Disruption of tubulin-alpha4a polyglutamylation prevents aggregation of hyper-phosphorylated tau and microgliactivation in mice

Torben Johann Hausrat✉1, Philipp C. Janiesch1, Petra Breiden1, David Lutz2,6, Sabine Hoffmeister-Ullerich3, Irm Hermans-Borgmeyer4, Antonio Virgilio Failla5 & Matthias Kneussel1✉

Dissociation of hyper-phosphorylated Tau from neuronal microtubules and its pathological aggregates, are hallmarks in the etiology of tauopathies. The Tau-microtubule interface is subject to polyglutamylation, a reversible posttranslational modification, increasing negative charge at tubulin C-terminal tails. Here, we asked whether tubulin polyglutamylation may contribute to Tau pathology in vivo. Since polyglutamylases modify various proteins other than tubulin, we generated a knock-in mouse carrying gene mutations to abolish Tuba4a polyglutamylation in a substrate-specific manner. We found that Tuba4a lacking C-terminal polyglutamylation prevents the binding of Tau and GSK3 kinase to neuronal microtubules, thereby strongly reducing phospho-Tau levels. Notably, crossbreeding of the Tuba4a knock-in mouse with the hTau tauopathy model, expressing a human Tau transgene, reversed hyper-phosphorylation and oligomerization of Tau and normalized microgliactivation in brain. Our data highlight tubulin polyglutamylation as a potential therapeutic strategy in fighting tauopathies.

1Department of Molecular Neurogenetics, Center for Molecular Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany. 2Department of Structural Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany. 3Bioanalytics Facility, Center for Molecular Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany. 4Transgenic Animal Unit, Center for Molecular Neurobiology, ZMNH, University Medical Center Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany. 5Microscopy Imaging Facility, University Medical Center Hamburg-Eppendorf, Martinistraße 52, 20251 Hamburg, Germany. 6Present address: Department of Neuroanatomy and Molecular Brain Research, Ruhr University Bochum, 44801 Bochum, Germany. ✉email: torben.hausrat@zmnh.uni-hamburg.de; matthias.kneussel@zmnh.uni-hamburg.de
A growing number of neurodegenerative diseases summarized as tauopathies are linked to the dysfunction of Tau, a microtubule-associated protein (MAP) also known as MAP-T1-4. They include Alzheimer’s disease (AD), corticobasal degeneration, or frontotemporal dementia, which are characterized by improper accumulation of hyperphosphorylated Tau, known as neurofibrillary tangles (NFTs) or Pick bodies. Tau accumulations are accompanied by microglia activation, which potentially induces gliosis and neurodegeneration in brain8-10. While microglial activation follows the aggregation of phosphorylated Tau in tauopathy models8, proinflammatory cytokines, released by activated microglia, contribute to Tau hyper-phosphorylation and pathology9. Glycogen synthase kinase-3 (GSK3), also associated with microtubules, is a major kinase catalyzing Tau phosphorylation10-12. Upon hyper-phosphorylation, axonal Tau detaches from microtubules, however, the physiological mechanisms that regulate their interaction are still incompletely understood5.

The microtubule cytoskeleton represents a complex and dynamic structure in eukaryotes, regulating a diversity of cellular functions. Microtubules maintain the structure of cells, form spindles in mitosis and meiosis and provide the tracks for intracellular transport12,13. In neurons, representing polarized and excitable cells, microtubules contribute to the formation and maintenance of axons and dendrites, regulate the polarized delivery and removal of cargos over long distances and transiently invade into actin-rich dendritic spines in a neuronal activity-dependent manner14-17. At the molecular level, microtubules are composed of alpha- and beta-tubulin isotypes that heterodimerize in a 1:1 stoichiometry18. Tubulin dimers polymerize into protofilaments in a GTP-dependent manner, with typically 13 protofilaments assembling into a hollow tube structure, representing the microtubule. So far, eight genes encoding alpha-tubulins and eight genes encoding beta-tubulins have been described, which are differentially expressed during development and in different brain regions to form functional microtubules12,19,20.

Microtubule-associated proteins (MAPs) regulate microtubule organization by different mechanisms and/or modulate the binding and function of interacting proteins21-23. For instance, Tau is a microtubule bundler that stimulates growth and inhibits shrinkage of microtubules24 through regulation of their labile domain25.

Microtubules are subject to posttranslational modifications (PTMs) such as phosphorylation, acetylation, de-tyrosination, polyglucination or polyglutamylation26-31. Polyglutamylation at tubulin C-termini differentially regulates the interaction of microtubules with MAPs in vitro32, modifies the function of severing enzymes or motor proteins33-35 and potentially alters dendritic and axonal transport33,36. Polyglutamylation is catalyzed in a reversible manner through a variety of TTL-type glutamylation (for tubulin tyrosine ligase-like) and CCP-type de-glutamylation (for cytoplasmic carboxypeptidase), respectively37. However, not all TTL members are glutamylases. In fact, TTL3, TTL8, and TTL10 represent polyglucylases whereas the specific function of TTL12 is presently unknown37. Several attempts to investigate the functional role of tubulin polyglutamylation have been limited by the fact that TTLs modify various protein substrates other than tubulin. This includes the nucleosome assembly proteins NAP1 and NAP2, the nucleophosmin B23, the microtubule +TIP protein EB1, the myosin light chain kinase (MLCK), and the retinitis pigmentosa GTPase regulator (RPGR)38-42. Consequently, loss-of-function approaches with respect to TTL enzymes lead to overlapping effects that are unspecific.

In order to unravel the functional role of tubulin polyglutamylation in the mouse brain, we developed a substrate-specific genetic approach. Generating a knock-in mouse that carries C-terminal Tuba4a point mutations (Tuba4aΔpolyGlu), we interfered with polyglutamylation specifically at Tuba4a-containing microtubules in vivo. Neuronal Tuba4a isoforms contain the longest polyglutamy side chains across the tubulin family43. The gene expression pattern of Tuba4a significantly increases at adult stages, leading to the most abundant levels of polyglutamylation, as compared to other tubulins43,44. Notably, Tuba4a is the only tubulin that lacks a C-terminal tyrosine residue and is therefore independent of another reversible tubulin PTM, known as de-tyrosination.

