochemical staining of human Aβ (A). The Aβ volume was estimated with *Cavalieri* method and adjusted by the relevant brain volume. IKKβ deficiency in neurons significantly reduced the cerebral Aβ volume (B and C; *t* test, $n \geq 7$ per group). The cerebral Aβ in APP^{tg} mice was also evaluated by detecting Aβ in the brain homogenate with quantitative Western blot (D). Normalization of Aβ against GAPDH shows reduced amount of dimeric Aβ but not monomeric Aβ after deletion of IKKβ in neurons (E and F; *t* test, $n \geq 5$ per group).

3.3 | Neuronal deficiency of IKKβ decreases neuroinflammation in APP-transgenic mice

Neuroinflammation is another key mechanism in AD pathogenesis.[4] We counted Iba1- and GFAP-positive cells in the hippocampus of 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates using our established protocol.[23] We observed that deletion of IKKβ in neurons significantly reduced the number of both Iba-1 and GFAP-positive cells compared to neuronal IKKβ-wild-type APP^{tg} mice (Figure 4A–D; *t* test, $p < .05$).

In further experiments, we measured inflammatory gene transcripts in the brains of 6-month-old APP-transgenic and non-APP-transgenic mice. IKKβ deficiency significantly decreased the transcription of *Ccl2* and *Il-10* genes (Figure 4E,H; *t* test, $p < .05$), but did not change the transcription of other inflammatory genes tested (e.g., *Tnf-α*, *Il-1β*, *Mrc1*, and *Chi3l3*; Figure 4F,G,I,J; *t* test, $p > .05$). IKKβ deficiency did not alter the transcription of *Cd200* and *Cx3cl1* genes in the brains compared with neuronal IKKβ-wild-type APP^{tg} mice (Figure 4K,L; *t* test, $p > .05$). CD200 and CX3CL1 are released by neurons and regulate microglial activation.[32]

3.4 | Neuronal deficiency of IKKβ attenuates cognitive deficits and synaptic impairments in APP-transgenic mice

After we observed that neuronal deficiency of IKKβ reduced Aβ and microglia/astrocytes in the brain of APP-transgenic mice, we asked whether IKKβ deficiency protected neurons. We used the Morris water maze test to

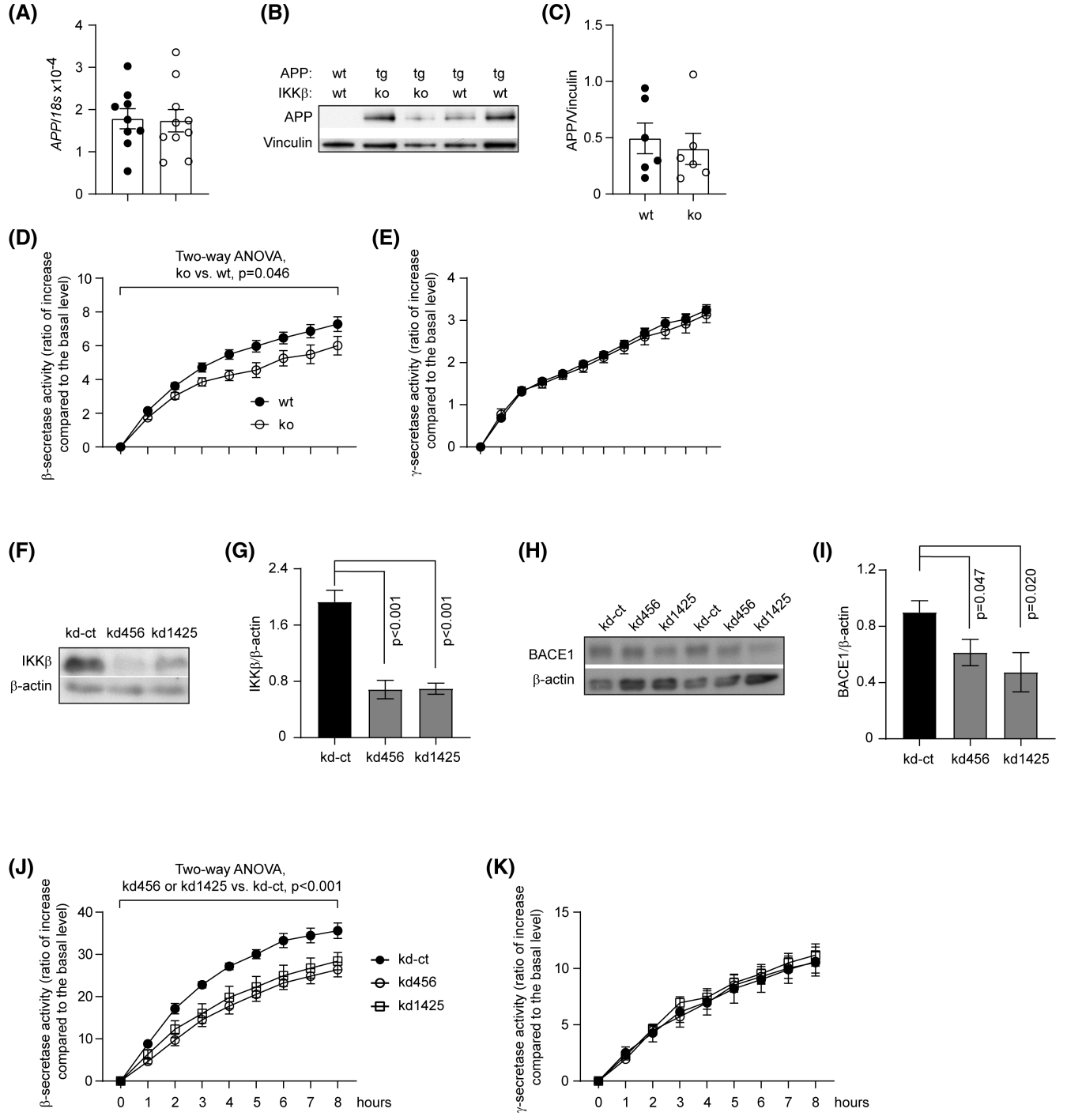
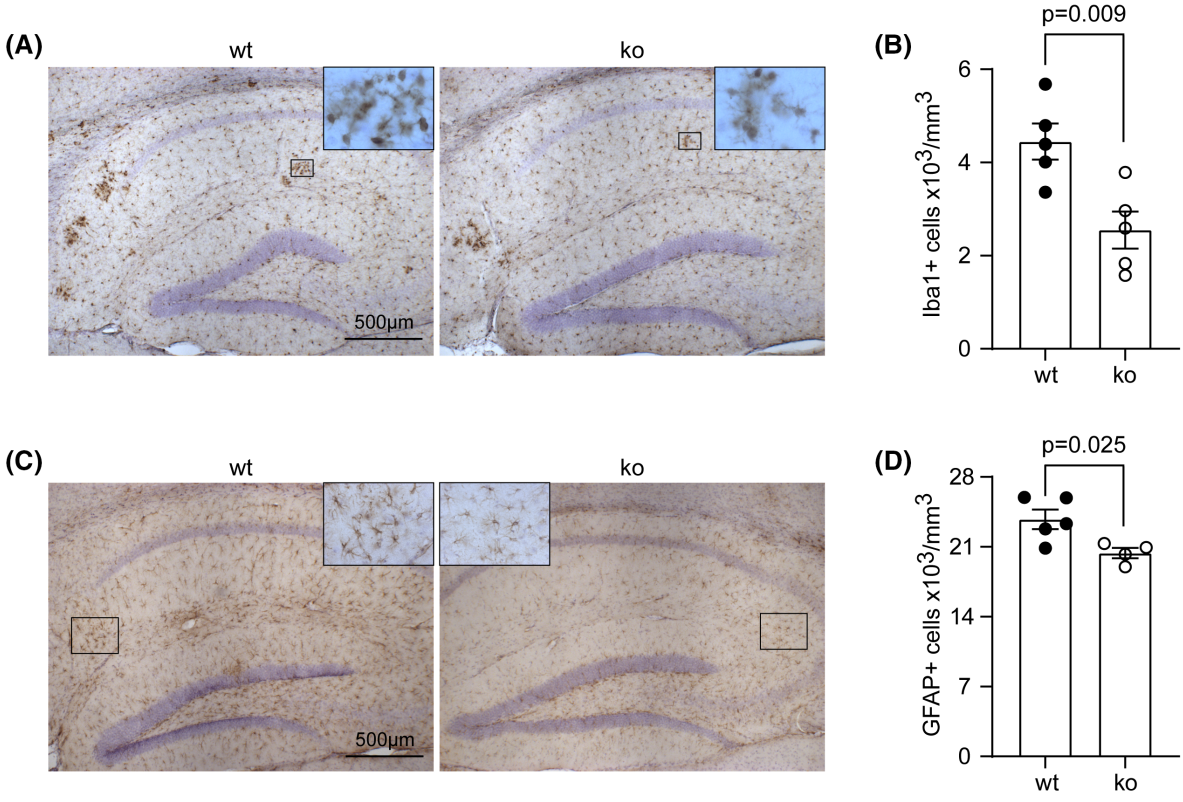


FIGURE 3 Deficiency of IKKβ reduces β-secretase activity in brains and cultured neuronal cells. Expression levels of APP in brains of 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were evaluated by RT-PCR (A; *t* test, $n \geq 9$ per group) and quantitative Western blot (B and C; *t* test, $n = 6$ per group). Membrane components were further prepared from IKKβ wt and ko AD mice and incubated with fluorogenic β- and γ-secretase substrates. IKKβ deficiency in neurons reduces β- but not γ-secretase activity in the brains of APP-transgenic mice (D and E; two-way ANOVA, $n = 9$ and 5 for wt and ko mice respectively). IKKβ-deficient cell lines were established by stably transfecting SH-SY5Y cells with kd456 and kd1425 knock-down vectors. Compared with control cells transfected with kd-ct vector, protein levels of IKKβ and BACE1 are significantly decreased (F–I; one-way ANOVA followed by Bonferroni *post-hoc* test, $n \geq 5$ per group). The following β- and γ-secretase assays showed that kd456 and kd1425 cells significantly decreases β- but not γ-secretase activity compared with kd-ct cells (J and K; two-way ANOVA followed by Bonferroni *post-hoc* test, $n \geq 4$ per group).



