

KIF2A downregulation links amyloid- β to Tau phosphorylation in Alzheimer's disease

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Abstract

Microtubules (MT) are essential components of the cytoskeleton. Dysfunctions of MT and MT-associated proteins are prominent features of neurodegenerative disorders. In Alzheimer's disease (AD), changes in MT composition and hyperphosphorylation of Tau are more closely related to neurodegeneration than amyloid plaque formation. However, the accumulation of amyloid beta (A β) species is the earliest event in AD pathology and induces Tau toxicity. KIF2A is a microtubule depolarizing kinesin with important roles during cortical development. KIF2A expression is maintained in the mature brain, where it is required for neuronal survival.

Here, we used a conditional approach to ablate KIF2A specifically in the adult mouse cortex and hippocampus to assess the impact of KIF2A deletion on neuronal survival and Tau phosphorylation.

We found that KIF2A deficiency leads to a reduction of dendritic spine density and maturation associated with cognitive decline, followed by an increase in Tau phosphorylation through ERK1/2 activation. We also studied KIF2A expression in 5xFAD mouse model and post-mortem human brain tissue. We report that A β accumulation alters KIF2A expression in neurons and most importantly, KIF2A protein levels are drastically reduced in AD patients but not in patients with other primary Tauopathies.

Our results shed light on the relationship between A β accumulation, KIF2A deregulation, microtubule dysfunction, and enhanced Tau phosphorylation in the context of AD.

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Introduction

Microtubules (MT) are important components of the cytoskeleton that play crucial roles in neuronal homeostasis, function, and plasticity. Nerve cells are highly dependent on functional MT. Alterations in MT dynamics or function trigger neurodevelopmental and/or neurodegenerative disorders¹⁻⁴. The plus end of MT is a dynamic region that undergoes cycles of rapid growth (polymerization) and shrinkage (depolymerization). The balance between labile and stable MT is regulated by MT-binding proteins, such as MT-associated proteins (MAPs) and plus-end-tracking proteins (+TIPs); tubulin posttranslational modifications (PTM) and destabilizing tubulin-binding factors. For a long time, it was believed that Tau, a MAP localized mainly in axons, plays an important role in MT stabilisation. However, recent studies have demonstrated that Tau binds the labile domain of MT preventing the binding of MAP6, a genuine MT stabilizer⁵. Tau depletion in neurons increases the stable MT mass, increasing MT stabilization. Inversely, increasing MT stabilization induces Tau dissociation from MT^{6,7}. Tauopathies are a heterogeneous group of neurodegenerative diseases characterized by the aggregation of misfolded Tau protein, including -but not limited to- Alzheimer's disease (AD), frontotemporal lobe degeneration (FTLD), Corticobasal degeneration (CBD) and Pick's disease (PiD). In these diseases, hyperphosphorylated Tau assembles into intraneuronal neurofibrillary tangles (NFTs), a causative factor for neurodegeneration. Changes in MT dynamics could be an important factor for Tau hyperphosphorylation and disease progression in Tauopathies. In AD, accumulation of insoluble A β deposits is more tightly correlated with disease progression compared to extracellular amyloid beta (A β) deposits, another neuropathological hallmark. According to the amyloid cascade hypothesis, A β deposits are the primary trigger of AD pathogenesis leading to hyperphosphorylated Tau accumulation and eventually neuronal death^{8,9}. Although the amyloid cascade hypothesis has been debated, a body of clinical and experimental evidence supports a link between soluble intermediates such as A β oligomers, changes in MT composition and Tauopathy^{6,10-15}. How an early deposition of A β species facilitates Tau phosphorylation and neurodegeneration remains unclear. Given that the malfunction of microtubules drives neuropathological modifications in AD, decoding the factors implicated in the normal function of MTs and the relationship between A β and MT dynamics will be key to understanding AD pathology.

KIF2A is the only member of the kinesin-13 subfamily of kinesins with MT depolymerizing activity expressed in postmitotic neurons. KIF2A forms homodimers that bind MT and move towards the plus end where it removes tubulin subunits in an ATP-dependent manner, thereby regulating MT dynamics¹⁶⁻¹⁹. Previous studies highlighted essential roles for KIF2A in brain development and neuronal function²⁰⁻²⁵. Specific deletion of KIF2A in progenitors of cortical glutamatergic neurons triggers early onset neurodegeneration and secondary microcephaly (i.e., 60% reduction of cortical and hippocampal volume at the age of 4 months). KIF2A-depleted neurons exhibit an increase in stable MTs, and this change in MT dynamics disrupts several microtubule-dependent processes, including neuronal polarity, neuritogenesis, synaptogenesis, and axonal transport²⁵. KIF2A expression persists in the cortex and hippocampus at adult stages. However, its function in mature neurons is poorly described. In this study, we used a tamoxifen-inducible system in mice to selectively inactivate KIF2A in the mature hippocampus and neocortex and analyse the functional consequences of its loss. Four weeks after tamoxifen injections, mutant mice presented a reduction in the density of dendritic spines associated with impaired spatial memory and hyperactivity, followed by increased endogenous Tau phosphorylation and progressive neurodegeneration. Deletion of KIF2A also triggered aberrant Tau hyperphosphorylation in the soluble and insoluble fractions of brain lysates from mice overexpressing mutated human Tau. Interestingly, analysis of postmortem brain tissue revealed a dramatic reduction of KIF2A protein levels in AD patients compared to controls, but not in patients suffering from Tauopathies with no A β accumulation. Using *in vitro* and *in vivo* approaches, we showed that A β oligomers reduce KIF2A expression in neurons, linking A β toxicity with changes in MT dynamics. Altogether, our findings emphasize the importance of KIF2A in the mature brain and provide mechanistic insights into how a reduction of MT depolymerization, due to KIF2A downregulation, increases MT stabilisation, impacting Tau phosphorylation in physiological and pathological contexts.

Materials and methods

Animals

All animal procedures were carried out in accordance with European guidelines (2010/63/UE) and approved by the animal ethics committee of the Université catholique de Louvain under agreement 2019/UCL/MD/006. The day of the vaginal plug was considered embryonic day 0.5 (E0.5). We used the following mouse lines: *Kif2a^{F/F}*²³, *Kif2a^{+/-}*²⁵, *CamKII-CreERT2* (Strain #:012362 The Jackson Laboratory)²⁶, *Thy1-YFP*²⁷, 5xFAD transgenic mice (Strain #034840 The Jackson Laboratory)²⁸ and PS19 (Strain #024841 The Jackson Laboratory)²⁹. To produce *CamKII-CreERT2 Kif2a^{F/F}* (*CamKII-cKO*) we crossed *CamKII-CreERT2 Kif2a^{F/F}* males with *Kif2a^{F/F}* females. *Kif2a^{F/F}* littermates were considered as control mice (*CamKII-control*). Tamoxifen (Sigma T5648) was diluted in 10% ethanol in sunflower seed oil (Sigma S5007) (30mg/ml) and intraperitoneally injected at 80mg/Kg/day for 5 consecutive days.

Postmortem human brain samples and Ethics

This study used human postmortem cerebral tissue from the Inferior frontal gyrus obtained from non-demented control participants, AD, FTLN and PiD patients, and the precentral gyrus obtained from CBD patients. Human brain tissue was obtained from The Netherlands Brain Bank (NBB) and the Université catholique de Louvain (UCLouvain). All Material and Data collected by the NBB were obtained on the basis of written informed consent and comply with the local ethical and legislative requirements. The UCLouvain brain sample collection was conducted after obtaining informed consent prior to death and family contact after death. It was approved by UCLouvain ethical committee under the number UCL-2020-355. Sample information is given in Supplementary Table 1.

Brain tissue sample processing and protein extract preparation

We followed a well-established protocol³⁰ for the extraction of soluble protein fractions from human samples. Briefly, 0.2 g of cortical brain sample was collected by dissection while still frozen, for each subject. The dissected tissue was diced into 2 × 2 mm pieces while thawing and

homogenized in 1 ml of low salt buffer (LS buffer: 50 mM HEPES, pH 7.0, containing 250 mM sucrose, 1 mM ethylene diamine tetra acetic acid (EDTA) and protease inhibitor cocktail (Roche) by ~15 strokes in a 2 ml Dounce homogenizer. The total homogenate volume was adjusted at 2 ml and mixed with 1% (w/v) Sarkosyl and 200 mM NaCl (final concentrations). The Sarkosyl-homogenates (H) were sonicated 3 × 5 s pulses at 30% amplitude on ice with a microtip probe and transferred to 4 mL ultracentrifuge tubes for centrifugation in a pre-cooled Beckman 50Ti fixed-angle rotor at 180,000 × g for 30 min at 4 °C. The supernatants, which corresponded to Sarkosyl-soluble protein fractions, were transferred to clean tubes. For all samples, protein concentration was performed with a NanoDrop using a Pierce™ BCA Protein Assay Kit (ThermoFisher 23227).