Our data show that the binding of Tau and GSK3 kinase to microtubules containing mutant Tuba4a is severely decreased, with Tau remaining in a hypo-phosphorylated state. This finding was unexpected, since pathologic Tau aggregating in tauopathies is strongly hyper-phosphorylated. Strikingly, crossbreeding of Tuba4a knock-in mutants with a tauopathy model, expressing a human Tau transgene, diminishes Tau pathology and microglia activation. Our data provide mechanistic insights into the Tau-microtubule interface and highlight isotype-specific polyglutamylation of tubulin as a potential molecular target to develop treatment strategies against tauopathies.

Results

Generation of knock-in mice characterized by a specific loss of Tuba4a C-terminal polyglutamylation. In order to investigate tubulin polyglutamylation in a substrate-specific manner, we eliminated the respective target sites at the C-terminal tail of a unique tubulin substrate. To this end, we generated gene-targeted knock-in mice, carrying a group mutation (glutamate (E) to aspartate (D)) in the mouse Tuba4a gene, encoding alpha4a-tubulin (Tuba4a) (Fig. 1A–C, green tails and Supplementary Fig. 1A, B). Gene-targeted mice were crossed with flip-Deleter and Cre-deleter mice to generate constitutive knock-in mutants (Supplementary Fig. 1C), hereafter referred to as Tuba4aΔpolyGlu. Southern blotting at the ES cell stage (Supplementary Fig. 1D–E) and a long-range PCR over the 3′integration site (Supplementary Figs. 1C, F) confirmed the correct insertion at the targeting vector. This result was verified using genomic DNA from knock-in mouse tissue (Supplementary Fig. 1G, H).

Microtubule-associated proteins (MAPs) regulate microtubule organization by different mechanisms and/or modulate the binding and function of interacting proteins21-23. For instance, Tau is a microtubule bundler that stimulates growth and inhibits shrinkage of microtubules24 through regulation of their labile domain25.

Several attempts to investigate the functional role of tubulin polyglutamylation have been limited by the fact that TTLs modify various protein substrates other than tubulin. This includes the nucleosome assembly proteins NAP1 and NAP2, the nucleophosmin B23, the microtubule +TIP protein EB1, the myosin light chain kinase (MLCK), and the retinitis pigmentosa GTPase regulator (RPGR)38-42. Consequently, loss-of-function approaches with respect to TTL enzymes lead to overlapping effects that are unspecific.
chains, is a major substrate of polyglutamylation enzymes. At the same time, other tubulin PTMs remain unaffected in Tuba4a mutants (Supplementary Fig. 2A–E). In neuronal cultures, detection of Tuba4a and polyGlu tubulin over in vitro development (DIV1-20), revealed a gradual increase in polyglutamylated tubulin (polyGlu, (+/+) Fig. 2E–G) that was also significantly reduced in neurons from homozygous Tuba4a mutants (polyGlu, (p/p), Fig. 2E–G). In line with this, reduced tubulin polyglutamylation in Tuba4a mutants was also detectable by immunostaining of hippocampal slices derived from adult mice (Fig. 2H, I), while Tuba4a signal intensities remained unchanged (Supplementary Fig. 2F). Furthermore, using an