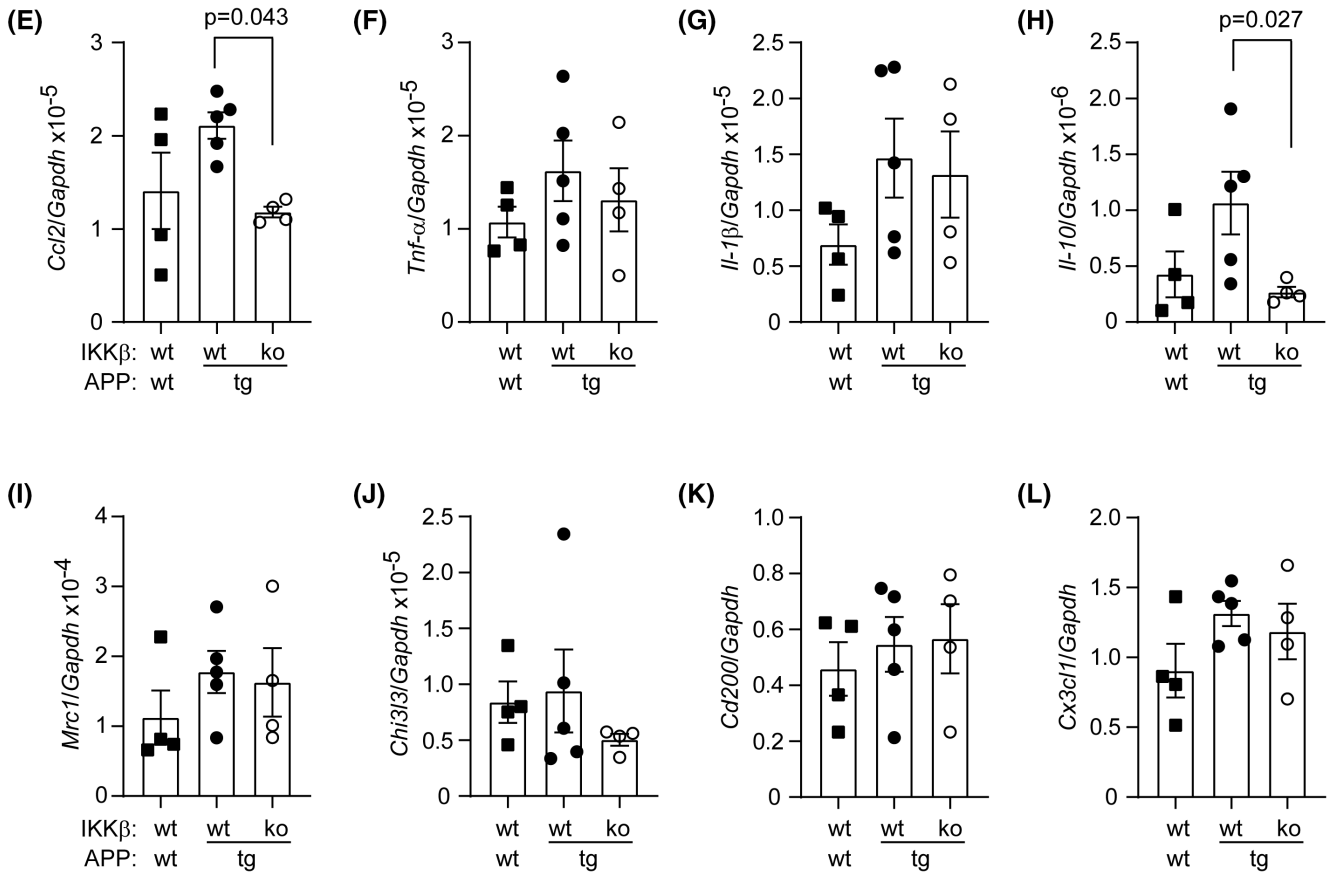


FIGURE 4 Deficiency of IKKβ in neurons inhibits inflammatory activation in the brains of APP-transgenic mice. Six-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice, and 6-month-old non-APP-transgenic mice (APP^{wt} $IKK\beta^{fl/fl}Cre^{-/-}$; APP^{wt}) as controls, were analyzed for the neuroinflammatory activation. Microglia and astrocytes were stained with immunohistochemistry using antibodies against Iba1 and GFAP (A and C; in brown color) and counted with the stereological probe, Optical Fractionator. Deficiency of IKKβ significantly reduces Iba1- and GFAP-positive cells in the hippocampus (B and D; *t* test, $n \geq 4$ per group). The transcripts of both pro- and anti-inflammatory genes, as well as *Cd200* and *Cx3cl1* genes, in the brain of 6-month-old APP^{tg} and APP^{wt} with different expression of IKKβ in neurons, were further detected with real-time PCR. Transcription of *Ccl-2* and *Il-10* genes, but not *Tnf-α*, *Il-1β*, *Mrc1*, *Chi3l3*, *Cd200*, and *Cx3cl1* genes was reduced by deficiency of IKKβ in APP-transgenic mice (E–L; *t* test, $n \geq 4$ per group).

examine the cognitive function of 6-month-old APP^{wt} and APP^{tg} littermate mice with and without deletion of IKKβ in neurons. As shown in Figure 5A–C, the swimming time and distance to reach the platform for all tested mice significantly decreased when the training time increased (two-way ANOVA, $p < .05$); the swimming velocity did not differ between IKKβ-deficient and wild-type APP-transgenic mice or for the same mice on different training dates (two-way ANOVA, $p > .05$).

Six-month-old APP^{tg} mice with normal IKKβ expression ($APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$) traveled longer distances than APP^{wt} mice to reach the escape platform during the acquisition phase (Figure 5A; two-way ANOVA, $p = .029$). Neuronal deficiency of IKKβ ($APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$) significantly improved APP^{tg} mice in searching for and finding the platform compared to $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (Figure 5A,B; two-way ANOVA followed by *post-hoc* test showing the difference in traveling distance and time between $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ and $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice: $p < .001$). However, in the 5-min probe trial, designed to test the memory of the mice, we did not detect a difference between any two groups of mice in the frequency that the mice visited the region where the escape platform was located (Figure 5D; one-way ANOVA, $p > .05$).

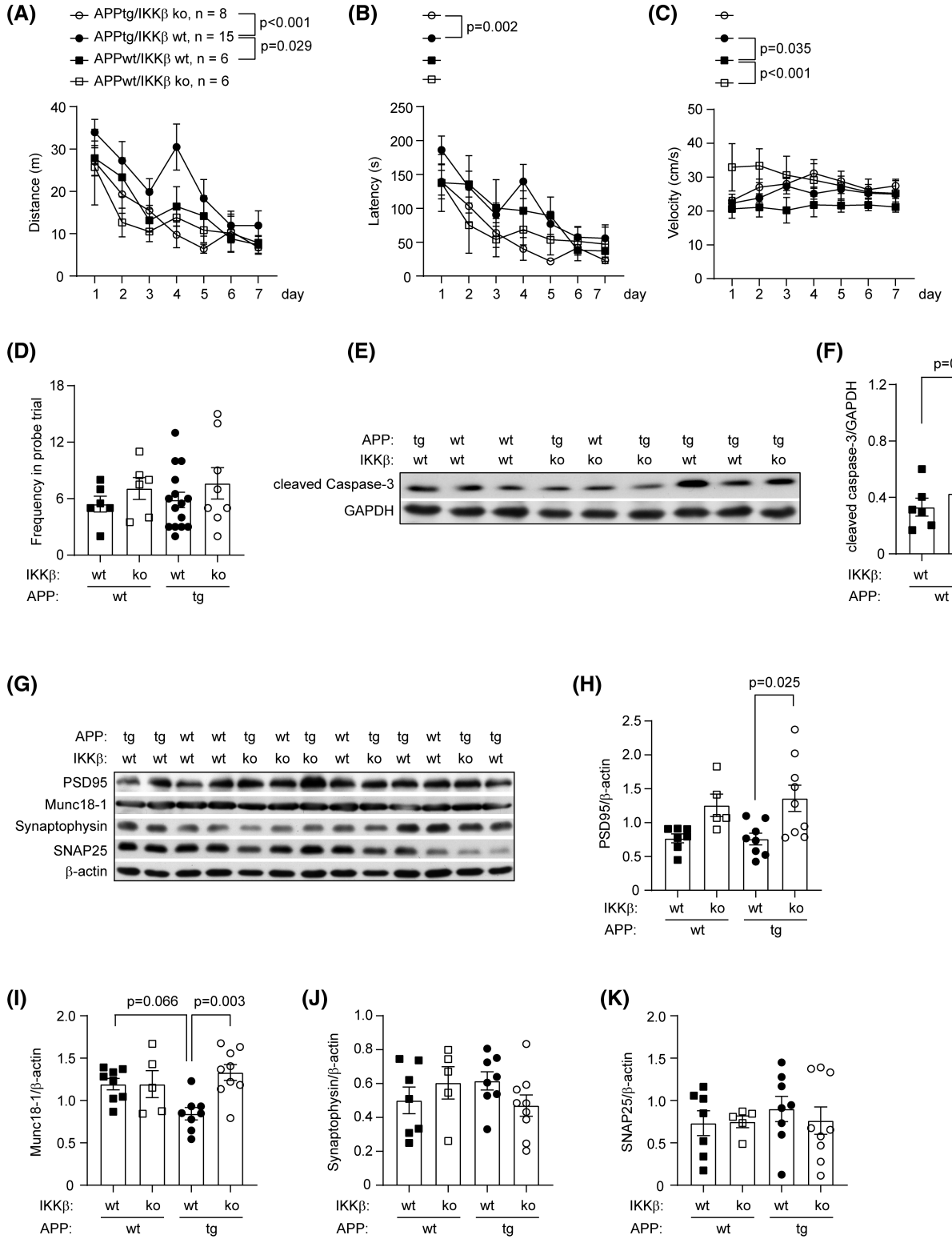
We further used Western blot analysis to quantify cleaved caspase-3 and evaluate the apoptosis of brain cells in 6-month-old APP-transgenic mice. As shown in Figure 5E,F, the protein level of cleaved caspase-3 was significantly higher in APP-transgenic mice than in APP-wild-type littermates (one-way ANOVA followed by *post-hoc* test; $p = .037$). Neuronal deficiency of IKKβ significantly decreased the protein level of cleaved caspase-3 in $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ controls (Figure 5F; one-way ANOVA followed by *post-hoc* test; $p = .034$).