Mouse brain cortices and hippocampi and primary cell culture were homogenized on ice in RIPA lysis buffer (ThermoFisher 89900), with Protease/Phosphatase Inhibitor Cocktail (Cell signaling #5872); and centrifuged at 16,000 g for 15 min at 4°C. To separate the soluble and insoluble Tau proteins from the mouse hippocampus, we homogenized the brain tissue in Lysis buffer (150mM NaCl, 20mM Tris, 1% NP40, 10% glycerol) with Protease/Phosphatase Inhibitors and centrifuged at 16,000G for 15 min at 4°C. We transferred the supernatant (soluble fraction) to new Eppendorf and we resuspended the pellets in Urea buffer (8M Urea, 5mM EDTA, 1mM EGTA) with Protease/Phosphatase Inhibitors. We centrifuged the samples at 16,000 G for 15 min at 4°C and we transferred the supernatant (insoluble fraction) to new Eppendorfs. Protein quantification from mouse brains was performed with a NanoDrop using a Pierce™ BCA Protein Assay Kit (ThermoFisher 23227) or with an Invitrogen Qubit Fluorometer machine using the Qubit Protein BR Assay kit (Thermofisher A50669).

RNA extraction from human samples and cDNA production

The RNA extraction protocol is based on Qiagen's RNeasy® Mini Kit (74104). Prior to RNA extraction, samples were allowed to thaw at room temperature. The RLT buffer, consisting of 500 µL of RLT and 5 µL of beta-mercaptoethanol, was prepared. Subsequently, 350 µL of the RLT buffer were added to each sample, and tissue homogenisation was achieved using pipettes and syringes. The samples were then centrifuged at 14,000 rpm for 3 minutes, and the supernatant was recovered. To further process the samples, 300 µL of 70% ethanol were added,

1 followed by mechanical disruption. The resulting mixture was placed in a column and
 2 centrifuged at 8000 rpm for 30 seconds. After discarding the contents of the collection tube, 700
 3 μL of buffer RW1 were added to the column and centrifuged again for 30 seconds at 8000 rpm.
 4 This step was repeated with 500 μL of RPE buffer. Following these washes, the column was
 5 centrifuged for 2 minutes at 8000 rpm and then for 1 minute at 14000 rpm after discarding the
 6 collection tube contents. The column was transferred to a 1.5 mL Eppendorf tube, and the
 7 remaining collection tube was discarded. To elute RNA, 30 μL of RNase-free water were added
 8 to the column and left for 1 minute. The Eppendorf was then centrifuged for 1 minute at 14,000
 9 rpm, and the RNA concentration was assessed using a NanoDrop spectrophotometer (Thermo
 10 Fisher Scientific, NanoDrop 2000 spectrophotometer).

11 The cDNA production protocol is based on Promega's GoScript™ Reverse Transcription Mix,
 12 Random Primer Protocol (A2801). To perform reverse transcription, the GoScript™ Reverse
 13 Transcription Mix components were thawed on ice, gently mixed, and briefly centrifuged. A 10-
 14 microlitre GoScript™ Reverse Transcription Mix was prepared for each cDNA reaction by
 15 sequentially combining 4 μL of Nuclease-Free Water, 4 μL of GoScript™ Reaction Buffer, and
 16 2 μL of GoScript™ Enzyme Mix on ice. Gentle mixing occurred by pipetting after each addition,
 17 and the overall mix was further blended by pipetting or vortexing. The cDNA reaction setup
 18 involved combining 10 μL of GoScript™ Reverse Transcription Mix with up to 10 μL of the
 19 RNA sample in each reaction tube or well of a reaction plate on ice. After pipetting to mix the
 20 reaction, the tubes or wells were closed or sealed. Subsequent incubation of the reverse
 21 transcription reactions was performed in controlled-temperature blocks or a programmed thermal
 22 cycler. Following incubation, the reaction products were centrifuged and stored at 4°C in the
 23 reaction tubes or wells for immediate analysis (within 24 hours) or at -80°C for long-term
 24 storage.

25 **RT-qPCR**

26 Human cDNA samples were prepared for quantitative polymerase chain reaction (qPCR)
 27 analysis following a standardised protocol. First, 20 μL of prepared human cDNA were mixed
 28 with 80 μL of sterilised water to create a solution with a concentration of 1 $\mu\text{g}/100 \mu\text{L}$ (Solution
 29 A). Subsequently, 20 μL of Solution A were combined with 980 μL of sterilised water to

generate a "sample" preparation with a concentration of 2 ng/10 μ L. The remaining 80 μ L of Solution A were added to the collective pool of human cDNA, resulting in a final concentration of 10 ng/ μ L. These steps were iterated for all human samples, encompassing 12 non-demented subjects and 15 Alzheimer's disease patients. For qPCR analysis, a SYBR GREEN mix was prepared, with each well of the qPCR plate containing a total of 15 microliters, including 12.5 μ L of SYBR GREEN (BioRad, 1708882), 1.25 μ L of Reverse Primer, and 1.25 μ L of Forward Primer. This mix was vortexed and centrifuged. Standards for calibration were established, with Standard 1 representing the cDNA pool at 10 nanograms/microlitre. Standard 2, Standard 3, and Standard 4 were prepared as 4-fold dilutions of the preceding standard. Dilutions were carried out using sterilised water. Subsequently, 10 μ L of either standard or prepared samples were added to the 15 μ L of SYBR GREEN mix, ensuring each well of the qPCR plate contained 25 μ L. The qPCR plate was then subjected to a specific program using a Real-Time qPCR detection system (BioRad, CFX96TM Optics Module Real-Time System). The programme used was as follows: the reaction mixture underwent an initial denaturation step at 95°C for 3 minutes. Subsequently, a cycling program was initiated, comprising denaturation at 95°C for 15 seconds, annealing at 65°C for 45 seconds, and extension at 72°C for 15 seconds. This cycling sequence was repeated 39 additional times to ensure the amplification of target sequences. Following the amplification cycles, a final step was executed, involving a brief denaturation at 95°C for 10 seconds. To assess the specificity of the amplified products, a melt curve analysis was conducted. This involved a gradual increase in temperature from 65°C to 95°C, with an increment of 0.5°C every 5 seconds. The genes analysed were: KIF2A forward primer TGTGTGTGTAAGAAAACGACCA, reverse primer CCATTTCATTAGGAGCTGAGTCA; GAPDH forward primer GTCTCCTCTGACTTCAACAGCG, reverse primer ACCACCCTGTTGCTGTAGCCAA; and human β -actin (ACTB) forward primer CACCATTGGCAATGAGCGGTTC, reverse primer AGGTCTTTGCGGATGTCCACGT. The housekeeping genes GAPDH and ACTB were used to normalize RNA expression³¹. KIF2A relative expression was calculated using the Pfaffl method.

Primary culture and A β 42 oligomers preparation

The primary culture was performed using an adapted protocol from³². Briefly, cortex and hippocampus from E15.5 CD1 wild-type embryos were carefully dissected in chilled L15

medium (Gibco #21083027) supplemented with glucose (6 g/L, Sigma), glutamine (2 mM, Gibco #25030081), and penicillin–streptomycin (1×, Gibco #15140122). The cortical and hippocampal explants were incubated in 500 µL of differentiation medium DMEM/F12 (Gibco #12634010), glucose (6 g/L Sigma), glutamine (2 mM, Gibco #25030081), penicillin–streptomycin (1×, Gibco #15140122), N2 (1:100, Gibco #17502048), FBS (5%, Gibco), and mechanically dissociated by repeated pipetting to isolate individual cells. A total of 250 000 cells/well were incubated on Poly-ornithine- (100µg/mL, Sigma # P8638) coated wells (Corning® Falcon® Cell Culture Plate # CLS353225) and maintained in a sterile incubator at 37 °C and 5% CO₂. After 2 hours of incubation at 37 °C, the medium was replaced by 1000µL of fresh Neurobasal (NB) medium NB (Gibco # 21103049), glucose (6 g/L Sigma), glutamine (2 mM, Gibco), penicillin–streptomycin (1×, Gibco), B27 (1:50, Gibco # 17504044), serum-free. The medium was replaced every two days by 1000µL of fresh NB medium at 37 °C. After 15 days in vitro (DIV), cells were incubated with a medium containing Aβ₄₂ oligomers or control medium for 1, 3 or 24 hours and cells were collected for western blot. For primary cell extraction, the medium was removed for later analysis, and the wells were washed three times with cold sterile PBS. 50µl of RIPA lysis buffer (ThermoFisher #89900), with Protease/Phosphatase Inhibitor Cocktail (Cell signaling #5872) was added per well and cells were collected using a cell scraper (Greiner # 541070).

Aβ₄₂ oligomer preparation was performed as previously described³³. Briefly, lyophilized recombinant Aβ₄₂ peptide (rPeptide, cat# A-1163-2) was resuspended to 5mM in DMSO (ThermoFisher, #85190) and stored at -20°C. To generate oligomers, recombinant Aβ₄₂ peptide was diluted to 100 µM in cold, 4 mM HEPES pH 8, and incubated on ice at 4 °C for 24 h. To prepare the medium with Aβ, Aβ oligomers were diluted in NB medium at a final concentration of 1µM. The same amount of DMSO was diluted in 4 mM HEPES pH 8 to prepare control medium.