**Fig. 1 Development of a knock-in mouse, carrying point mutations to preclude polyglutamylation of Tuba4a.** A Microtubules assemble from heterodimers of alpha- and beta-tubulin. C-terminal tubulin tails are subject to different posttranslational modifications (PTMs). B, C To preclude Tuba4a polyglutamylation at glutamate residues (green circles), 7 final glutamate residues (red circles) are genetically substituted by aspartate (blue circles). PTMs of other tubulin isotypes remain unaffected. D, E DNA sequencing. Amino acid substitutions are indicated (red letters). F Representative Nissl staining of coronal brain sections from adult mice, derived from three independent experiments. Scale bar, 1 mm. G, H Magnification of hippocampal regions shown in (F). Scale bar, 250 μm. I–K Immunohistochemical analysis of cortical layering based on NeuN and CTIP2 in cortical brain sections derived from 1-year-old Tuba4aΔpolyGlu (+/+), (+/p) and (p/p) mice. Scale bar, 100 μm. Quantification of (J) individual cortical layer thickness and (K) CTIP2 signal intensities normalized to the area analyzed, as indicated. n = 3 mice per genotype. L, M Open field test to study locomotor activity in 6-month-old Tuba4aΔpolyGlu (+/+), (+/p) and (p/p) mice. No differences regarding latency to fall over 5 trials and mean latency to fall over 5 trials on the rotarod between genotypes. Main effect for genotype: F(2,56) = 0.501; p = 0.608; Main effect for bins × genotype: F(10,280) = 0.408; p = 0.942; n(+/+), n(+/p) = 28, n(p/p) = 13, n(p/p) = 21. N, O Accelerating rotarod test. Latency to fall off the rod measures balance and motor learning. No differences regarding latency to fall over 5 trials and mean latency to fall over 5 trials on the rotarod between genotypes. Main effect for genotype: F(2,58) = 0.549; p = 0.610. Two-sided unpaired Student’s t-test (J, K) and ANOVA (M, O) and two-way repeated measure ANOVA (L, N) were used to assess statistical significance. Data represent mean ± SEM. Source data, including exact p-values, are provided as a Source Data file.
Fig. 2 The Tuba4a mutation reduces overall tubulin polyglutamylation levels but is still incorporated into microtubules. A Relative Tuba4a mRNA expression levels. (+/+) set to 1 (gray dotted line) in adult mice. Median (center), interquartile range (bounds of boxes), and minima and maxima (whiskers) are indicated. n = 11 samples per genotype. Two-sided alternative hypothesis (H1) testing was used to assess statistical significance. B Representative western blot analysis depicting tubulin polyglutamylation (polyGlu), Tuba4a, total alpha-tubulin, NSE and Actin in adult Tuba4a+/+ (+/+), (+/p) and (p/p) mice. Please note that the remaining levels of polyglutamylation are due to tubulins other than Tuba4a. C, D Quantification of Tuba4a normalized to NSE (tubulin-independent loading control) (C) and polyglutamyalted tubulin normalized to total alpha-tubulin (D) signal intensities shown in (B). (+/+) set to 100%. C n = 4 (+/+) and p/p), 3 (+/p) and D n = 5 (+/+) and p/p), 4 (+/p) experiments per genotype. E Developmental expression of polyGlu, Tuba4a, Tubb3, and Actin in hippocampal neurons at different DIV, as indicated. F, G Quantification of Tuba4a (F) and polyglutamylated tubulin (G) signal intensities shown in (E) over time. Two-way ANOVA (DIV × genotype) with p = 0.2 (Tuba4a) and p = 0.0004 (polyGlu), n = 3 independent cultures per genotype per time point. H Immunohistochemical analysis of Tuba4a (green) and polyGlu (greyscale or blue) in CA1 region of hippocampal brain sections derived from 12-month-old Tuba4aΔpolyGlu (+/+) and (p/p) mice. Scale bar, 50 µm. I Quantification of polyGlu normalized to the area analyzed, shown in (H), +/+ set to 1, n = 3 mice per genotype. J Representative coIP of Tuba4a and Tubb3 from hippocampal lysates derived from adult mice. In addition, polyglutamyalted tubulin levels were analyzed. K Quantification of signal intensities of precipitated polyGlu normalized to signal intensities of precipitated Tuba4a, shown in (J), n = 3 experiments. L Coinmunostaining of Tuba4a (green) and AnkyrinG (blue; axonal marker) using DIV14 neurons. n = 3 experiments. Scale bars, 20 µm (overview), 5 µm (soma), 2.5 µm (axon). M High-resolution immunogold electron microscopy (EM) of Tuba4a in the medulla oblongata (rich in parallel axons) from adult Tuba4aΔpolyGlu (+/+) and (p/p) mice. Micrographs on the left show magnifications of the white rectangles. The presence of myelin sheets (#) identifies axons. Note, the pearl necklace-like distribution of gold particles at microtubules. Scale bars, 500 nm (overview), 200 nm (magnification), n = 3 mice per genotype. Two-sided unpaired Student’s t-test (A, C, D, I, K) and ANOVA (F, G) were used to assess statistical significance. *p < 0.05, **p < 0.001. Data represent mean ± SEM, if not stated otherwise. Source data, including exact p-values, are provided as a Source Data file.
In contrast, the Tuba4a C-terminal mutations had no effect on microtubule dynamics is sensitive to Tuba4a polyglutamylation. 17 to 23 cells from three independent cultures per genotype: Axon, neuronal somata of both genotypes (Fig. 2L), confirming, expected, Tuba4a signals decorated microtubule dendrites and in Ankyrin-G (AnkG) -positive axons (Fig.2L). As tubulin. Using immunostaining, we detected Tuba4a in neuronal polyglutamylation is not a prerequisite to dimerize with beta-tubulin. This could be corroborated by Tuba4a signals in hippocampal neurons derived from Tuba4aΔpolyGlu (+/+ ) and (p/p) mice. Quantification of EB3-GFP growth length 24 h after transfection at DIV4 (A), DIV5 (B) and DIV12 (D). Representative kymographs acquired from dendrites at DIV5 are shown in (C). Upper panels: GFP signal, lower panels: tracked EB3-GFP. Analyzed comets from 19 to 24 cells from three independent cultures per genotype per DIV: DIV4, n(+/+ ) = 32, n(p/p) = 48; DIV5, n(+/+ ) = 49, n(p/p) = 53; DIV12, n(+/+ ) = 30, n(p/p) = 40. E-G Quantification of KIF5C motility (velocity in µm/s) in axons (E) and dendrites (G) at DIV13 using KIF5C without a cargo-binding domain fused to tdTomato and pex26 (peroxisome binding domain) expressed for 24 h in hippocampal neurons derived from Tuba4aΔpolyGlu (+/+ ) and (p/p) mice. Representative kymographs of KIF5C in dendrites are shown in (F). Analyzed tracks from 17 to 23 cells from three independent cultures per genotype: Axon, n(+/+ ) = 40, n(p/p) = 26; Dendrite, n(+/+ ) = 68, n(p/p) = 89. Two-sided Mann-Whitney test was used to assess statistical significance. ***p < 0.001. Data represent mean ± SEM. Source data, including exact p-values, are provided as a Source Data file.

Immunoprecipitation experiment to enrich Tuba4a from hippocampal brain lysate, we confirmed a strong reduction of polyglutamylation specifically for the precipitated tubulin isotypes (Fig. 2J, middle (p/p), 2 K). Notably, Tubb3 co-immunoprecipitated with Tuba4a using brain lysate from mutant (p/p) mice (Fig. 2I), lower), indicating that C-terminal Tuba4a polyglutamylation is not a prerequisite to dimerize with beta-tubulin. Using immunostaining, we detected Tuba4a in neuronal dendrites and in Ankyrin-G (AnkG) -positive axons (Fig. 2L). As expected, Tuba4a signals decorated microtubule filaments in neuronal somata of both genotypes (Fig. 2L), confirming the incorporation of wild-type and mutant Tuba4a into the microtubule polymer. This could be corroborated by Tuba4a immunogold electron microscopy, which labeled tubulin-alpha4a along individual microtubules of both genotypes at ultrastructural resolution (Fig. 2M). Together, we conclude that the Tuba4aΔpolyGlu mouse lacks tubulin polyglutamylation at Tuba4a C-termini (Figs. 1E and 2B, D, E, G–K), while mutant Tuba4a still dimerizes with beta-tubulin (Fig. 2J) and integrates into microtubules (Fig. 2L, M).