In our previous study, we observed that protein levels of synaptic proteins, PSD-95 and Munc18-1, in the brain homogenate of 6-month-old TgCRND8 APP-transgenic mice are significantly lower than that in APP-wild-type controls.[23] In this study, we did observe that the protein level of Munc18-1 tended to decrease in APP-transgenic mice compared with APP-wild-type littermates (Figure 5I; one-way ANOVA followed by *post-hoc* test; $p = .066$). Deficiency of IKKβ in neurons significantly increased the protein levels of PSD-95 and Munc18-1, but not synaptophysin and SNAP-25, in 6-month-old $APP^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $APP^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermate controls (Figure 5G–K; one-way ANOVA followed by *post-hoc* test; $p < .05$). Deficiency of IKKβ in neurons did not change protein levels of all tested synaptic proteins in APP-wild-type mice (Figure 5G–K; one-way ANOVA followed by *post-hoc* test; $p > .05$).

3.5 | Neuronal deficiency of IKKβ reduces phosphorylated tau protein in the brain of tau-transgenic mice

APP-transgenic mice cannot model all pathological changes of AD, such as those associated with p-tau. It was reported that p-tau mediates toxic effects of Aβ in AD pathogenesis.[33,34] To investigate the effects of neuronal IKKβ on tau-associated pathologies, we cross-bred $IKK\beta^{fl/fl}$, Nex-$Cre^{+/-}$, and P301S tau-transgenic (Tau^{tg}) mice[3] to create neuronal IKKβ-deficient and wild-type Tau^{tg} AD mice. We counted AT8-positive cells in cortex and hippocampus of 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ and $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice. The total number of AT8-immunoreactive cells adjusted to the investigated area in neuronal IKKβ-deficient $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice ($2.25 \pm .39/mm^2$ in cortex, and $3.94 \pm 0.65/mm^2$ in hippocampus) was significantly fewer than that in IKKβ-wild-type $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice ($3.73 \pm 0.55/mm^2$ in cortex and $8.13 \pm 0.88/mm^2$ in hippocampus; Figure 6A–C; *t* tests, $p = .044$ and .001 respectively).

We also extracted tau proteins from 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice with RAB, RIPA, and FA buffers as we did in previous studies.[26,27] Western blots revealed that ratios of p-tau/t-tau were significantly lower in RAB and RIPA fractions derived from $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice, than from $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 6D–F; *t* test, $p < .05$). In the FA fraction, deletion of neuronal IKKβ tended to decrease the ratios of p-tau/t-tau in Tau^{tg} mice, although the difference was not significant (Figure 6G; *t* test, $p = .051$). Moreover, we observed that the protein levels of p-tau adjusted by β-actin in RIPA and FA fractions of $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice were also decreased



by IKKβ deficiency compared with $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 6D,H,I; *t* test, $p < .05$). As β-actin was not detectable in RAB fraction, we could not determine the protein levels of p-tau in RAB fractions. Additionally, we found that the protein level of t-tau in RIPA fraction of brain homogenate was significantly higher in $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice than in $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ littermates (Figure 6K,L; *t* test, $p < .05$); however, IKKβ deficiency did not change the transcription of *TAU* gene (Figure 6J; *t* test, $p > .05$), which suggested that deficiency of IKKβ might also increase the degradation of tau protein.

FIGURE 5 Deficiency of IKKβ in neurons improves cognitive function and attenuates synaptic impairments in APP-transgenic mice. Six-month-old APP-transgenic (APP^{tg}) and non-transgenic (APP^{wt}) mice with (ko) and without (wt) deletion of neuronal IKKβ were examined for cognitive function with Morris water maze test. During the training phase, APP^{tg}IKKβ-wt mice reached the escape platform with significantly longer traveling distance than APP^{wt}IKKβ-wt littermate mice (A; two-way ANOVA followed by Bonferroni *post-hoc* test, *n* is shown in the figure). Deletion of IKKβ in neurons (APP^{tg}IKKβ-ko) significantly reduced the traveling time and distance to the escape platform compared with APP^{tg}IKKβ-wt mice (A, B; two-way ANOVA followed by Bonferroni *post-hoc* test). IKKβ deficiency affected the swimming speed neither of APP^{tg} mice, nor for each mouse at different training time points (C; two-way ANOVA, $p > .05$). However, APP^{tg}IKKβ-wt mice swam much faster than non-APP transgenic (APP^{wt}IKKβ-wt) mice with unknown reasons (C; two-way ANOVA followed by Bonferroni *post-hoc* test). In the probe trial, APP^{tg} and APP^{wt} mice with and without IKKβ deficiency did not differ in the frequency, with which the mice visited the region where the platform was previously located (D; one-way ANOVA, $p > .05$, $n \geq 6$ per group). Western blotting was used to detect cleaved caspase-3, and the amount of synaptic structure proteins, Munc18-1, SNAP25, synaptophysin, and PSD-95 in the brain homogenate of 6-month-old APP^{tg} and APP^{wt} mice (E–K). Transgenic expression of APP increases cleaved caspase-3, and neuronal deficiency of IKKβ recovers it (F; one-way ANOVA, $n \geq 7$ per group). Deficiency of IKKβ in neurons was associated with a higher level of PSD-95 and Munc18-1 in APP-transgenic mice (H and I; one-way ANOVA followed by Bonferroni *post-hoc* test, $n \geq 5$ per group).

3.6 | Neuronal deficiency of IKKβ potentially increases the dephosphorylation of p-tau in tau-transgenic mice

As IKKβ deficiency in neurons attenuated p-tau in the brain of tau-transgenic mice, we hypothesized that IKKβ regulated the phosphorylation of tau protein in AD mice. We quantified phosphorylation levels of GSK3β and p38-MAPK, two important kinases phosphorylating tau proteins in neurons.[26,35] As shown in Figure 7A–D, neuronal IKKβ-deficient and wild-type 9-month-old tau-transgenic mice did not differ in phosphorylation of any of the enzymes (*t* test, $p > .05$), suggesting that the decrease in p-tau in IKKβ-deficient tau mice was probably not due to the reduction in p-tau generation. Interestingly, we observed that IKKβ deficiency significantly up-regulated the transcription of *Ppp2ca*, but not *Ppp2cb*, *Ppp3ca*, and *Ppp3cb* in the brain of tau-transgenic mice (Figure 7E–H; *t* test, $p < .05$), which was in line with a previous observation that NF-κB activation inhibits expression of catalytic subunit of PP2A (PP2Ac).[36] Western blot experiments verified the finding by showing that the cerebral protein level of PP2Ac was significantly higher in 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice than in $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (Figure 7I,J; *t* test, $p < .05$). Thus, neuronal deficiency of IKKβ potentially increases the dephosphorylation of p-tau in tau-transgenic mice.

3.7 | Neuronal deficiency of IKKβ promotes autophagy in the brains of both APP- and tau-transgenic mice

We have recently observed that activated autophagy is a mechanism mediating the degradation of BACE1 in APP-transgenic mice[37] and p-tau in tau-transgenic mice.[27] Interestingly, we observed that neuronal deficiency of IKKβ significantly increased the ratios of LC3B-II/I in RIPA-soluble brain homogenates of both 6-month-old APP-transgenic mice and 9-month-old tau-transgenic mice, indicating that IKKβ deficiency enhanced autophagy in the brains of AD mice (Figure 8A–D; one-way ANOVA followed by *post-hoc* test for APP mice and *t* test for tau mice; $p < .05$ for both mice), which was in accordance with a previous finding that inhibition of NF-κB in neurons promotes autophagy in the brains of TDP-43-transgenic mice.[38] Compared with non-APP-transgenic mice, APP-transgenic mice inhibited autophagy in the brain (Figure 8A,B; one-way ANOVA followed by *post-hoc* test; $p < .05$). However, the protein levels of Beclin1 and SQSTM1/p62 did not differ between $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (Figure 8E,F; *t* test, $p > .05$).

3.8 | Neuronal deficiency of IKKβ regulates neuroinflammation in tau-transgenic mice

As we did for APP-transgenic mice, we counted Iba1 and GFAP-immunoreactive cells in the brains of 9-month-old Tau^{tg} mice. Similarly, we observed that deficiency of neuronal IKKβ significantly decreased the numbers of microglia and astrocytes in the hippocampus and cortex of $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (Figure 9A–E; *t* test, $p < .05$).