Data analysis and image processing

Images were captured with a digital camera coupled with a Laser Scanning confocal microscope (Olympus Fluoview FV1000). Figures were prepared using Adobe Photoshop and Adobe Illustrator CC 2019, and 2D mosaic reconstructions were produced when needed using the

Photomerge tool of the Photoshop software package. Cell counting was conducted manually using Fiji software (ImageJ). Cell quantification and western blot data and graphs were constructed using Prism 9 (GraphPad, San Diego, CA, United States) software. A minimum of 3 animals per genotype was used for all analyses and quantifications. The exact sample size is specified in the Figure legends. For each genotype, a mean value was calculated from 3 sections. Error bars represent the standard error of the mean (SEM). We performed a Shapiro-Wilks test to evaluate the distribution of the data. We then used a two-tailed Student's t-test when the data followed a normal distribution and the Mann-Whitney test when it did not (n.s. not significant, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$). Outliers were calculated with the GraphPad outlier calculator and the positive outliers were discarded from the analysis.

Results

Conditional deletion of KIF2A in mature neurons leads to progressive neurodegeneration in mice

KIF2A expression and function during development have been extensively studied²⁰⁻²⁵. However, little information is available regarding the expression dynamics and function of KIF2A in the mature brain. To evaluate whether KIF2A protein levels change over time, we performed western blot analysis from cortex and hippocampal protein extracts from wild-type mice at different time points. At embryonic day E15.5, KIF2A was present in different isoforms (Fig. S1A,³⁴). In adult stages, only the full-length isoform was detected (Fig. S1B, C). KIF2A expression level peaked around 6 months in both cortex and hippocampus (Fig. S1B-D). To analyse the consequences of KIF2A deletion in the mature brain, we designed a mouse model in which the gene can be deleted in a temporal manner²⁵. Mice expressing an inducible Cre (*CreERT2*) under the promoter of *CamKIIa* (calcium/calmodulin-dependent protein kinase II alpha, expressed, mainly, in the cerebral cortex, hippocampus, and hypothalamus) were crossed with *Kif2a^{F/F}*. We injected both *CamKII-cKO* (*CamKII-CreERT2;Kif2a^{F/F}*) and *CamKII-control* mice (*Kif2a^{F/F}* littermates), with tamoxifen (TAM) for 5 consecutive days starting from postnatal day 40 (P40) to delete KIF2A in mature glutamatergic neurons and analysed the cortex and

hippocampus. We confirmed the proper recombination by analysing the relative amount of KIF2A 1 month and 2 months after tamoxifen injections (Fig. S2). For cortical analysis, we selected the somatosensory area (S1) as a representative region of the cerebral cortex. One month after TAM injections, we did not observe any difference in cortical thickness or the number of cortical neurons (Fig. 1A, B). However, there was a noticeable reduction of the cortex starting two months after injection ($-16.7\% \pm 0.87$, $P=0.016$; Fig. 1A, B). The reduction was more pronounced after four months ($-35.2\% \pm 5.18$, $P=0.0095$) and plateaued by 6 months ($-38.1\% \pm 15.45$, $P=0.0002$) (Fig. 1A, B). We observed a concomitant reduction in the number of NeuN positive cells at 2 months ($-18.94\% \pm 2.74$, $P=0.0005$), 4 months ($-28.6\% \pm 5.35$, $P=0.006$), and 6 months after TAM injections ($-47.22\% \pm 6.33$, $P=0.0007$) (Fig. 1C, D). In the hippocampus, we found a similar progressive reduction in size accompanied by neuronal loss in mutant mice compared with control littermates (Fig. 1E). Together, these results underscore the important role of KIF2A in neuronal survival in the mature cortex and hippocampus.

KIF2A conditional KO mice exhibit behavioural defects associated with loss of dendritic spines at pre-degenerative stages

To assess whether KIF2A deletion affects cognitive functions, we performed behavioural tests at pre-degenerative stages (i.e., 1 month after TAM injection). In the open field test, *CamKII-cKO* mice travelled a longer distance ($+76 \pm 21.7\%$ $P=0.0014$, Fig. 2A) and avoided the central area ($-52.56 \pm 17.6\%$, $P=0.0067$; Fig. 2A, B) compared with control littermates, suggesting hyperactivity and anxiety-like behaviours. For learning and memory, we did not observe any difference in the Y-maze test or the novel object recognition test, evaluated with an inter-trial interval of 50 min (Fig. 2C, D). However, mutant mice needed more time to find the hidden platform in the Morris water maze (Fig. 2E, F), suggesting a deficit in spatial learning and reference memory, but not in short-term working and recognition memories. Given that spatial reference memory is linked to the number and maturation of dendritic spines^{35,36}, we evaluated the density and characteristics of dendritic spines in control and mutant mice *in vivo*. We crossed *CamKII-Ctrl* and *CamKII-cKO* mice with the transgenic line *Thy1-YFP*, which labels pyramidal neurons of the cortical layer V and hippocampus²⁷ (Fig. 2G). Neurons with downregulated

KIF2A expression exhibited an overall lower density of dendritic spines compared with control littermates in the cortex 1 month after tamoxifen injections ($-30.2 \pm 10.5\%$, $P=0.028$; Fig. 2G, H). Additionally, we observed a significant increase in the ratio of immature stubby and filopodia-like spines in mutant mice (Stubby: $+34.3 \pm 13.5\%$, $P=0.0438$, Filopodia: $+70.8 \pm 17.9$, $P=0.0075$; Fig. 2G, H) and a slight increase in dendritic spine length ($+34.6 \pm 15.7\%$) but no difference in spine width (Fig. 2H). Compared to the cortex, the dendritic spines were more numerous and shorter in the hippocampus, with thin spines being the most predominant. We found a significant reduction in the dendritic spine density in the mutant compared to control hippocampus ($-22\% \pm 4.1$, $P=0.0018$; Fig. 2G, I). A slight increase in filopodia-like spine's ratio was also observed ($+1.806 \pm 0.6\%$ in mutant mice, $P=0.011$; Fig. 2G, I) and no difference in dendritic spine length or width (Fig. 2I). These results suggest that KIF2A is necessary for dendritic spine formation and maturation and its deletion in mature neurons leads to hyperactivity and impaired spatial learning in mice.

KIF2A modulates endogenous Tau phosphorylation

KIF2A regulates MT dynamics, and *KIF2A* KO neurons exhibit an increase in stable MT at the expense of dynamic MT²⁵. Given the relationship between MT (over) stability and Tau (hyper) phosphorylation⁶, we analysed the phosphorylation status of Tau in *KIF2A* cKO mice one month after TAM injections. Mutant mice showed a significant reduction in the total amount of Tau compared to control littermates ($-27.8 \pm 9\%$, $P=0.004$; Fig. 3A, B and Fig. S3). Analysis of the ratio of phosphorylated Tau (p-Tau) to total Tau revealed a significant increase in p-Tau at Thr181 ($62.5 \pm 10\%$, $p<0.0001$) and Ser422 ($39.4 \pm 14\%$, $P=0.011$) residues (Fig. 3A, C and Fig. S3). However, when normalized to the protein load control GAPDH, we observed a significantly increased of p-Tau only in epitope Thr181 ($+24.2 \pm 9\%$, $P=0.014$) (Fig. 3A, C and Fig. S3). Two months after Tamoxifen injections, we observed a more prominent decrease in total Tau in *Kif2a*-cKO mice ($-42.5 \pm 7.1\%$, $p<0.0001$; Fig. 3D, E and Fig. S4A, B). The phosphorylation status of Tau was enhanced, particularly at Ser422 ($+120 \pm 42\%$, $P=0.0007$), Thr181 ($113 \pm 27\%$, $P=0.0004$) and Thr231 ($148 \pm 30\%$, $p<0.0001$) when normalized to total Tau, and at Thr181 ($+52 \pm 22.3\%$, $P=0.0296$) and Thr231 ($+23.9\%$, $P=0.0173$) when normalized to GAPDH (Fig. 3D, F and Fig. S4A, B). To untangle the signalling pathway involved in p-Tau alterations, we analysed the protein levels of the kinases ERK1/2 and AKT,

which are implicated in Tau phosphorylation³⁷. We detected a significant increase in total ERK1/2 and phospho-ERK1/2 (ERK1 +54.3 ± 8.1%, $p < 0.0001$; ERK2 +23 ± 6.9, $P = 0.0031$; p-ERK1 +91.5 ± 18.2%, $p < 0.0001$; pERK2 +49 ± 19.9%, $P = 0.0208$; Fig. 3G and S4C) indicating a gain in ERK activity. The total amount of the phospho-AKT did not change (Fig. 3H). Interestingly, the ratio of p-AKT to total AKT was increased in mutant mice (+45.8 ± 15.1%, $P = 0.0063$; Fig. 3H), suggesting an increase in the activation status of the protein (Fig. 3H and Fig. S4D). These results suggest that KIF2A deletion affects the composition of MT, which in turn triggers endogenous Tau phosphorylation.