Loss of Tuba4a C-terminal polyglutamylation alters microtubule growth but not KIF5C-mediated transport. To ask whether polyglutamylation at the Tuba4a C-terminus regulates microtubule growth and/or kinesis-mediated transport, we performed neuronal live-cell imaging with cultured hippocampal neurons. The use of a fluorescent microtubule + TIP protein EB3 (EB3-GFP) revealed a significant reduction in the growth length and growth duration of microtubules, using neurons from Tuba4aΔpolyGlu (p/p) mice at stage DIV5 and DIV12 (Fig. 3B–D, Supplementary Fig. 3D–I). Consistent with Tuba4a being hardly expressed at earlier stages (compare with Fig. 2E, F), this effect was not detectable at DIV4 (Fig. 3A, Supplementary Fig. 3A–C). These data, therefore, suggest that the regulation of microtubule dynamics is sensitive to Tuba4a polyglutamylation. In contrast, the Tuba4a C-terminal mutations had no effect on KIF5C-mediated transport velocities along axonal or dendritic microtubules in mutant (p/p) neurons, following expression of a KIF5C-PEX construct that connects the motor to peroxisomes (Fig. 3E–G).

Analysis of MAP- and Tau-microtubule binding in the absence of Tuba4a C-terminal polyglutamylation. Previous biochemical in vitro studies had suggested that polyglutamylation acts like a rheostat regulating the affinity between tubulin and Tau or MAP232,46. To test this hypothesis in the Tuba4aΔpolyGlu mouse, we initially performed a soluble tubulin extraction assay using hippocampal neurons. Tau, MAP2a/b, and polyglutamylated tubulin (polyGlu) were prominent in the wildtype (+/+ ) in-soluble fraction enriched in polymerized microtubules (Fig. 4A, left). In contrast, neurons from mutant mice (p/p), characterized by a strongly reduced polyGlu signal (Fig. 4A, bottom right, compare with Fig. 2B and J), displayed a >50% reduction in Tau-microtubule (Fig. 4A top right and B) and MAP2-microtubule (Fig. 4A, C) co-occurrence in the in-soluble fraction. This result was even more striking in a microtubule pelleting experiment, following repolymerization of microtubules. Whereas Tau was highly abundant in pellets from wild-type (+/+ ) fractions, containing strong polyGlu signals (Fig. 4D left and E), Tau binding to microtubules was hardly detectable in the pellet of fractions derived from Tuba4a mutant (p/p) mice (Fig. 4D right and E), as characterized by weak polyglutamylation (Fig. 4D, bottom, right) but equal tubulin levels (Supplementary Fig. 4A). Likewise, MAP2a/b binding to microtubules was severely reduced under these conditions (Fig. 4D, F), whereas MAP1a binding to microtubules remained unchanged (Supplementary Fig. 4B, C), indicating a differential effect of Tuba4a C-terminal polyglutamylation on MAP/microtubule interaction. These effects were not due to changes in gene expression, since the relative expression levels of Tau, MAP2a/b and MAP1a remained equal in both genotypes (Fig. 4G–J and Supplementary Fig. 4D–G).

Immunocytochemical analysis revealed equal Tau levels in axons of wild-type and mutant neurons (Supplementary Fig. 4H, I), suggesting that the axonal localization of Tau is independent of Tuba4a polyglutamylation. In contrast, super-resolution STED
microscopy confirmed a loss of colocalization between endogenous Tuba4a and endogenous Tau at microtubule filaments. The signal intensities for the colocalization of both proteins were significantly reduced in neurons from Tuba4a mutant (p/p) mice, as compared to wild-type (+/+ -) controls (Fig. 4K (yellow signal), Fig. 4L-N, Supplementary Fig. 4J). In contrast, the signal intensities of Tuba4a (red channel) remained equal, confirming that Tuba4a itself was not downregulated (Fig. 4O). We, therefore, conclude that efficient Tau binding to microtubules requires a polyglutamylated Tuba4a C-terminus.

Analysis of Tau phosphorylation in the absence of Tuba4a C-terminal polyglutamylatation. The inability of Tau to efficiently bind and colocalize with non-polyglutamylated Tuba4a prompted us to analyze the Tau phosphorylation status, since Tau hyperphosphorylation and microtubule-detachment are hallmarks in Alzheimer’s disease (AD) and other tauopathies.2,47,48 Interestingly, western blotting with a pan Tau antibody, using hippocampal extracts derived from wild-type (+/+ -) or Tuba4aΔPolyGlu mutant (p/p) mice, led to the detection of different molecular weights of individual Tau variants with or without Tuba4a C-terminal polyglutamylatation, respectively (Fig. 5A). This result was due to changes in the phosphorylation status of Tau, since phosphatase treatment, resulting in the dephosphorylation of Tau, led to an equal pattern in both genotypes (Fig. 5B). Notably, application of a phospho-specific Tau