In following experiments, we quantified transcripts of inflammatory genes in 9-month-old Tau^{tg} and Tau^{wt} littermate mice. As shown in Figure 9F,J, the transcription of pro-inflammatory *Tnf-α* gene was significantly down-regulated, while the transcription of anti-inflammatory *Mrc1* gene was up-regulated in $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice

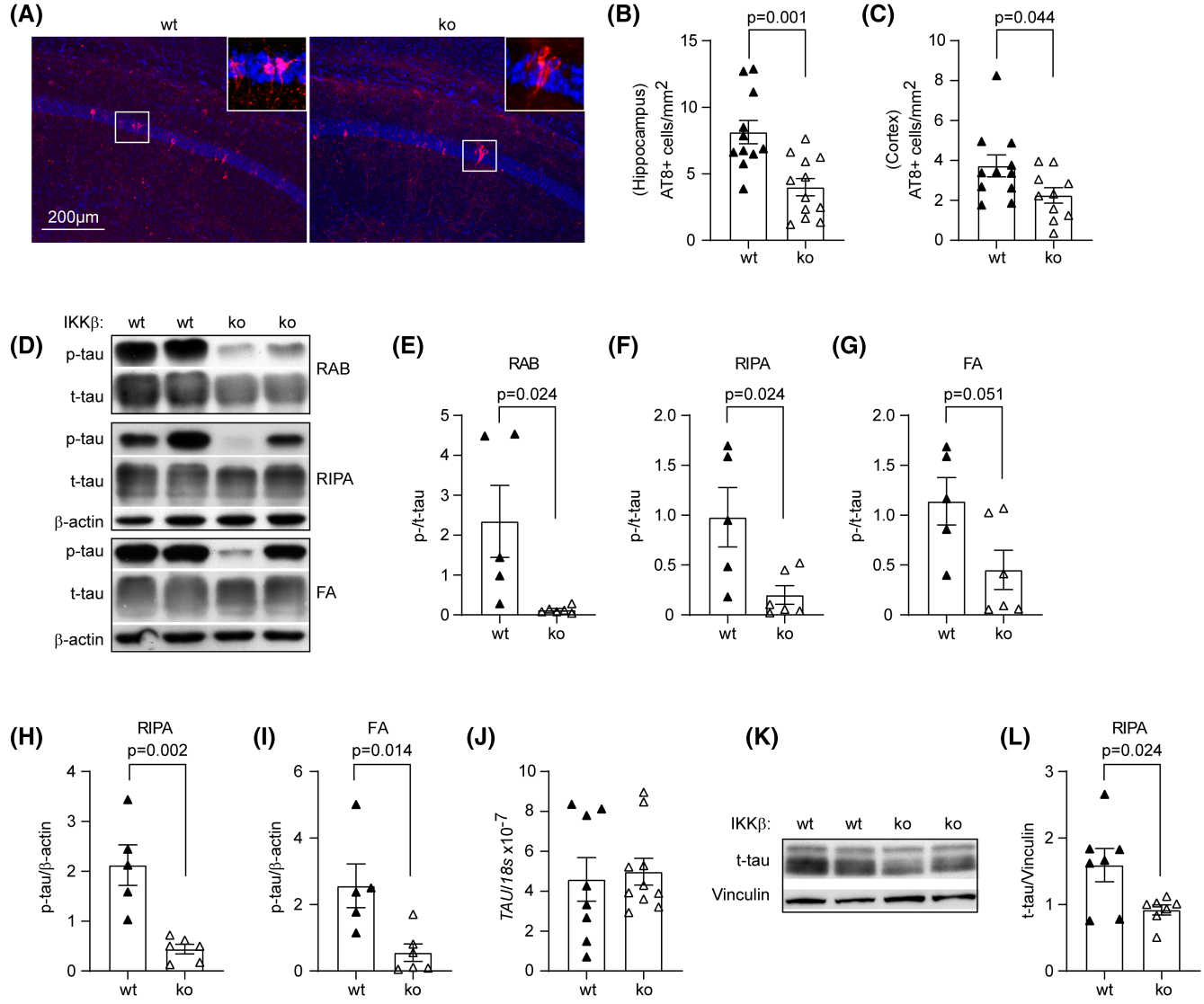


FIGURE 6 Deficiency of IKKβ in neurons reduces cerebral p-tau in tau-transgenic mice. Nine-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were analyzed for cerebral p-tau load after immunofluorescent labeling with AT8 antibody (A). The p-tau-positive cells were counted and adjusted by the relevant brain area. IKKβ deficiency in neurons significantly reduces the cerebral p-tau-positive cells (B and C; *t* test, $n \geq 10$ per group). Tau proteins were extracted from 9-month-old tau-transgenic mice with RAB, RIPA, and FA buffers and detected with Western blots for both phosphorylated and total tau (p-tau and t-tau, respectively) (D–I). The ratios of p−/t-tau are significantly lower in RAB and RIPA fractions of IKKβ-ko mice than in IKKβ-wt littermates (E and F; *t* test, $n \geq 5$ per group). When adjusted to β-actin in the same sample, the protein levels of p-tau in RIPA and FA fractions were also significantly reduced by deficiency of IKKβ, compared with IKKβ-wt tau-transgenic mice (H and I; *t* test, $n \geq 5$ per group). In additional experiments, expression levels of tau were determined by quantitative RT-PCR assay of *TAU* gene transcripts and quantitative Western blot of total tau protein in RIPA-soluble brain homogenate fractions. Neuronal deficiency of IKKβ does not change *TAU* transcription (J; *t* test, $n \geq 8$ per group), but significantly decreases protein level of t-tau (K and L; *t* test, $n = 7$ per group).

compared with $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (*t* test, $p < .05$). However, neuronal deficiency of IKKβ did not change the transcription of *Tnf-α* and *Mrc1* gens in Tau^{wt} mice (Figure 9F,J; *t* test, $p > .05$). The transcription of other tested genes (e.g., *Il-1β*, *Ccl-2*, *Il-10*, and *Chi3l3*) was not changed by IKKβ deficiency in neurons in both Tau^{tg} and Tau^{wt} mice (Figure 9G–I,K; *t* test, $p > .05$). It is known that active and healthy neurons inhibit microglial activation through releasing CD200 and CX3CL1.[32] Interestingly, we observed that the transcriptional level of *Cd200* but not *Cx3cl1* was significantly higher in $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice than in $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermate mice (Figure 9L,M; *t* test, $p < .05$).

CD8-positive lymphocytes have been shown to exacerbate AD pathology in tau-transgenic mice.[39] We continued to stain CD8-positive cells in brains of 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermate mice. CD8-positive cells were mainly located in the dentate

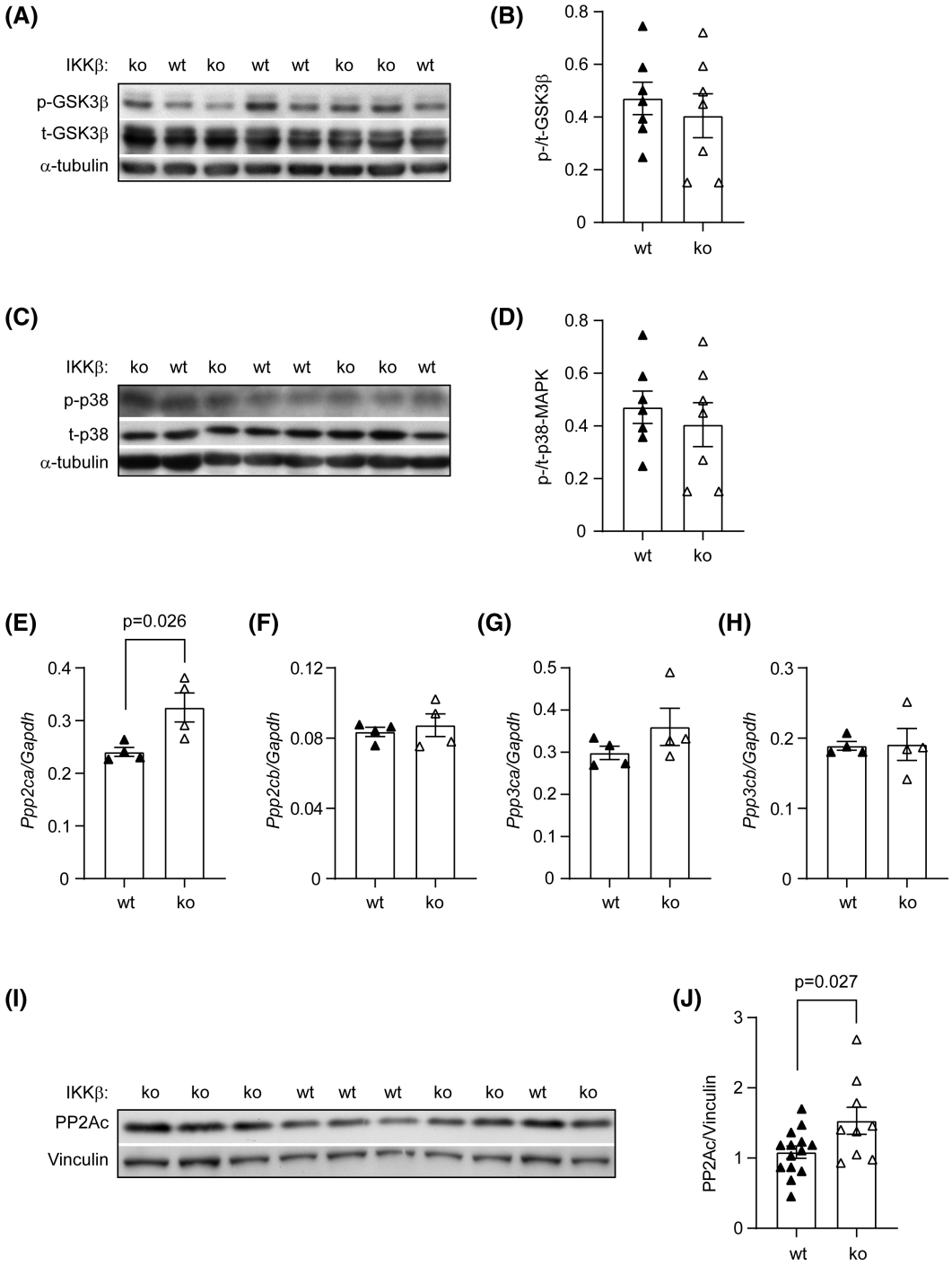


FIGURE 7 Deficiency of IKKβ in neurons increases PP2Ac expression in the brain of tau-transgenic mice. Brains from 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were homogenized in RIPA lysis buffer. Phosphorylated (p-) and total (t-) GSK3β and p38-MAPK were detected with Western blot (A and C). The ratios of p−/t-GSK3β and p−/t-p38-MAPK are not different between IKKβ-wt and ko tau-transgenic mice (B and D; *t* test, $p > .05$, $n = 7$ per group). Transcripts of *Ppp2ca*, *Ppp2cb*, *Ppp3ca*, and *Ppp2cb* genes were also measured in the brain of tau-transgenic mice with quantitative PCR (E–H), showing that IKKβ deficiency significantly up-regulates the transcription of *Ppp2ca* gene, but not other genes tested (E–H; *t* test, $n = 4$ per group). Quantitative Western blot of PP2Ac in RIPA fraction of brain homogenate verified that IKKβ deficiency in neurons increases the cerebral protein level of PP2Ac in tau-transgenic mice (I and J; *t* test, $n \geq 9$ per group).

gyrus, especially in the perivascular space (Figure S2B). After counting cells, we found that neuronal deficiency of IKKβ tended to decrease the density of CD8-positive lymphocytes in the dentate gyrus compare to IKKβ-wild-type tau-transgenic mice; however, the reduction was not statistically significant (Figure S2A–C).