KIF2A deletion triggers the aggregation of mutated hyperphosphorylated Tau

In humans, aberrant Tau hyperphosphorylation led to NFT, a causative factor for neurodegeneration. However, mice do not develop NFT, likely due to the sequence and isoform differences between humans and mice^{38,39}. To analyse the consequence of KIF2A downregulation in aberrant Tau hyperphosphorylation, we crossed two different *Kif2a* mutant mice with the PS19 transgenic line, a tauopathy mouse model that expresses the mutant human Tau with the P301S mutation²⁹. First, we analysed whether an increase in mutant human Tau (h-Tau) in the mouse brain affects KIF2A levels. We performed western blot analysis in 6-month-old PS19 and WT animals in the cortex and hippocampus, and we did not find any difference in the relative amount of KIF2A in PS19 brains compared with control littermates (Fig. S5). Then, we assessed if hemizygous reduction of KIF2A expression, using *Kif2a*^{+/-} mice, is sufficient to impact aberrant human Tau phosphorylation. We analysed the relative amount of total human-Tau (h-Tau) and human phosphorylated-Tau (h-p-Tau) at different epitopes including AT8 antibody (p-Ser202/Thr205 Tau), a well-known marker of human hyperphosphorylated-Tau, in the soluble and insoluble fraction of hippocampal lysates from 6-month-old PS19 and PS19-*Kif2a*^{+/-} mice (Fig. 4A and Fig. S6). *PS19;Kif2a*^{+/-} mice had no changes in the amount of total human Tau in the soluble or insoluble fractions compared to *PS19* mice (Fig. S6A-D). However, we found a significant increase in the relative amount of human p-Tau at Thr231, Thr181 and Ser422 epitopes in the soluble fraction (Fig. S6A, B). In the insoluble protein fraction, we did not detect any difference between *PS19* and *PS19;Kif2a*^{+/-} mice in the relative amount of p-Tau at

any of the analysed epitopes when they were normalized to GAPDH (Fig. S6C, D), indicating that 50% reduction of KIF2A levels is not sufficient to trigger an increase in insoluble Tau phosphorylation in PS19 mice. We asked whether a greater reduction of KIF2A in PS19 mice would impact insoluble hyperphosphorylated Tau. We crossed the PS19 line with *CamKII* mutant mice (PS19-*CamKII*-Ctrl and PS19-*CamKII*-cKO) and analysed the phosphorylation status of Tau in 6-month-old control and mutant mice, which received tamoxifen injections only one month earlier (at 5 months), ensuring no apparent neuronal death. We did not find any difference in the relative amount of total human Tau in the soluble or insoluble fractions from the hippocampus of PS19-*CamKII*-cKO mice (Fig. 4B-F and Fig S6E, F). However, we observed a significant increase of human Tau phosphorylation at Thr231 ($+113 \pm 50.7\%$, $P=0.0463$) and Ser413 ($+170 \pm 39.9\%$, $P=0.001$) in the soluble fraction and at Ser202/Thr205 ($+234 \pm 52.2\%$, $P=0.0008$), Thr181 ($+86 \pm 21.4\%$, $P=0.0017$) and Ser413 ($+75.1 \pm 28.2\%$, $P=0.0205$) in the insoluble fraction, indicating enhanced hyperphosphorylation of Tau when KIF2A is deleted in mature neurons (Fig. 4B, D, E, G and Fig. S6E, F). These results suggest that a substantial reduction in neuronal KIF2A levels increases Tau pathology in a transgenic mouse model of Tauopathy.

KIF2A expression is considerably downregulated in AD patients but not in other Tauopathies

To analyse KIF2A levels in the brain of patients suffering from different Tauopathies, we first assessed the relative amount of α -tubulin in brain soluble protein extracts. Patients with frontotemporal lobe degeneration (FTLD), Corticobasal degeneration (CBD) and Pick's disease (PiD) presented no changes in the relative amount of α -tubulin (Fig. S7A-C, E). In contrast, AD patients showed a significant reduction ($-47\% \pm 13.3$, $P=0.017$; Fig. S7D, E)⁴⁰ consistent with the reported reduction of MT in pyramidal neurons⁴¹. Given that KIF2A levels would depend on the amount of MT, we analysed the relative amount of KIF2A normalized to α -tubulin. KIF2A was reduced in AD patients but not in patients with other Tauopathies ($-68.36 \pm 7.5\%$, $p<0.0001$; Fig. 5A-D, S7A-D). We also analysed the relative amount of KIF2A in relation to protein loading control (GAPDH) and found a reduction of 84% in AD patients compared with control individuals (Fig. 5E, $p<0.0001$). We measured the relative amount of KIF2A transcripts by RT-

1 *qPCR* and detected a marked reduction in *KIF2A* mRNA in AD patients ($-73.12\% \pm 18.7$,
 2 $P=0.0007$; Fig. 5F). We then analysed *KIF2A* expression in males and females. The relative
 3 amount of *KIF2A* did not change between men and women in the control group (Fig. 5G), but
 4 interestingly, we found a *KIF2A* reduction in AD-affected females (Fig. 5H; $-44.9\% \pm 32.9$,
 5 $P=0.196$), even though this difference was not statistically significant. Finally, we investigated
 6 the relationship between age and *KIF2A* expression and did not detect any significant correlation
 7 in control individuals, AD patients (Fig. 5I, J), or patients with other Tauopathies (Fig. S7F-H).
 8 These results indicate that *KIF2A* levels are specifically downregulated in AD patients as
 9 compared to controls.

11 **Accumulation of A β 1-42 oligomers reduces *KIF2A* levels in neurons**

12 Our results above show a specific reduction of *KIF2A* protein levels in AD patients. While
 13 aberrant Tau hyperphosphorylation is a common feature of all Tauopathies, only AD patients
 14 present additional accumulation and aggregation of the amyloid beta peptide (A β). To investigate
 15 plausible relationships between A β and *KIF2A*, we analysed *KIF2A* levels in 5xFAD mice, a
 16 well-characterized model of amyloid pathology which exhibits increased production of A β 42 and
 17 amyloid plaque formation²⁸. Public transcriptomic datasets from this model⁴² uncovered a mild
 18 decrease in *KIF2A* expression in the cortex at 8 months, and a prominent reduction of *KIF2A* in
 19 the hippocampus of mutant mice starting from 8 months (Fig. S8A, B). We assessed *KIF2A*
 20 protein levels in 12-month 5xFAD mice and control littermates and observed a reduction of
 21 *KIF2A* in the cortex (Cx) and hippocampus (HC) of mutant mice when the amounts were
 22 normalised to the protein loading control GAPDH (Cx: $-21.8 \pm 4.7\%$, $P=0.0003$; HC: $-11.7 \pm$
 23 5% , $P=0.0371$; Fig. S8C, D). However, 5xFAD mice displayed a reduction in the amount of α -
 24 tubulin in the cortex ($-23.9\% \pm 4$ p<0.0001; Fig. S8C) but not in the hippocampus (Fig. S8D).
 25 When we normalized the amount of *KIF2A* to the amount of α -tubulin, we observed a significant
 26 reduction only in the mutant hippocampus compared with control mice ($-10.46 \pm 4.4\%$,
 27 $P=0.0297$; Fig. 6A, B). To evaluate whether acute exposition to toxic A β oligomers affects
 28 *KIF2A* levels in neurons, we performed primary culture from wild-type embryonic hippocampus
 29 and cortex and added A β 42 oligomers or control vehicle to the medium after 15 days in vitro

(DIV) (Fig. 6C). Neurons were cultured in control medium or medium supplemented with 1 μ M A β 42 oligomers for 1, 3 and 24 hours before being collected for analysis (Fig. 6C, D). We did not observe any change in the amount of total MT, as indicated by stable α -tubulin levels (Fig. 6E, F). However, the relative amount of KIF2A to α -tubulin was reduced after 1 hour ($-23.6 \pm 18.5\%$; $P=0.235$), 3 hours ($-23.8 \pm 14.6\%$; $P=0.185$), and 24 hours ($-41.84 \pm 10.4\%$; $P=0.0069$) in neurons exposed to A β 42 oligomers compared to vehicle-treated neurons (Fig. 6E, F). These results suggest that A β affects KIF2A expression in neurons at transcriptional and translational levels.