---

**Fig. 4 Loss of Tuba4a C-terminal polyglutamylatation significantly decreases Tau binding to microtubules.** A-C Soluble-tubulin extraction assay using DIV15 neurons derived from Tuba4aΔPolyGlu (+/+ -) and (p/p) mice. A Western blot. Soluble fraction (S): un-polymerized tubulin and dissociated MAPs. Insoluble fraction (Ins): polymerized tubulin (microtubules) and associated MAPs. Fractions were probed for pan Tau, MAP2a/b, total alpha-tubulin, and polyGlu. Quantification of pan Tau (B) and MAP2a/b (C) in-soluble/soluble levels. Ratios smaller than 1 indicate higher protein abundance in the insoluble fraction. n = 6 independent cultures per genotype. D-F Microtubule pelleting assay after repolymerization of hippocampal tubulin derived from adult mice. D Western blot. Supernatant (SN): un-polymerized tubulin and dissociated MAPs. Pellet (P): polymerized tubulin (microtubules) and associated MAPs. Fractions were probed for pan Tau, MAP2a/b, total alpha-tubulin and polyGlu. Quantification of pan Tau (E) and MAP2a/b (F) pellet/supernatant levels. Ratios smaller than 1 indicate higher protein abundance in the supernatant fraction. n = 3 mice per genotype. G-J Representative western blot analysis depicting Tau (G) and MAP2a/b (H) normalized to γ-Adaptin protein expression levels in the hippocampus. Respective quantifications are shown in (I, J). (+/+) set to 1. n = 5-7 experiments. K Representative super-resolution STED images of microtubules in axonal regions from DIV14 hippocampal neurons, derived from three independent experiments. Tuba4a (red), pan Tau (green). L M Line scans: relative intensities of signals along 1µm of microtubule length (boxed regions in (K)). Scale bar, 500 nm. Arbitrary units (arb. units). N Mean pan Tau signal intensities normalized to Tuba4a. n = 45(+/-), 60(p/p) axonal regions. O Total Tuba4a signal intensities. n = 45(+/-), 60(p/p) axonal regions. Two-sided unpaired Student’s t-test (B, C, E, F, I, J) and Mann–Whitney test (N, O) were used to assess statistical significance. *p < 0.05, **p < 0.01, ***p < 0.001. Data represent mean ± SEM. Source data, including exact p-values, are provided as a Source Data file.
antibodies AT8 and AT270, known to detect individual phospho-
residues within the protein (Supplementary Table 1), revealed 
significantly reduced Tau phosphorylation levels in Tuba4a 
mutant (p/p) fractions at both epitopes (Fig. 5C–E), whereas Tau 
extpression per se remained unaltered (Fig. 5C, pan Tau detection 
and Fig. 4G, I and Supplementary Fig. 4F). Together, these data 
suggest that a non-polyglutamylated Tuba4a C-terminus not only 
prevents Tau binding (Fig. 4), but also interferes with Tau 
phosphorylation.

Therefore, we analyzed two major Tau kinases GSK3α and β in 
wild-type (+/+) and Tuba4aΔpolyGlu mutant (p/p) mice. Protein 
expression levels of GSK3α and β were found to be 
equal in hippocampal lysates derived from either genotype 
(Fig. 5F, G, Supplementary Fig. 5A). On the other hand, 
atachment of GSK3α and β at microtubules was significantly 
reduced in a soluble-tubulin extraction assay derived from 
mutant (p/p) neurons (Fig. 5H, I, Supplementary Fig. 5B), an 
effect that was not due to changes in tubulin levels (Supple-
mental Fig. 5C, D). This result was confirmed using an independent 
 microtubule pelleting assay from the hippocampal lysate, 
following repolymerization of microtubules. In this experiment, 
we also detected significantly less GSK3β associated with 
microtubules, as compared to a tubulin loading control 
(Fig. 5I–L).

Together, our data reveal that the Tuba4a mutation in this 
study (Fig. 1A–E), which interferes with C-terminal tubulin

---

**Fig. 5 Loss of Tuba4a C-terminal polyglutamylation significantly decreases phosphorylation of mouse Tau and binding of GSK3β to microtubules.**

**A** Representative western blot analysis of pan Tau in hippocampal lysates (S1 extract) from adult Tuba4aΔpolyGlu (+/+ ) and (p/p) mice. 
**B** Representative western blot analysis of pan Tau in hippocampal lysates (S1 extract) after phosphatase treatment from adult mice. 
**C** Western blot analysis of Tau phosphorylation in hippocampal lysates (S1 extract) from adult mice. AT8 and AT270: phosphorylated Tau-specific antibodies. pan Tau: total Tau. polyGlu: polyglutamylated tubulin. GAPDH: loading control. The representative western blots shown in (A–C) are derived from three independent experiments. 
**D–E** Quantification of relative AT8 (D) and AT270 (E) signal intensities normalized to total Tau. (+/+ ) set to 100%. n = 3 experiments per genotype. 
**F** Western blot analysis of GSK3α/β in hippocampal lysates (S1 extract) from adult mice. NSE: loading controls. 
**G** Quantification of GSK3β signal intensities shown in (F). n = 3 mice per genotype. 
**H** Western blot. Soluble fraction (SN): un-polymerized tubulin and dissociated MAPs. Insoluble fraction (Ins): polymerized tubulin (microtubules) and associated MAPs. Fractions were probed for GSK3α/β and alpha-tubulin. 
**I** Quantification of GSK3β insoluble/soluble levels. 
**J–L** Microtubule pelleting assay following re-polymerization of hippocampal tubulin from adult mice. 

**Note:** Two-sided unpaired Student’s t-test was used to assess statistical significance. *p < 0.05, **p < 0.01. Source data, including exact p-values, are provided as a Source Data file.
polyglutamylation in mice (Fig. 2), significantly alters (i) Tau binding to microtubules (Fig. 4), (ii) Tau phosphorylation (Fig. 5A–E) and (iii) GSK3-microtubule interactions (Fig. 5H–L). They highlight the critical importance of Tuba4a polyglutamylation in regulating the Tau-microtubule interface in vivo.

Loss of Tuba4a C-terminal polyglutamylation leads to reduced phosphorylation levels of human Tau. To investigate whether the Tuba4a C-terminus may be a potential target sequence in developing treatment strategies against tauopathies, we aimed to study human Tau and its tendency to form hyper-phosphorylated oligomers, in Tuba4aΔpolyGlu (p/p) mice.