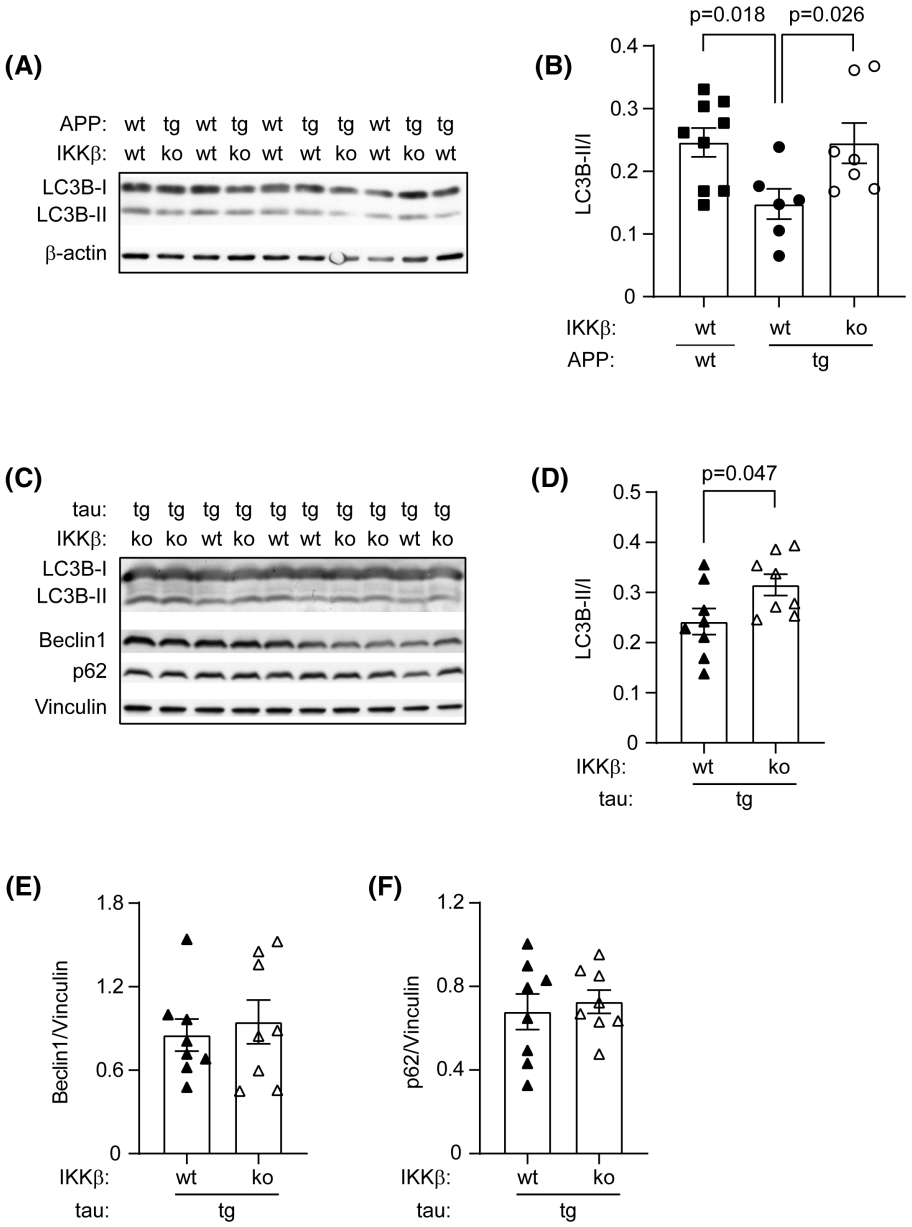


FIGURE 8 Deficiency of IKKβ in neurons increases autophagic activity in brains of both APP-and tau-transgenic mice. Brain homogenates were prepared from 6-month-old APP-transgenic mice and 9-month-old tau-transgenic mice with (IKKβ ko) and without (IKKβ wt) deletion of IKKβ in neurons. Six-month-old non-APP-transgenic (APPwt) mice were used as controls. Quantitative Western blot was used to detect LC3B, beclin1, and SQSTM1/p62 (A and C). IKKβ deficiency significantly increases ratios of LC3B-II/I, but not protein levels of p62 and Beclin1 in both AD mouse models (B, D–F; one-way ANOVA followed by Bonferroni *post-hoc* test for APP mice, $n \geq 6$ per group; and *t* test for tau mice, $n = 8$ per group).

3.9 | Neuronal deficiency of IKKβ does not improve cognitive function and neuroprotection in tau-transgenic mice

We have observed that the cognitive function of tau-transgenic mice is impaired in the Morris water maze test,[27] so we used water maze to evaluate whether neuronal deficiency of IKKβ improves the cognitive function of 9-month-old tau mice. As shown in Figure 10A,B, the swimming time and distance to reach the platform for all tested mice significantly decreased when the training time increased (two-way ANOVA testing the effect of training time, $p < .05$). However, $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermate mice differed in neither the traveling time nor distance to reach the escaping platform in the training phase (two-way ANOVA testing effect of genotypes, $p > .05$). Similarly, in the probe trial, IKKβ deficiency did not change the frequency with which $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice visited the initial region for platform during the training phase compared to $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ littermates (Figure 10C; *t* test, $p > .05$).