Discussion

Deregulation of MT dynamics by KIF2A disruption is associated with loss of dendritic spines and cognitive decline

Dendritic spines are protrusions in the dendrites of neurons where synapses are formed. The development and maturation of dendritic spines depend on actin filaments and the entry of dynamic MT, which in turn is regulated by neuronal activity⁴³. Dynamic MT are important for head and length formation promoting the maturation of dendritic spines⁴⁴. Knocking down the +TIP protein EB3 impairs dendritic spine formation and morphology, increasing the percentage of filopodia-like spines⁴⁵⁻⁴⁷. EB3 is implicated in MT dynamics regulating the binding of other +TIPS and MAPs^{48,49}. KIF2A knockdown causes a reduction in +TIP proteins EB3 and CLASP1 and in EB3 positive comets movement, indicating a decrease in MT extension^{23,25}. We believe this is the main cause of dendritic spine reduction in *Kif2a* cKO mice. Moreover, the percentage of immature dendritic spines (filopodia and stubby-like) is increased in the mutant cortex indicating impaired spine maturation efficiency. Loss of dendritic spines is directly associated with deficits in spatial learning and precedes cognitive decline in neurodegenerative diseases^{35,36,50,51}. A β accumulation decreases dynamic MT entry in dendritic spines and was proposed to cause synapse loss at early stages of AD⁵². EB3 overexpression rescues the dendritic spine loss caused by A β toxicity⁴⁷. Moreover, increasing MT tyrosination prevents the loss of dendritic spines induced by oligomeric A β in neuronal primary culture⁵³. Overall, these

data suggest a direct relation between reduced EB3 levels, decreased dynamic MTs in dendritic spines, and synapse loss in AD. We propose that a reduction of MT depolymerization due to KIF2A deletion decreases dynamic MT entry in dendritic spines, lowering dendritic spine density and maturation and thereby impacting learning and memory in mice.

Linking KIF2A to MT stabilization and Tau phosphorylation

It has been broadly believed that Tau stabilises MT. However, this view has been challenged in the last few years and evidence pointed to the opposite scenario⁵. Tau binds preferably the labile domain of MT preventing their stabilization and promoting MT assembly⁷. In Tauopathies, Tau is hyperphosphorylated averting its binding to MT and forming intraneuronal NFT, a causative factor of neurodegeneration. Interestingly, AD brains present more stable MT characterized by an increase in polyglutamylated-tubulin, delta 2-tubulin and acetylated tubulin^{40,53,54}. Whether enhanced MT stabilization is a causal factor in Tau phosphorylation and NFT formation remains unclear. Previous studies revealed that stabilization of dynamic MT increases Tau phosphorylation and Tau-dependent loss of dendrites^{6,55}. MT destabilization exerts the opposite effect, reducing Tau phosphorylation at AT8 and PHF-1⁵⁶. We have previously reported an increase in polyglutamylated-tubulin and acetylated tubulin in *Kif2a*-KO brains, and a concomitant decrease in +TIPS proteins EB3 and CLASP1, indicating a reduction of MT dynamics in the absence of KIF2A²⁵. In the current study, we observed an increase in endogenous phospho-Tau when KIF2A was deleted in adult mouse brains, suggesting a link between MT stabilization and Tau phosphorylation likely mediated by ERK1/2 activation. ERK1/2, are kinases involved in many biological functions including phosphorylation of Tau⁵⁷. ERK1/2 have been associated with Tau phosphorylation in AD^{37,58,59}. To analyse whether the reduction of KIF2A impacts hyperphosphorylated-Tau aggregates, we crossed the transgenic mouse line PS19 (expressing mutated human Tau) with two different *Kif2a* mutant mice. Heterozygous reduction of KIF2A did not increase in insoluble p-Tau. However, homozygous conditional loss of KIF2A in mature cortical glutamatergic neurons of *PS19* mice dramatically increased the phosphorylation of insoluble Tau at epitopes Ser202/Thr205 (AT8), pSer413 and Thr181, suggesting that only a marked reduction of KIF2A levels can increase tangle-like aggregates in *Tau* transgenic mice. These results are in line with our observations in human brain tissue, which showed a reduction of KIF2A protein levels by approximately 84%, likely

contributing to Tau hyperphosphorylation and NFT formation in AD patients. Interestingly, Genome-wide association studies linked KIF2A rare variants to higher AD risk in humans⁶⁰. We did not find a decrease in KIF2A protein levels in the brains of patients suffering from Tauopathies with no evidence of A β accumulation such as FTLD, CBD or PiD. These diseases are classified as primary Tauopathies in which Tau pathology is the main neuropathological landmark. In contrast, AD is considered as a secondary Tauopathy since Tau aggregation is downstream of other factors such as A β deposition as posited by the amyloid cascade hypothesis⁸. Therefore, the mechanisms by which Tau is hyperphosphorylated and aggregated can be different. In primary Tauopathies, alterations in the Tau gene (*MAPT*) or in activity of Tau kinases and phosphatases are often linked to Tau deposition and neurodegeneration⁶¹. In AD, Tau pathology is not directly caused by mutations in *MAPT* but by the accumulation of A β oligomers. The aberrant production and accumulation of A β -42 triggers a molecular cascade, including KIF2A downregulation, contributing to aberrant Tau hyperphosphorylation and aggregation. The Different Tau hyperphosphorylation mechanisms results in variations in the pathological features, such as the predominant Tau isoform involved, the Tau filament structure, and the spatiotemporal pattern of neuronal Tau deposition⁶².

AB accumulation and MT dynamics

An important question in AD aetiology is whether and how the accumulation of A β leads to Tau hyperphosphorylation. Previous studies suggested that in the presence of A β 42 oligomers, Tau detaches from MT and is more prompt to phosphorylation⁶³. A possible explanation is that the accumulation of A β , either directly or indirectly, affects MT composition, which in turn triggers Tau hyperphosphorylation. The exposition of neurons to A β reduces MT dynamics and increases the levels of stable MT independently of Tau^{6,12,14}. Moreover, A β exposition reduces MT catastrophe indicating a decline in MT dynamics⁶. Increasing MT stabilization would reduce the dynamic domain of MT, where Tau binds MT, and promote MT-unbound Tau and Tau hyperphosphorylation⁶⁴⁻⁶⁶. We found that A β -42 oligomers reduce the relative amount of KIF2A related to MT in cultured neurons. In 5xFAD mice, which exhibit elevated A β -42 production, there is a reduction of KIF2A at transcriptomic and protein levels, although this reduction is only significant in the hippocampus. How A β could impact KIF2A levels is not known. In dividing cells, A β inhibits different kinesins, such as motor kinesins Eg5 and KIF4A and the MT

depolymerizing kinesin KIF2C (MCAK), leading to aneuploidy associated with AD pathology⁶⁷. In addition, A β can bind MT-associated proteins and degrade MAP1A, MAP1B, and MAP2^{68,69}.

Overall, this study showed that A β decreases KIF2A expression in neurons and that KIF2A downregulation affects MT function leading to Tau hyperphosphorylation and neurodegeneration. Hence, our results identify KIF2A dysfunction as an important molecular event in the pathological cascade that links A β deposition to Tau hyperphosphorylation and aggregation.

Data availability

Mouse lines and other resources used in the study are available through material transfer agreements. All data generated during this study have been included in the manuscript and supplementary material.

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Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Conditional deletion of KIF2A in mature neurons leads to progressive neurodegeneration. (A) Representative images of Nissl-stained coronal sections of CamKII-control (Ctrl) and CamKII-cKO mice (cKO). Oranges lines depict the measured S1 thickness. (B) Quantification of the S1 thickness at 1 month (1M) (CamKII-Ctrl: $1257 \pm 3.6 \mu\text{m}$ n=4; CamKII-cKO $1262 \pm 50.9 \mu\text{m}$ n=3, $P=0.906$) 2 months (2M) (CamKII-Ctrl: $1182 \pm 19.9 \mu\text{m}$ n=5; CamKII-cKO $985.1 \pm 10.3 \mu\text{m}$ n=4, $P=0.016$), 4 months (4M) (CamKII-Ctrl: 1210 ± 13.7