To this end, we virally expressed CFP-tagged human Tau (hTau40-CFP) in neurons derived from wild-type (+/+) or Tuba4a mutant (p/p) mice. Due to its CFP-tag, human Tau40 displayed a higher molecular weight (*), compared to endogenous mouse Tau (#) (Fig. 6A). Infected wild-type (+/+) and Tuba4a mutant (p/p) neurons expressed the hTau40-CFP fusion protein and mouse Tau at similar levels (Fig. 6A, lower pan-Tau detection, 6B and Supplementary Fig. 6A). In contrast, hTau40 phosphory-levels normalized to total hTau40-CFP levels were significantly reduced in Tuba4a mutant (p/p) neurons (Fig. 6A, upper AT8 detection, Fig. 6C), suggesting that the loss of Tuba4a C-terminal polyglutamylation might protect against hyper-phosphorylation of human Tau. Using immunocytochemistry, reduced Tau phosphory-levels could also be confirmed in axons of Tuba4a mutant (p/p) neurons expressing human Tau (hTau40-CFP). In wild-type neurons, Tau phosphorylation (red AT8 signals) in the axon was relatively high after hTau40-CFP expression, but significantly reduced in Tuba4a mutant (p/p) neurons under the same conditions (Fig. 6D, E).

Loss of Tuba4a C-terminal polyglutamylation normalizes Tau hyper-phosphorylation and its abnormal oligomerization in a tauopathy model. In a further approach, we crossbred wild-type (+/+) or Tuba4a mutant mice (p/p) with a tauopathy mouse model (hTau), known for its Alzheimer’s disease-like pathology, as characterized by the pathologic oligomerization of hyper-phosphorylated Tau in cell bodies and dendrites. Genetically, hTau mice represent homozygous knockouts for mouse Tau, carrying one allele of a human Tau40 transgene. As expected, hTau (dark gray) and hTau/Tuba4aΔpolyGlu (black) animals containing an hTau40 allele (+/+) overexpressed hTau40 in the hippocampus (Fig. 7A, Y9 detection) at equal levels (Supplementary Fig. 7H, I). Similar to Tuba4aΔpolyGlu (p/p) mice (Fig. 2D–I, orange conditions), hTau/Tuba4aΔpolyGlu animals were also characterized by significantly reduced polyglutamylated signal levels (Fig. 7D, E, black condition), whereas related tubulins or other tubulin PTM levels were equal across genotypes (Supplementary Fig. 7A–G). Strikingly, following crossbreeding of Tuba4aΔpolyGlu (p/p) mice with hTau tauopathy mice, the phosphorylation of Tau (AT8 and AT270) was significantly reduced in the resulting hTau/Tuba4aΔpolyGlu condition (Fig. 7A, black condition and Fig. 7B, C), as compared to the tauopathy hTau condition only (dark gray condition). We, therefore, conclude that the loss of Tuba4a C-terminal polyglutamylation (Tuba4aΔpolyGlu) is sufficient to normalize the hyper-phosphorylation Tau phenotype, characteristic of the hTau tauopathy model, in vivo.

To confirm this result, we stained cortical and hippocampal brain slices using the phosphorylated Tau-specific antibody AT8. According to the literature, we detected a significant increase in cortical cells containing hyper-phosphorylated Tau, using the tauopathy model (hTau), as compared to wild-type (WT) controls. This increase was most prominent in layer V of the cortex (Supplementary Fig. 8A, B, dark gray header). Notably, cells from hTau/Tuba4aΔpolyGlu mice (black header), lacking Tuba44a polyglutamylation, resembled those from wild-type mice (white header), indicating a significant normalization of the pathologic hTau phenotype in vivo (Supplementary Fig. 8A, B). We also analyzed phosphorylated Tau levels at higher magnification within neuronal somata. Cells in the tauopathy model (hTau), were characterized by prominent hyper-phosphorylation in perinuclear regions of their somata (Fig. 7F–H, Supplementary Fig. 9A–C, gray header), which was significantly lower in wild-
revealed a normal thickness of cortical layers (Supplementary Fig. 10), suggesting that at this stage of analysis neurodegeneration is not yet prominent.

We, therefore, asked whether the abnormal oligomerization of human hyper-phosphorylated Tau, often referred to as pre-tangles, a premature state of neurofibrillary tangles (NFTs)\(^5\), might also be normalized in hTau/Tuba4a\(\text{-}\)polyGlu mice. To this end, we applied TOMA-1 antibodies specific for the detection of oligomeric forms of Tau\(^3\). Similar to the normalization of hyper-phosphorylated Tau (Fig. 7F–H and Supplementary Fig. 9A–C), hTau/Tuba4a\(\text{-}\)polyGlu mice, lacking Tuba4a polyglutamylation revealed normalization of Tau oligomeric assemblies, as compared to hTau tauopathy mice (Fig. 8A–C). Human Tau oligomerization could further be confirmed in a western blot-based experiment using the hTau antibody. As expected, WT and mTauKO animals did not display any hTau expression (Fig. 8D, white and light gray condition), whereas hTau animals displayed bands at the higher molecular weight (MW) above 75 kDa (Fig. 8D, dark gray condition), indicative of Tau oligomers. Consistent with the TOMA-1 immunostainings (Fig. 8A–C), the intensity of these hTau-specific high MW signals, was significantly decreased in hTau/Tuba4a\(\text{-}\)polyGlu animals lacking Tuba4a polyglutamylation revealed (Fig. 8D, E, dark gray condition), indicative of Tau oligomers. Finally, the use of the TOMA-1 antibody in dot blot assays corroborated Tau oligomers in hTau animals (Fig. 8F–H, dark gray condition) that were significantly reduced in hTau/Tuba4a\(\text{-}\)polyGlu mice (Fig. 8F–H, black condition).

Together, our data point to Tuba4a polyglutamylation as a potential parameter to preclude the hyper-phosphorylation and oligomerization of Tau (Fig. 10).