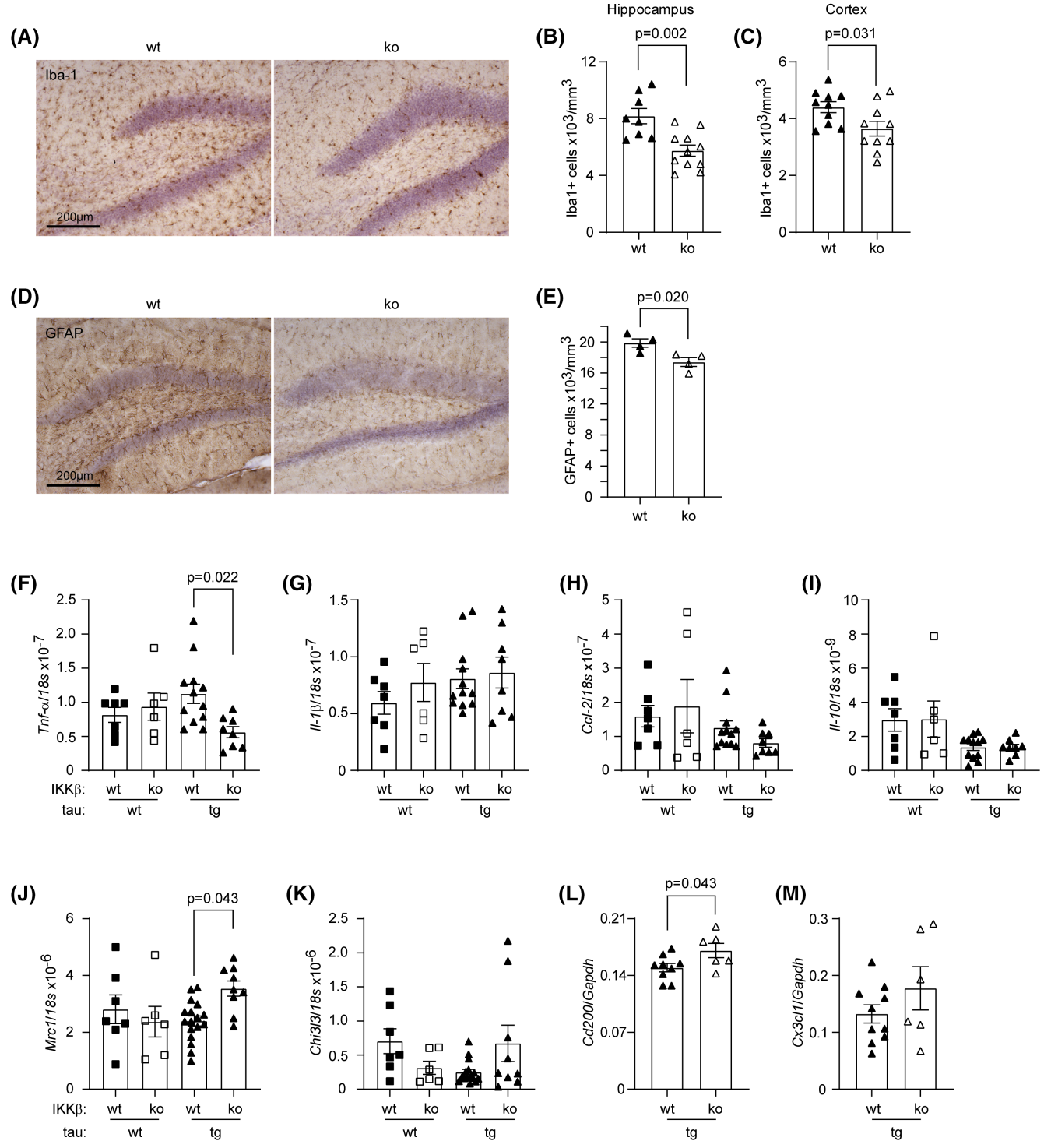


FIGURE 9 Deficiency of IKKβ in neurons inhibits inflammatory activation in the brain of tau-transgenic mice. Brain sections from 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) mice were stained with immunohistochemistry for Iba1 and GFAP (A and D, in brown color). Deficiency of IKKβ significantly reduces the number of Iba1-positive cells in both the hippocampus and cortex (B and C; *t* test, $n \geq 8$ per group) and GFAP-positive cells in the hippocampus (E; *t* test, $n = 4$ per group). The transcripts of both pro- and anti-inflammatory genes, as well as *Cd200* and *Cx3cl1* genes, in brains of tau-transgenic (tg) and non-transgenic (wt) mice with (ko) and without (wt) deficiency of neuronal IKKβ were further detected with real-time PCR (F–M). Transcription of *Tnf-α* gene is significantly down-regulated, while the transcription of *Mrc1* gene is up-regulated in $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ mice compared with $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ mice (F and J; one-way ANOVA followed by Bonferroni *post-hoc* test, $n \geq 6$ per group). Moreover, transcription of *Cd200* is also increased in tau^{tg} mice by neuronal deficiency of IKKβ (L; *t* test, $n \geq 6$ per group).

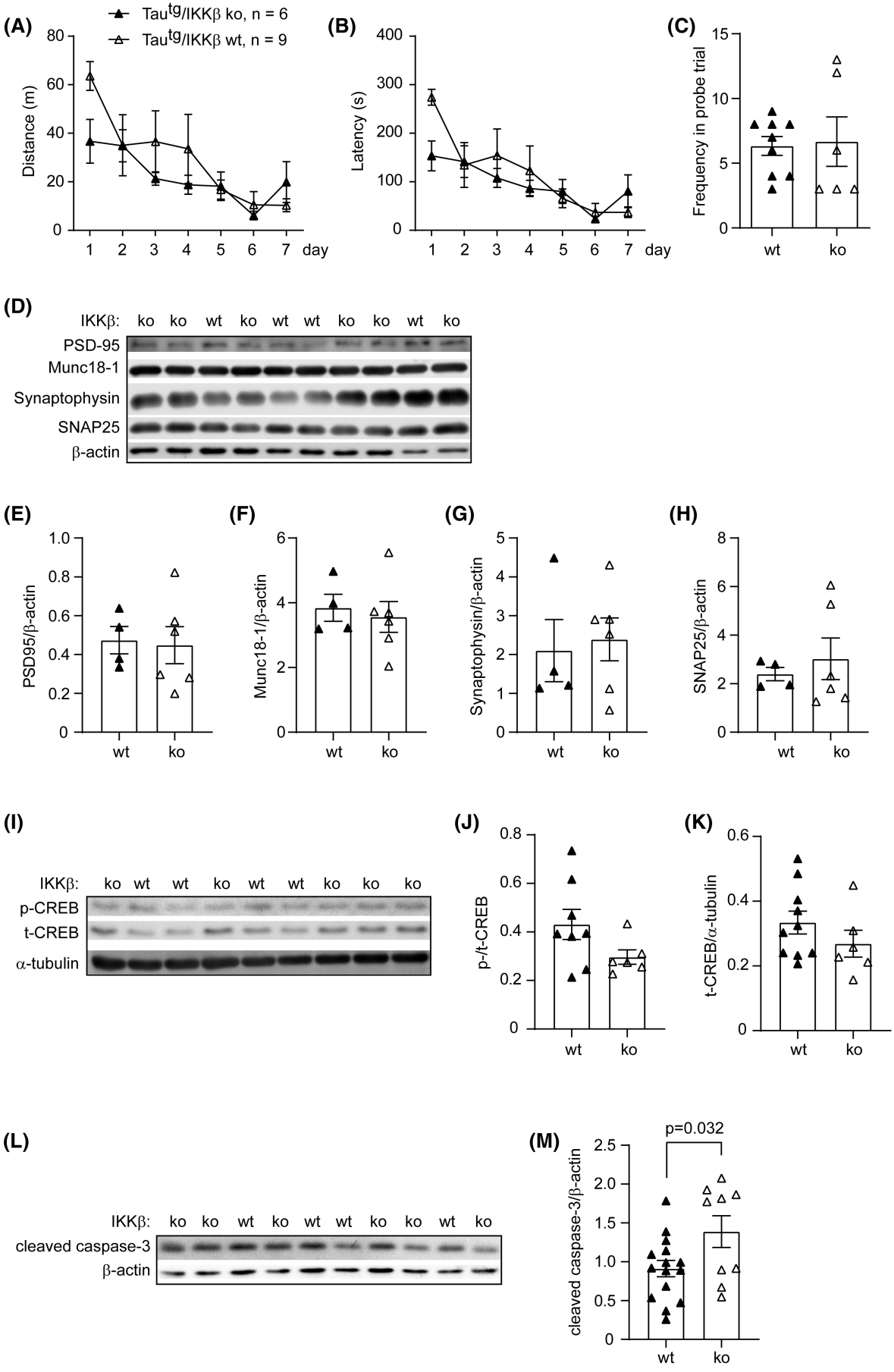


FIGURE 10 Deficiency of IKKβ in neurons does not affect cognitive function and even increases apoptosis in the brains of tau-transgenic mice. In the water maze test, 9-month-old $Tau^{tg}IKK\beta^{fl/fl}Cre^{+/-}$ (IKKβ ko) and $Tau^{tg}IKK\beta^{fl/fl}Cre^{-/-}$ (IKKβ wt) littermate mice did not differ in traveling latency and distance to reach the escape platform during the training phase (A and B; two-way ANOVA, $p > .05$, $n \geq 6$ per group), nor in the frequency with which the mice visited the region where the platform was previously located during the probe trial (C; *t* test, $p > .05$, $n \geq 6$ per group). Western blotting was used to detect cleaved caspase-3, phosphorylation level of CREB, and the amount of synaptic structure proteins, Munc18-1, SNAP25, synaptophysin, and PSD-95 in the brain homogenate of tau-transgenic mice (D–M). Deficiency of IKKβ in neurons does not alter the protein levels of various synaptic proteins (E–H; *t* test, $p > .05$, $n \geq 4$ per group), nor the ratio of phosphorylated (p-) to total (t-) CREB (J and K; *t* test, $p > .05$, $n \geq 6$ per group). Surprisingly, IKKβ deficiency significantly increases the protein level of cleaved caspase-3 in the brains of 9-month-old tau-transgenic mice (M; *t* test, $n \geq 9$ per group).

Moreover, we observed that neuronal deficiency of IKKβ altered neither the protein levels of Munc18-1, PSD-95, SNAP25, and synaptophysin, nor the phosphorylation level of cAMP response element-binding protein (CREB) as detected with quantitative Western blot (Figure 10D–K; *t* test, $p > .05$). CREB activation promotes neuronal survival and plasticity.[40] Surprisingly, we observed that neuronal deficiency of IKKβ increased the protein level of cleaved caspase-3 in the brain homogenate of 9-month-old tau-transgenic mice (Figure 10L,M; *t* test, $p < .05$), suggesting that IKKβ deficiency in neurons promotes apoptosis in the brain of tau mice.

4 | DISCUSSION

Neuroinflammation is a hallmark of AD pathology. Uncontrolled inflammatory activation exacerbates amyloid pathology and tauopathy, leading to neurodegeneration in the AD brain. However, the molecular mechanisms by which neurons respond to neuroinflammation are still unclear. In this project, we knocked out IKKβ-encoding gene, *Ikbkb*, specifically in neurons of both APP- and tau-transgenic mice and observed that neuronal deficiency of IKKβ attenuates Aβ and p-tau load, and modifies inflammatory activation in the brain. Deficiency of IKKβ improves cognitive function and prevents neurodegeneration in APP-transgenic mice; however, it does not confer an efficient neuroprotection in tau-transgenic mice.

The effect of neuroinflammation on Aβ production has been extensively studied. Activation of BACE1 coincides with focal glial inflammatory activation in APP-transgenic mice.[41] In APP-transgenic mice or the systemically lipopolysaccharide-administered mice, pharmacological treatments with acetyl-11-keto-β-boswellic acid,[42] bee venom,[43] and carbon monoxide[44] simultaneously suppress BACE1 expression and NF-κB activation in the brain, indicating the correlation between BACE1 and NF-κB. We deleted IKKβ specifically in neurons, which inhibited NF-κB activation in the brain of APP-transgenic mice. Using this approach, we directly demonstrated the role of IKKβ/NF-κB in BACE1 activation and Aβ production in the brain. Our SH-SY5Y cell culture experiments showed that deficiency of IKKβ decreases protein levels of BACE1, verifying our in vivo finding.

Aβ and inflammatory activation induce tau phosphorylation,[5,45] and trigger tau spreading along axonal projections in AD brains.[46] Our experiments showed that deletion of IKKβ in neurons attenuates both phosphorylated and total tau in brains of tau-transgenic mice. Because neuronal deficiency of IKKβ did not alter transcription of *TAU* gene, or activation of p38α-MAPK and GSK3β, two key kinases that phosphorylate tau in AD brain,[26,35] we hypothesized that the reduction in tau proteins might be due to increased dephosphorylation of tau and/or degradation of tau. PP2A and -2B are the enzymes dephosphorylating p-tau.[47] NF-κB activation inhibits the expression of the catalytic subunit of PP2A (PP2Ac) in pancreatic cancer cells.[36] We observed that IKKβ deficiency in neurons upregulates both the transcription of *Ppp2ca* gene, which encodes isoform A of PP2Ac, and the protein level of PP2Ac in the brain of tau-transgenic mice. Thus, the reduction of p-tau in IKKβ-deficient AD mice may result from the increased dephosphorylation of p-tau.