μm $n=6$; CamKII-cKO $784.6 \pm 62.65 \mu\text{m}$ $n=4$, $P=0.0095$) and 6 months (6M) (CamKII-Ctrl: $1226 \pm 35 \mu\text{m}$ $n=7$; CamKII-cKO $758.5 \pm 77.5 \mu\text{m}$ $n=3$, $P=0.0002$) after Tam injections. **(C)** Number of NeuN-positive cells per 390- μm wide stripes at 1 month (CamKII-Ctrl: 1206 ± 26 $n=4$; CamKII-cKO $1114 \pm 102 \mu\text{m}$ $n=3$, $P=0.355$) 2 months (CamKII-Ctrl: 1179 ± 20.3 $n=4$; CamKII-cKO $956 \pm 25.2 \mu\text{m}$ $n=4$, $P=0.0005$), 4 months (CamKII-Ctrl: 1214 ± 28.8 $n=3$; CamKII-cKO $866.4 \pm 58.3 \mu\text{m}$ $n=3$, $P=0.0059$) and 6 months (CamKII-Ctrl: 1248 ± 46.5 $n=4$; CamKII-cKO $658.6 \pm 67.9 \mu\text{m}$ $n=3$, $P=0.0007$) after TAM injections. **(D, E)** NeuN immunostaining in S1 (D) and the hippocampus (E) of control and cKO mice. Scale bars (A) 1mm, (D) 100 μm and (E) 200 μm . Data are represented as mean $\pm$ SEM. Values were obtained by unpaired Student's t-test for S1 thickness at 1M and 6M and NeuN quantification and Mann-Whitney test for S1 thickness at 2M and 4M since data did not follow a normal distribution; n.s., not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Figure 2 KIF2A cKO mice present cognitive decline and loss of dendritic spines. (A) Graphs showing the travelled distance (CamKII-Ctrl: $8311 \pm 728.3 \text{ cm}$ $n=15$; CamKII-cKO $14630 \pm 1800 \text{ cm}$ $n=11$, $P=0.0014$) and time in the field's central area (CamKII-Ctrl: 230.8 ± 29.7 seconds $n=14$; CamKII-cKO 109.5 ± 25.9 seconds $n=11$, $P=0.0067$) in the open field test. **(B)** An illustrative example of mice movement in the open field test. **(C)** Y-maze test to assess short-term spatial working memory (CamKII-Ctrl: $69.5 \pm 2.6 \%$ $n=15$; CamKII-cKO $67.9 \pm 4.6 \%$ $n=10$, $P=0.757$) **(D)** Novel object recognition (NOR) test with an inter-trial interval (ITI) of 50 min to assess short-term recognition memory. The data is represented as the percentage of time the mice spend recognizing the familiar object (FO) and the new object (NO) (CamKII-Ctrl: FO $35.6 \pm 6.2 \%$, NO $64.4 \pm 6.2 \%$ $n=14$ $P=0.0029$; CamKII-cKO FO $32.2 \pm 4.7 \%$ NO $67.8 \pm 4.7 \%$ $n=12$, $P<0.0001$). **(E)** Morris water maze (MWM) test. The time latency is the needed time for mice to locate the escape platform in seconds (sec) for each training day; day 1 (CamKII-Ctrl: 44.1 ± 3.6 ; CamKII-cKO 55.9 ± 2.1 , $P=0.0236$), day 2 (CamKII-Ctrl: 22.3 ± 3.7 ; CamKII-cKO 47.4 ± 3.7 , $P<0.0001$), day 3 (CamKII-Ctrl: 21.2 ± 3.4 ; CamKII-cKO 40.6 ± 4.5 , $P=0.002$), day 4 (CamKII-Ctrl: 13.9 ± 1.8 ; CamKII-cKO 28.7 ± 3.9 , $P=0.0017$) $n=13$ for CamKII-Ctrl and $n=14$ for CamKII-cKO. **(F)** An illustrative example of mouse track plots in the MWM **(G)** Representative images of dendritic spines from pyramidal neurons of the cortical layer 5 (L5) and the CA1 region of the hippocampus labelled with YFP in control and mutant adult mice. **(H)** Quantification of the number of dendritic spines per μm in the cortex (CamKII-Ctrl: 1.35 ± 0.09

n=36 dendrites from 4 animals; CamKII-cKO: 0.94 ± 0.11 ; n=33 dendrites from 4 animals, $P=0.0279$) the percentage of dendritic spine morphology (Thin-like: CamKII-Ctrl 62.1 ± 4.1 %; CamKII-cKO 43.5 ± 7.1 %, $P=0.0643$; Mushroom-like: CamKII-Ctrl 24 ± 3.9 %; CamKII-cKO 22.8 ± 3.6 %, $P=0.824$; Stubby-like: CamKII-Ctrl 7.2 ± 1.2 %; CamKII-cKO 11 ± 0.9 %, $P=0.0438$; Filopodia-like: CamKII-Ctrl 6.7 ± 0.8 %; CamKII-cKO 22.7 ± 4 %, $P=0.0075$) dendritic spine length (CamKII-Ctrl 1.23 ± 0.07 ; CamKII-cKO 1.57 ± 0.15) and width (CamKII-Ctrl 0.53 ± 0.05 ; CamKII-cKO 0.59 ± 0.07) n=694 and 536 dendritic spines from 4 control and 4 mutant mice, respectively. (I) Quantification of the number of dendritic spines per μm in the hippocampus (HC) (CamKII-Ctrl: 1.99 ± 0.05 n=45 dendrites from 4 animals; CamKII-cKO: 1.55 ± 0.07 n=39 dendrites from 4 animals, $P=0.0018$) the percentage of dendritic spine morphology (Thin-like: CamKII-Ctrl 87.4 ± 1.5 %; CamKII-cKO 79.7 ± 3.8 %, $P=0.0749$; Mushroom-like: CamKII-Ctrl 7.6 ± 1.4 %; CamKII-cKO 8.6 ± 1.2 %, $P=0.686$; Stubby-like: CamKII-Ctrl 5 ± 0.6 %; CamKII-cKO 10 ± 2.2 %, $P=0.0695$; Filopodia-like: CamKII-Ctrl 0 %; CamKII-cKO 1.8 ± 0.6 %, $P=0.0113$) dendritic spine length (CamKII-Ctrl 0.80 ± 0.04 ; CamKII-cKO 0.88 ± 0.12) and width (CamKII-Ctrl 0.44 ± 0.02 ; CamKII-cKO 0.46 ± 0.04) n=680 and 642 dendritic spines from 4 control and 4 mutant mice respectively). Scale bar (G) $5 \mu\text{m}$. Data from the NOR test for CamKII-cKO and MWM day 1 did not follow a normal distribution, and we performed the Mann-Whitney test. The rest of the values were obtained by unpaired Student's t-test; n.s., not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Figure 3 KIF2A deletion in the adult cortex triggers endogenous Tau phosphorylation. (A-C) Western blot analysis and quantification of the relative amount of Total Tau (Tau-1) (B) and phosphorylated Tau at different epitopes (C) in cortical extracts of control (CamKII-Ctrl) and Kif2a-cKO mice (CamKII-cKO) one month after tamoxifen injections at P40 (TAM P40-1M) and normalized with Tau-1 and GAPDH (n = 12 for CamKII-Ctrl and n=11 for CamKII-cKO). **(D-F)** Western blot analysis and quantification of the relative amount of Total Tau (Tau-1) (E) and phosphorylated Tau at different epitopes (F) in cortical extracts of control and Kif2a-cKO mice two months after tamoxifen injections at P40 (TAM P40-2M) and normalized with Tau-1 and GAPDH (n = 12 for each genotype). **(G, H)** Western blot analysis and quantification of the relative amount of phospho-ERK1/2 (G) and phospho-AKT normalized with GAPDH and phospho-AKT normalized with total AKT (H) in cortical extracts of control (CamKII-Ctrl) and Kif2a-cKO mice (CamKII-cKO) two months after tamoxifen injections at P40 (n = 12 for each

genotype). The levels in the control group were set as 1. Data are represented as mean $\pm$ SEM. Values were obtained by unpaired Student's t-test; n.s., not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Dashed lines separate non-adjacent bands. Original full blots are displayed in Supplementary Figures 3 and 4.

Figure 4 Conditional deletion of KIF2A triggers Tau hyperphosphorylation in the hippocampus. (A) Schematic illustration showing the extraction of soluble (Lysis buffer) and insoluble Tau fractions (Urea buffer) fractions and from hippocampal lysates. (B-G) Western blot analysis and quantification of the relative amount of total human Tau (h-Tau-1) and different phospho-human-Tau (p-h-Tau) normalized with GAPDH in hippocampal lysates from PS19 CamKII-Ctrl (n=8) and PS19 CamKII-cKO mice (n=6) 1 month after tamoxifen injections at 5 months in the soluble fraction (B-D) and the insoluble fraction (E-G). The levels in the control group were set as 1. Data are represented as mean $\pm$ SEM. Values were obtained by unpaired Student's t-test; n.s., not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Dashed lines indicate non-adjacent bands. Full blots are displayed in Supplementary Figure 6.

Figure 5 KIF2A expression is downregulated specifically in Alzheimer's disease patients. (A-D) Western blot analysis and quantification of the relative amount of KIF2A normalized with α -Tub from frontotemporal lobe degeneration (FTLD) (n= 13) (A), Corticobasal degeneration (CBD) (n=4) (B), Pick's disease (PiD) (n=5) (C) Alzheimer's disease (AD) (n=15) (D) and control (n=12) cortical extracts. (E) Western blot quantification of the relative amount of KIF2A normalized with GAPDH from Alzheimer's disease (AD) (n=15) and control cortical extracts (n=11). (F) KIF2A mRNA levels determined by qPCR analysis from AD (n=13) and control cortical extracts (n=12). For analysis in A-F, the levels in the control group were set as 1. (G, H) Comparative analysis between KIF2A relative amount in men and women in the control group (G) and AD group (H). (I, J) Correlation of KIF2A protein levels and age in the control (I) and AD group (J). Data are represented as mean $\pm$ SEM. Values were obtained by unpaired Student's t-test; n.s., not significant, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$. Full blots are displayed in Supplementary Figure 7.