**Fig. 7 Hyper-phosphorylation of human Tau in a tauopathy mouse model is normalized upon the loss of Tuba4a C-terminal polyglutamylation.**

A Western blot analysis of hTau phosphorylation using hippocampal lysates from 12-month-old mice. Tuba4a\(\text{-}\)polyGlu (+/−) and (p/p) mice were crossed with a tauopathy mouse model (hTau (+/+); mTau4a (d/d)). The mouse Tau knockout condition (mTauKO (d/d)) indicates antibody specificity. AT8 and AT270: phosphorylated Tau. Y9; total human tau. hTau positive area

B Quantification of phosphorylated Tau signal intensities, probed with AT8 (B) or AT270 (C), normalized to hTau, as shown in (A). hTau mice (dark gray condition) set to 1; (B) n = 5 experiments, C n = 3 (+/−), 6 (p/p) experiments.

D Western blot analysis depicting polyglutamylation tubulin (polyGlu). GAPDH: loading control.

E Quantification of polyGlu signal intensities normalized to GAPDH. hTau mice (dark gray condition) set to 1, n = 6 mice per genotype. Note, human Tau hyper-phosphorylation is normalized in Tuba4a (p/p) as compared to Tuba4a (+/+) derived hippocampal lysates. F Immunohistochemical analysis of subcellular phosphorylated Tau-positive accumulations in layer V of cortical brain sections derived from 12-month-old Tuba4a (+/+) and (p/p) mice crossed with hTau (+/+) mice. Wild-type mice not expressing hTau, served as controls. AT8 (red); phospho-Tau, DAPI (blue); nuclei. Scale bar, 10 μm.

G Legend indicating group (condition)/genotype assignment.

H Quantification of AT8-positive accumulations size per area analyzed, shown in (F). WT set to 1; n = 3 (WT), 4 (+/+) and (p/p) mice per genotype. Note, Tau hyper-phosphorylation is normalized in the genetic background of Tuba4a\(\text{-}\)polyGlu mice.

I Immunohistochemical analysis of subcellular Tau aggregates in layer V of cortical brain sections derived from 12-month-old Tuba4a (+/+) and (p/p) mice crossed with hTau (+/+) mice. Wild-type sections not expressing hTau, served as antibody control. hTau (green): total human Tau. DAPI (blue): nuclei. Scale bar, 10 μm.

J, K Quantification of hTau-positive accumulations size per area analyzed (J) and signal intensities per area (K) analyzed, shown in (I). WT set to 1; n = 4 mice per genotype. Two-sided unpaired Student’s t-test (B, E, J–K) and one-way ANOVA (H) were used to assess statistical significance. **p < 0.01, ***p < 0.001. Data represent mean ± SEM. Source data, including exact p-values, are provided as a Source Data file.
Loss of Tuba4a C-terminal polyglutamylation normalizes microglia activation in a tauopathy model. Microglia activation contributes to gliosis in response to neuronal damage and is a common pathological feature in tauopathies and AD, accompanied by the aggregation of hyper-phosphorylated Tau. As expected, the hTau tauopathy model displayed strong levels of and actively phagocytic cells. In contrast, hTau/Tuba4a mice revealed that activated microglial cells had formed long branching processes following a Sholl analysis in cortical layer V (Fig. 9C, E), turned out to be normalized upon the loss of Tuba4a C-terminal polyglutamylation.

It is therefore plausible to conclude that blocking polyglutamylation at the Tuba4a C-terminus protects neuronal tissue against the consequences of reactive microglia in a tauopathy model (Fig. 10).

Discussion

In this study, we aimed to investigate how polyglutamylation of tubulin affects microtubules and their downstream functions in neurons and the brain. Since the enzymes that reversibly catalyze polyglutamylation (TTLLs and CCPs) modify multiple substrates, genetic modification or functional inhibition of these proteins leads to a variety of unspecific effects. In the present study, we, therefore, applied a substrate-specific approach, generating a knock-in mouse to study the loss of polyglutamylation at a selected tubulin isotype. We identified C-terminal polyglutamylation of Tuba4a as a critical factor, in regulating the Tau-microtubule interface. Loss of Tuba4a C-terminal polyglutamylation impaired Tau phosphorylation and severely reduced Tau binding to microtubules. Following crossbreeding with a tauopathy mouse model, the Tuba4a mutation prevented hyper-phosphorylation and oligomerization of Tau and normalized microglia activation and gliosis within the cortex, a major indicator preceding neurodegeneration. These data point to Tuba4a polyglutamylation as a potential target in fighting tauopathies.

Eight individual isotypes of alpha- and beta-tubulin are described. They are differentially expressed during development and in individual brain regions, mediating unique isotype-specific functions. The unique identity of individual tubulin isotypes is extended through different posttranslational modifications, such as polyglutamylation, acetylation, and/or de-tyrosination. Our data support and extend this view providing a specific role of...
Tuba4a C-terminal polyglutamylation in regulating the microtubule-associated protein Tau under pathological conditions. Tuba4a is a major tubulin isotype, highly expressed in the postnatal nervous system. It is the only tubulin isotype that lacks a tyrosine residue at its very C-terminal end and is therefore independent of de-tyrosination, another tubulin PTM. Among the different tubulin isotypes expressed in the brain, Tuba4a carries the longest polyglutamyl side chains, which is a unique characteristic within the family of tubulins. Since TTLL enzymes are suggested to tolerate amino acid substitutions within tubulin C-termini and seem to modify neighboring glutamate residues instead, we substituted seven C-terminal glutamate residues in Tuba4a, as compared to Tuba4a (WT). Normalized CD68 / γ-Adaptin expression

**Fig. 9 Microglia activation and expansion in a tauopathy mouse model is normalized upon the loss of Tuba4a C-terminal polyglutamylation.**

A Western blot analysis of CD68 (marker for activated microglia) in cortical lysates derived from 12-month-old Tuba4a (WT) and Tuba4aΔpolyGlu mice crossed with a tauopathy mouse model (hTau (+/–)). Wild-type mice not expressing hTau, served as controls.