It was interesting to find that deficiency of IKKβ in neurons enhances autophagy in the brains of our APP- and tau-transgenic mice. IKKβ/NF-κB signaling mediates the expression of autophagy-associated proteins, for example, Beclin1, LC3B, and Atg5, and is thought to promote autophagy[20,48,49]; however, IKKβ/NF-κB activation also induces the expression of Bcl-2 and Bcl-xL,[50] which bind to Beclin1 and block autophagy.[49] In TDP-43-transgenic mice, neuron-specific expression of a super-repressor form of IκB ($I\kappa B^{S32A, S36A}$) enhances autophagy, decreases TDP-43 accumulation, and improves motor performance.[38] Thus, enhancing autophagy in IKKβ-deficient neurons could promote degradation of BACE1 and tau in neurons, as we observed in previous studies.[26,27,37]

Deficiency of IKKβ in neurons reduces microglia and astrocytes in APP- and tau-transgenic mice; however, how it regulates inflammatory activation in AD is unclear. Not surprisingly, deficiency of IKKβ decreases Aβ and subsequently inhibits both pro- and anti-inflammatory activation (e.g., down-regulation of *Ccl-2* and *Il-10* transcription) in APP-transgenic mice. As we previously observed, Aβ is a ligand of CD14 and TLR2, which induces

both types of inflammatory activation in microglia.[51,52] Tauopathy can primarily damage neurons and then triggers microglial activation,[53] although tau proteins also activate microglia.[54] In our tau-transgenic mice, IKKβ deficiency decreases pro-inflammatory *Tnf-α* transcription while increasing anti-inflammatory *Mrc1* transcription, in which IKKβ-regulated neuronal status may be one of the determinants. CD200 and CX3CL1 released by healthy and active neurons control microglial activation.[32] IKKβ deficiency increases transcription of *Cd200* gene in the brains of tau mice, but not APP-transgenic mice, perhaps providing further evidence that neuronal activity regulates neuroinflammation in tau mice.

We observed that IKKβ deficiency protects neurons in APP- but not in tau-transgenic mice, which may also be due to the different pathogenic mechanisms of Aβ and p-tau in AD. In APP-transgenic mice, Aβ oligomers activate N-methyl-D-aspartate receptor (NMDARs) directly,[55] and indirectly by blocking astrocytic re-uptake of glutamate and accumulating glutamate in the perisynaptic space,[56] both of which lead to calcium overload of neurons. Elevated calcium activates NF-κB possibly by interaction between CaMKII and IKKβ.[57,58] The rapid, particularly sustained, accumulation of p65/NF-κB and IκBα in the nucleus is associated with neuron death.[59] IKKβ deficiency may prevent calcium-induced NF-κB activation and neurotoxicity in APP-transgenic mice. In tau-transgenic mice, p-tau destabilizes the cytoskeleton and disrupts the axonal transport, leading to axonal degeneration and neuronal death.[60] Tau accumulation damages mitochondria, induces endoplasmic reticulum stress and dysregulates neuronal GABAergic and cholinergic signaling.[53] IKKβ deficiency may not prevent these pathogenic processes; however, compromise NF-κB activation-mediated neuroprotection in tau-transgenic mice.

Our study has shown that neuronal deficiency of IKKβ serves diverse effects on neuroprotection in APP and tau-transgenic AD mice. It is obviously a limitation of our study that we studied pathogenic role of neuronal IKKβ in APP- or tau-transgenic mice, instead of in an AD model with both Aβ pathology and tauopathy. It is known that Aβ and p-tau synergistically contribute to AD pathogenesis. Tau appears to mediate the neurotoxic effects of Aβ. Deletion of endogenous tau abolishes Aβ-induced neurotoxicity.[33,34,61] Thus, further studies are needed to clarify the pathogenic role of neuronal IKKβ, particularly in the context of interaction between Aβ and p-tau.

In summary, deficiency of IKKβ in neurons reduces Aβ and p-tau pathologies in APP- and tau-transgenic mice. Possible mechanisms are that IKKβ deficiency: (1) decreases BACE1 expression and Aβ generation, (2) increases PP2Ac expression and p-tau dephosphorylation, and (3) enhances neuronal autophagy, which promotes BACE1 and p-tau degradation. However, IKKβ has also neuroprotective effects. Although our study deciphered the pathophysiological role of neuronal IKKβ/NF-κB in AD, it remains unclear whether IKKβ can serve as a therapeutic target for AD patients. A following study is needed to address the pathogenic role of neuronal IKKβ in an AD model with both amyloid pathology and tauopathy.

AUTHOR CONTRIBUTIONS

Yang Liu conceptualized and designed the study, acquired funding, conducted experiments, acquired and analyzed the data, and wrote the manuscript. Laura Schnöder, Wenqiang Quan, Ye Yu, Inge Tomic, Qinghua Luo, and Wenlin Hao designed and conducted the experiments, acquired data, and analyzed the data. Guoping Peng and Dong Li provided advice and edited the manuscript. Klaus Fassbender provided laboratory equipment and supervised the study. All authors read and approved the final manuscript.

ACKNOWLEDGMENTS

The authors thank D. Westaway (University of Toronto, Canada) for providing TgCRND8 APP-transgenic mice, M. Pasparakis (University of Cologne, Germany) for providing *Ikbkb*$^{fl/fl}$ mice, and K. Nave (Max-Planck-Institute for Medicine, Germany) for providing Nex-Cre mice. The authors appreciate Andrea Schottek for her excellent technical assistance. This work was supported by grants from the National Natural Science Foundation of China (81771371; to YL), Alzheimer Forschung Initiative e.V. (#18009; to YL), Youth Science Foundation of Jiangxi Province (20181BAB215016; to YY), and Medical Faculty of Universität des Saarlandes through HOMFOR2022 (to LS). Qinghua Luo holds a scholarship from China Scholarship Council (CSC; 201906820011). Open Access funding enabled and organized by Projekt DEAL.

DISCLOSURES

The authors have declared that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article. Raw data are available upon reasonable request.

ETHICS APPROVAL

All animal experiments were performed in accordance with relevant national rules and authorized by Landesamt für Verbraucherschutz, Saarland, Germany (permission number: 13/2018).