Figure 6 KIF2A expression is downregulated in the presence of A β -42 oligomers. (A, B) Western blot analysis and quantification of the relative amount of KIF2A normalized with α -

Tubulin in wild-type mice (WT) (n=8) and 5xFAD mice (n=9) in the cortex (Cx) and hippocampus (HC). **(C)** Schematic illustration of the experimental design in which embryonic wild-type neurons after 15 days in vitro (DIV) were incubated with a control vehicle or 1 μ M A β -42 oligomers, and cells were collected after 1, 3- or 24-hours of exposition. **(D)** Western blot analysis of the presence of A β -42 high molecular weight (MW) and low MW oligomers in the control and A β culture medium. **(E, F)** Western blot analysis **(E)** and quantification **(F)** of the relative amount of α -Tubulin and KIF2A in neurons from primary culture incubated with 1 μ M A β -42 oligomers or control medium for 1 hour (α -Tub $P=0.792$; KIF2A $P=0.235$; n=6 embryos), 3 hours (α -Tub $P=0.677$; KIF2A $P=0.185$; n=6 embryos) and 24 hours (α -Tub $P=0.209$; KIF2A $P=0.007$; n=4 embryos). The levels in the control group were set as 1. Data are represented as mean $\pm$ SEM. Values were obtained by unpaired Student's t-test; n.s., not significant, * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Full blots are displayed in Supplementary Figure 8.

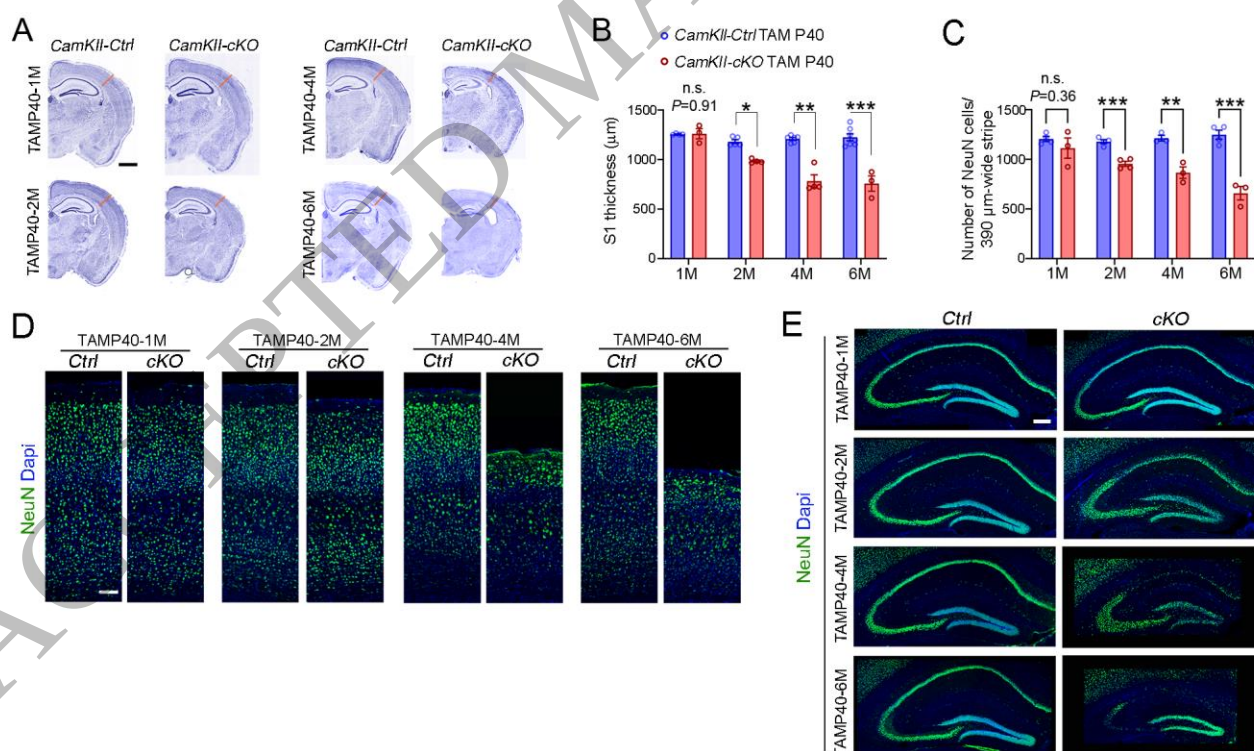


Figure 1
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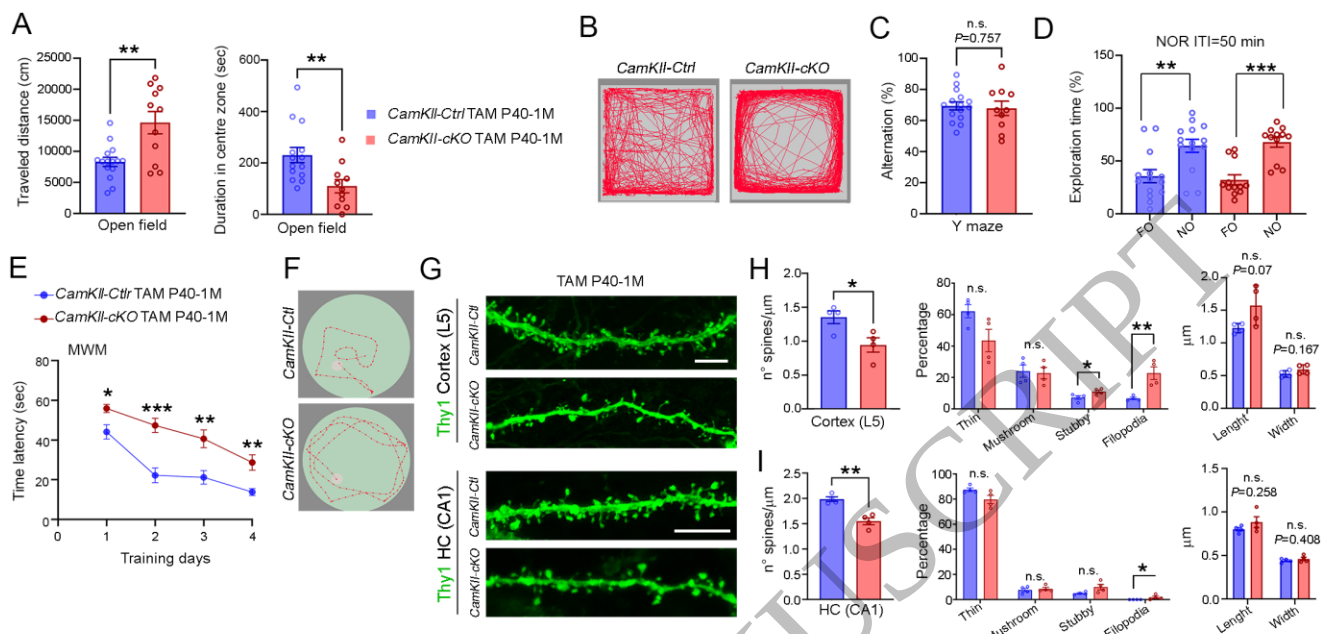


Figure 2
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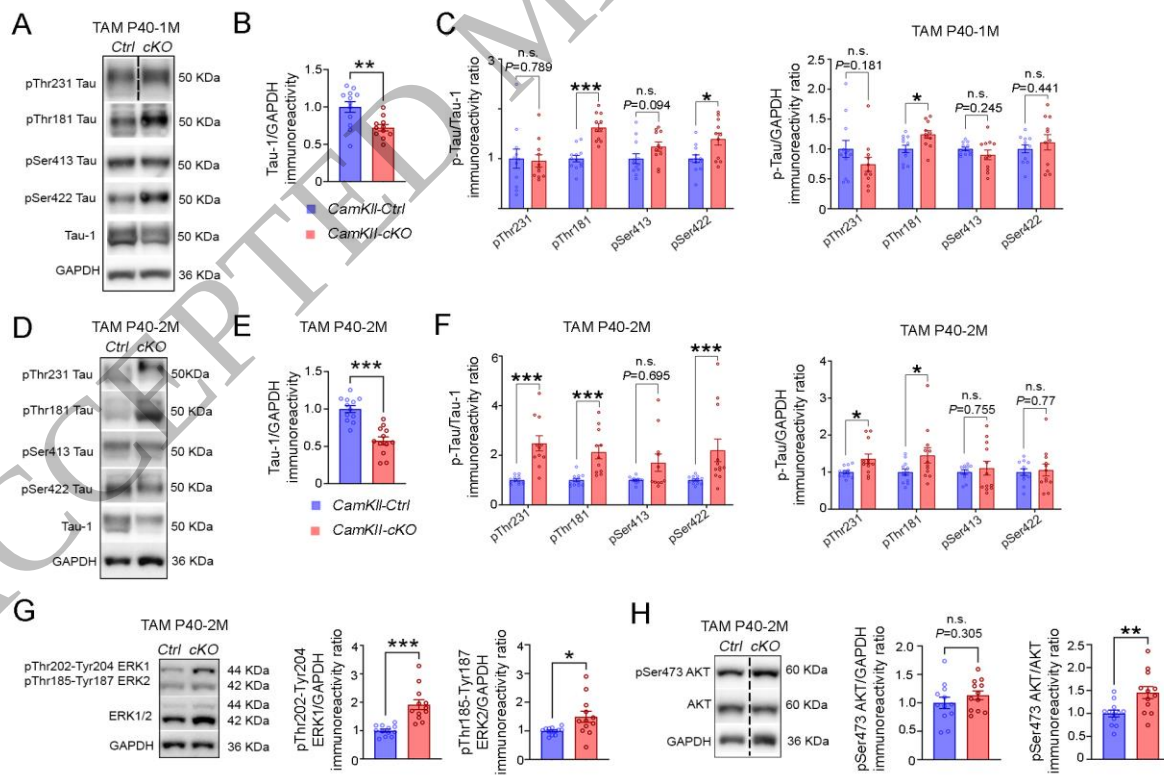


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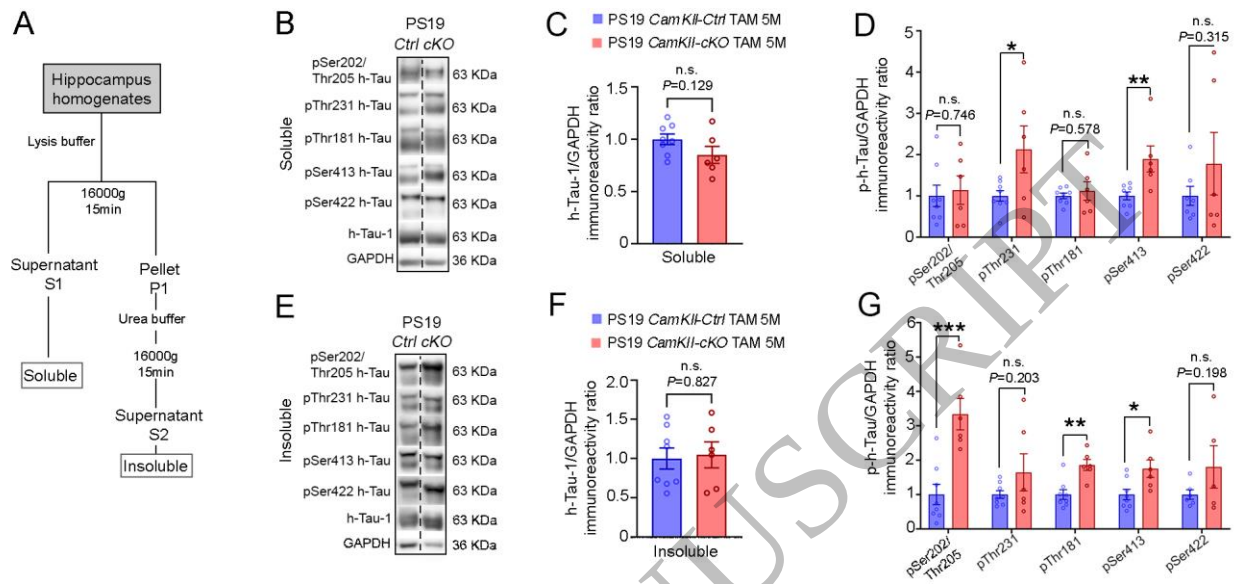


Figure 4
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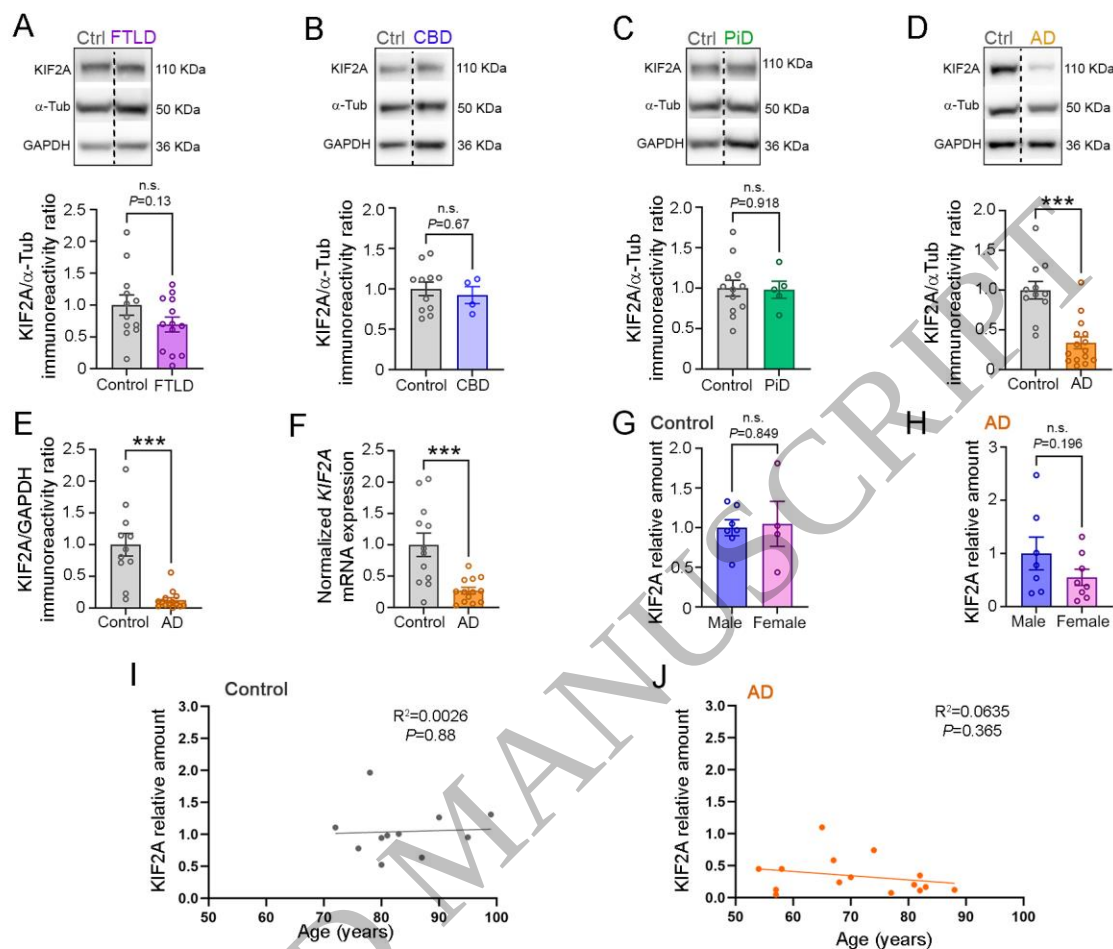


Figure 5
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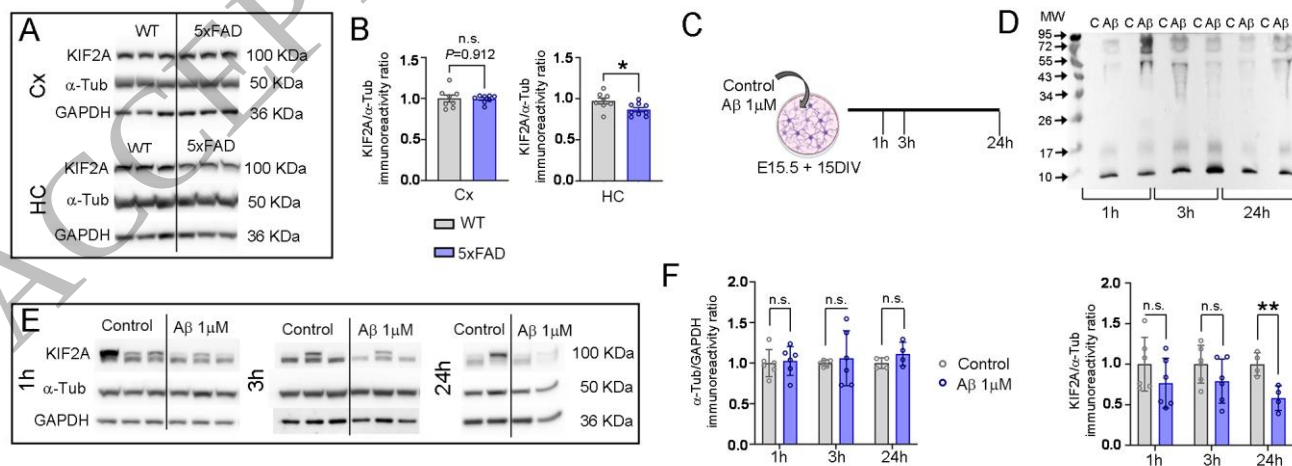


Figure 6
185x64 mm (x DPI)