REFERENCES

1. Querfurth HW, LaFerla FM. Alzheimer's disease. *N Engl J Med*. 2010;362:329-344.
2. Shankar GM, Li S, Mehta TH, et al. Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med*. 2008;14:837-842.
3. Yoshiyama Y, Higuchi M, Zhang B, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron*. 2007;53:337-351.
4. Heneka MT. Microglia take centre stage in neurodegenerative disease. *Nat Rev Immunol*. 2019;19:79-80.
5. Ghosh S, Wu MD, Shaftel SS, et al. Sustained interleukin-1beta overexpression exacerbates tau pathology despite reduced amyloid burden in an Alzheimer's mouse model. *J Neurosci*. 2013;33:5053-5064.
6. Maphis N, Xu G, Kokiko-Cochran ON, et al. Reactive microglia drive tau pathology and contribute to the spreading of pathological tau in the brain. *Brain*. 2015;138:1738-1755.
7. Pascoal TA, Benedet AL, Ashton NJ, et al. Microglial activation and tau propagate jointly across Braak stages. *Nat Med*. 2021;27:1592-1599.
8. Sheng JG, Bora SH, Xu G, Borchelt DR, Price DL, Koliatsos VE. Lipopolysaccharide-induced-neuroinflammation increases intracellular accumulation of amyloid precursor protein and amyloid beta peptide in APPswe transgenic mice. *Neurobiol Dis*. 2003;14:133-145.
9. Kaltschmidt B, Uherek M, Volk B, Baeuerle PA, Kaltschmidt C. Transcription factor NF-kappaB is activated in primary neurons by amyloid beta peptides and in neurons surrounding early plaques from patients with Alzheimer disease. *Proc Natl Acad Sci U S A*. 1997;94:2642-2647.
10. Terai K, Matsuo A, McGeer PL. Enhancement of immunoreactivity for NF-kappa B in the hippocampal formation and cerebral cortex of Alzheimer's disease. *Brain Res*. 1996;735:159-168.
11. Gutierrez H, Davies AM. Regulation of neural process growth, elaboration and structural plasticity by NF-kappaB. *Trends Neurosci*. 2011;34:316-325.
12. Grilli M, Goffi F, Memo M, Spano P. Interleukin-1beta and glutamate activate the NF-kappaB/Rel binding site from the regulatory region of the amyloid precursor protein gene in primary neuronal cultures. *J Biol Chem*. 1996;271:15002-15007.
13. Grilli M, Ribola M, Alberici A, Valerio A, Memo M, Spano P. Identification and characterization of a kappa B/Rel binding site in the regulatory region of the amyloid precursor protein gene. *J Biol Chem*. 1995;270:26774-26777.
14. Thonda S, Puttapaka SN, Kona SV, Kalivendi SV. Extracellular-signal-regulated kinase inhibition switches APP processing from beta- to alpha-secretase under oxidative stress: modulation of ADAM10 by SIRT1/NF-kappaB signaling. *ACS Chem Nerosci*. 2021;12:4175-4186.
15. Chen C-H, Zhou W, Liu S, et al. Increased NF-κB signalling up-regulates BACE1 expression and its therapeutic potential in Alzheimer's disease. *Int J Neuropsychopharmacol*. 2012;15:77-90.
16. Maqbool A, Lattke M, Wirth T, Baumann B. Sustained, neuron-specific IKK/NF-kappaB activation generates a selective neuroinflammatory response promoting local neurodegeneration with aging. *Mol Neurodegener*. 2013;8:40.
17. Herrmann O, Baumann B, de Lorenzi R, et al. IKK mediates ischemia-induced neuronal death. *Nat Med*. 2005;11:1322-1329.
18. Kaltschmidt C, Kaltschmidt B, Neumann H, Wekerle H, Baeuerle PA. Constitutive NF-kappa B activity in neurons. *Mol Cell Biol*. 1994;14:3981-3992.
19. Mettang M, Reichel SN, Lattke M, et al. IKK2/NF-kappaB signaling protects neurons after traumatic brain injury. *FASEB J*. 2018;32:1916-1932.
20. Ochaba J, Fote G, Kachemov M, et al. IKKbeta slows Huntington's disease progression in R6/1 mice. *Proc Natl Acad Sci U S A*. 2019;116:10952-10961.
21. Kaltschmidt B, Uherek M, Wellmann H, Volk B, Kaltschmidt C. Inhibition of NF-kappaB potentiates amyloid beta-mediated neuronal apoptosis. *Proc Natl Acad Sci U S A*. 1999;96:9409-9414.
22. Yankner BA, Duffy LK, Kirschner DA. Neurotrophic and neurotoxic effects of amyloid beta protein: reversal by tachykinin neuropeptides. *Science*. 1990;250:279-282.
23. Liu Y, Liu X, Hao W, et al. IKKbeta deficiency in myeloid cells ameliorates Alzheimer's disease-related symptoms and pathology. *J Neurosci*. 2014;34:12982-12999.
24. Chishti MA, Yang DS, Janus C, et al. Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *J Biol Chem*. 2001;276:21562-21570.
25. Pasparakis M, Courtois G, Hafner M, et al. TNF-mediated inflammatory skin disease in mice with epidermis-specific deletion of IKK2. *Nature*. 2002;417:861-866.
26. Schnöder L, Gasparoni G, Nordstrom K, et al. Neuronal deficiency of p38alpha-MAPK ameliorates symptoms and pathology of APP or tau-transgenic Alzheimer's mouse models. *FASEB J*. 2020;34:9628-9649.
27. Qin Y, Liu Y, Hao W, et al. Stimulation of TLR4 attenuates Alzheimer's disease-related symptoms and pathology in tau-transgenic mice. *J Immunol*. 2016;197:3281-3292.
28. Liu Y, Hao W, Dawson A, Liu S, Fassbender K. Expression of amyotrophic lateral sclerosis-linked SOD1 mutant increases the neurotoxic potential of microglia via TLR2. *J Biol Chem*. 2009;284:3691-3699.
29. Xie K, Liu Y, Hao W, et al. Tenascin-C deficiency ameliorates Alzheimer's disease-related pathology in mice. *Neurobiol Aging*. 2013;34:2389-2398.
30. Goebbels S, Bormuth I, Bode U, Hermanson O, Schwab MH, Nave KA. Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. *Genesis*. 2006;44:611-621.
31. Mucke L, Selkoe DJ. Neurotoxicity of amyloid beta-protein: synaptic and network dysfunction. *Cold Spring Harb Perspect Med*. 2012;2:a006338.
32. Kierdorf K, Prinz M. Factors regulating microglia activation. *Front Cell Neurosci*. 2013;7:44.
33. Ittner LM, Ke YD, Delerue F, et al. Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models. *Cell*. 2010;142:387-397.
34. Vossel KA, Zhang K, Brodbeck J, et al. Tau reduction prevents Abeta-induced defects in axonal transport. *Science*. 2010;330:198.
35. Zhou Q, Li S, Li M, et al. Human tau accumulation promotes glycogen synthase kinase-3beta acetylation and thus upregulates the kinase: a vicious cycle in Alzheimer neurodegeneration. *EBioMedicine*. 2022;78:103970.
36. Tao M, Liu L, Shen M, et al. Inflammatory stimuli promote growth and invasion of pancreatic cancer cells through

NF-kappaB pathway dependent repression of PP2Ac. *Cell Cycle*. 2016;15:381-393.
37. Schnöder L, Hao W, Qin Y, et al. Deficiency of neuronal p38alpha MAPK attenuates amyloid pathology in Alzheimer disease mouse and cell models through facilitating lysosomal degradation of BACE1. *J Biol Chem*. 2016;291:2067-2079.
38. Dutta K, Thammisetty SS, Boutej H, Bareil C, Julien JP. Mitigation of ALS pathology by neuron-specific inhibition of nuclear factor kappa B signaling. *J Neurosci*. 2020;40:5137-5154.
39. Laurent C, Dorothee G, Hunot S, et al. Hippocampal T cell infiltration promotes neuroinflammation and cognitive decline in a mouse model of tauopathy. *Brain*. 2017;140:184-200.
40. Jancic D, Lopez de Armentia M, Valor LM, Olivares R, Barco A. Inhibition of cAMP response element-binding protein reduces neuronal excitability and plasticity, and triggers neurodegeneration. *Cereb Cortex*. 2009;19:2535-2547.
41. Heneka MT, Sastre M, Dumitrescu-Ozimek L, et al. Focal glial activation coincides with increased BACE1 activation and precedes amyloid plaque deposition in APP[V717I] transgenic mice. *J Neuroinflammation*. 2005;2:22.
42. Wei C, Fan J, Sun X, et al. Acetyl-11-keto-beta-boswellic acid ameliorates cognitive deficits and reduces amyloid-beta levels in APPswe/PS1dE9 mice through antioxidant and anti-inflammatory pathways. *Free Radic Biol Med*. 2020;150:96-108.
43. Gu SM, Park MH, Hwang CJ, et al. Bee venom ameliorates lipopolysaccharide-induced memory loss by preventing NF-kappaB pathway. *J Neuroinflammation*. 2015;12:124.
44. Kim HJ, Joe Y, Chen Y, Park GH, Kim UH, Chung HT. Carbon monoxide attenuates amyloidogenesis via down-regulation of NF-kappaB-mediated BACE1 gene expression. *Aging Cell*. 2019;18:e12864.
45. Ryan SD, Whitehead SN, Swayne LA, et al. Amyloid-beta42 signals tau hyperphosphorylation and compromises neuronal viability by disrupting alkylacylglycerophosphocholine metabolism. *Proc Natl Acad Sci U S A*. 2009;106:20936-20941.
46. Shimada H, Kitamura S, Shinotoh H, et al. Association between Abeta and tau accumulations and their influence on clinical features in aging and Alzheimer's disease spectrum brains: a [11C] PBB3-PET study. *Alzheimers Dement (Amst)*. 2017;6:11-20.
47. Wang JZ, Gong CX, Zaidi T, Grundke-Iqbal I, Iqbal K. Dephosphorylation of Alzheimer paired helical filaments by protein phosphatase-2A and -2B. *J Biol Chem*. 1995;270:4854-4860.
48. Criollo A, Senovilla L, Authier H, et al. The IKK complex contributes to the induction of autophagy. *EMBO J*. 2010;29:619-631.
49. Salminen A, Hyttinen JM, Kauppinen A, Kaarniranta K. Context-dependent regulation of autophagy by IKK-NF-kappaB signaling: impact on the aging process. *Int J Cell Biol*. 2012;2012:849541.
50. Tamatani M, Che YH, Matsuzaki H, et al. Tumor necrosis factor induces Bcl-2 and Bcl-x expression through NFkappaB activation in primary hippocampal neurons. *J Biol Chem*. 1999;274:8531-8538.
51. Liu S, Liu Y, Hao W, et al. TLR2 is a primary receptor for Alzheimer's amyloid beta peptide to trigger neuroinflammatory activation. *J Immunol*. 2012;188:1098-1107.
52. Liu Y, Walter S, Stagi M, et al. LPS receptor (CD14): a receptor for phagocytosis of Alzheimer's amyloid peptide. *Brain*. 2005;128:1778-1789.
53. Yang Y, Wang JZ. Nature of tau-associated neurodegeneration and the molecular mechanisms. *J Alzheimers Dis*. 2018;62:1305-1317.
54. Wang C, Fan L, Khawaja RR, et al. Microglial NF-kappaB drives tau spreading and toxicity in a mouse model of tauopathy. *Nat Commun*. 2022;13:1969.
55. Texido L, Martin-Satue M, Alberdi E, Solsona C, Matute C. Amyloid beta peptide oligomers directly activate NMDA receptors. *Cell Calcium*. 2011;49:184-190.
56. Zott B, Simon MM, Hong W, et al. A vicious cycle of beta amyloid-dependent neuronal hyperactivation. *Science*. 2019;365:559-565.
57. Martin TP, McCluskey C, Cunningham MR, Beattie J, Paul A, Currie S. CaMKIIdelta interacts directly with IKKbeta and modulates NF-kappaB signalling in adult cardiac fibroblasts. *Cell Signal*. 2018;51:166-175.
58. Meffert MK, Chang JM, Wiltgen BJ, Fanselow MS, Baltimore D. NF-κB functions in synaptic signaling and behavior. *Nat Neurosci*. 2003;6:1072-1078.
59. Schwamborn R, Dussmann H, Konig HG, Prehn JHM. Time-lapse imaging of p65 and IkappaBalpha translocation kinetics following Ca(2+)-induced neuronal injury reveals biphasic translocation kinetics in surviving neurons. *Mol Cell Neurosci*. 2017;80:148-158.
60. Salvadores N, Geronimo-Olvera C, Court FA. Axonal degeneration in AD: the contribution of Abeta and tau. *Front Aging Neurosci*. 2020;12:581767.
61. DeVos SL, Corjuc BT, Commins C, et al. Tau reduction in the presence of amyloid-beta prevents tau pathology and neuronal death in vivo. *Brain*. 2018;141:2194-2212.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Schnöder L, Quan W, Yu Y, et al. Deficiency of IKKβ in neurons ameliorates Alzheimer's disease pathology in APP- and tau-transgenic mice. *The FASEB Journal*. 2023;37:e22778. doi:10.1096/fj.202201512R