B CD68 signal intensities normalized to γ-Adaptin, shown in (A). Tuba4a (WT) set to 1; n = 4 mice per genotype. C Iba1-positive cells (marker for microglia), in cortical brain sections derived from 12-month-old Tuba4a (WT) and Tuba4aΔpolyGlu mice, crossed with hTau (+/–) mice. Wild-type mice not expressing hTau, served as controls. Upper panels: Iba1 (grayscale) within layer V, scale bar, 50 μm. Middle and lower panels: magnification of boxed regions in upper panels; Iba1 (green), CD68 (red); scale bar, 25 μm. Lower panels: Sholl analysis with 6 μm intervals (circles).

D Normalized cell number of Iba1-positive cells per area analyzed in cortical layer V. n = 6(WT), 8(+/+) and p/p) per genotype. E Quantification of Sholl analysis: the number of microglia branch intersections that occur from the soma in concentric circles was analyzed. Higher values reflect complex microglia processes, indicating activated microglia. Note that gliosis, as detectable in hTau cortical sections, is significantly reduced in the background of Tuba4aΔpolyGlu, as compared to Tuba4a (WT).

Observe a reduction but not a total loss of tubulin polyglutamylation in Tuba4aΔpolyGlu derived tissues. Early work suggested that proteolytic removal of the complete C-terminal tubulin tail by subtilisin decreases the mobility of kinesin and dynein motor proteins that move along such microtubules. In vitro studies with engineered chains of glutamate residues confirmed and extended this view, reporting differential effects on motor protein processivity. In fact, long chains of glutamate reduced the processivity of kinesin-1 but not kinesin-2, whereas dynein processivity was increased. Moreover, a cellular study reported that increased tubulin polyglutamylation, induced by synaptic activity, correlated with inhibition of kinesin-1, but not kinesin-3 processivity. These data were supported by reduced mitochondrial transport in a double knockout of the de-glutamylases CCP1/CCP6, which induced tubulin hyper-glutamylation. Although previous cellular and in vivo investigations suggest a functional relevance for the polyglutamylation of “tubulin”, none of them has in fact been substrate specific, taking into account that other subcellular and cytoskeletal regulators, such as the +TIP protein EB1 or the myosin light chain kinase (MLCK), also undergo polyglutamylation by the same family of enzymes. Genetic approaches at the substrate level, such as the present study, are therefore required to pinpoint open questions and to distinguish between overlapping effects.

Pathologic tubulin mutations are further linked to neurodevelopmental and neurodegenerative disorders summarized as tubulopathies. Among them, eight point-mutations in the Tuba4a gene have been associated with ALS in patients, connecting this tubulin isotype to neurodegeneration, however, none of them are located within the C-terminal tail affecting Tuba4a polyglutamylation. The relatively late expression of Tuba4a in neurons (Fig. 2H, I) matches with a potential role in late-onset disease.

Within the family of microtubule-associated proteins, neuronal Tau mainly binds to axonal microtubules. Mechanistically, Tau is thought to regulate the long labile domains of microtubules, stimulating their growth and inhibiting their shrinkage.
pathological conditions, hyper-phosphorylated Tau detaches from microtubules and forms aggregates, known as neurofibrillary tangles. However, it is controversially discussed, whether Tau accumulation represents a cause or consequence of tauopathies1–3. The binding site between Tau and microtubules and the regulation of their interaction has been characterized by liquid-liquid phase separation in solution using NMR and other biochemical methods67. Previous biochemical in vitro data suggested C-terminal tubulin polyglutamylation as a potential regulator of Tau-microtubule interactions32. Cryo-electron microscopy further characterized different full-length and truncated Tau constructs with respect to their microtubule affinity68. Based on these data, computational modeling predicts that Tau interacts with microtubules through four conserved tubulin-binding repeats that span over three tubulin monomers along a protofilament. Notably, the adjacent C-terminal tail of alpha-tubulin is located in a perfect position to regulate this interaction68. In combination with the results of the present study, it is plausible to conclude that Tuba4a polyglutamylation might be a critical determinant in this scenario. However, the loss of Tuba4a C-terminal polyglutamylation also reduced the binding of MAP2a/b to microtubules and both MT-associated proteins, MAP2a/b and Tau, have been shown to regulate microtubule dynamics24,62. In line with this, we observed a decrease in microtubule growth in Tuba4aΔpolyGlu derived neurons at the onset of Tuba4a expression. On the other hand, microtubule
They suggest that the Tau phosphorylation state, which to this effect,1 might contribute to the degeneration of motor axons. However, the specific loss of Tuba4a C-terminal polyglutamylation described in our study, revealed no effect on KIF5 velocities in dendrites or axons and did not induce neurodegeneration, suggesting that the regulation of kinesin-microtubule interactions might be mediated by specific tubulin isotypes other than Tuba4a. Alternatively, the depletion of TTTL1, which modifies substrates other than tubulin38–42, might lead to secondary effects that are presently unknown.

Hyper-phosphorylation is thought to induce Tau detachment from microtubules and its subsequent aggregation into cytosolic neurofilibrillary tangles. In the present study, our Tuba4aPolyGlu mouse was characterized by a major loss of Tau–microtubule binding, however, with Tau remaining in an un-phosphorylated state. The hyper-phosphorylation of Tau differs from pathological conditions characterized by hyper-phosphorylated Tau. Notably, in both settings, Tau binding to microtubules is markedly diminished. According to current knowledge, hyper-phosphorylation of Tau reduces its affinity for microtubules. Subsequently, abnormally hyper-phosphorylated Tau misfolds, leading to self-aggregation into NFTs. In the present study, analyzing hTau mice at one year of age, we detected oligomerization of hyper-phosphorylated Tau, but not yet aggregation of the protein. Consistent with the late onset of AD-like Tau pathology in this particular hTau mouse model, these observations likely reflect early disease conditions. In addition, reduced hTau expression due to a shortening of the cortical layer 5 (compare with Supplementary Figs. 8 and 11).

To gain a better understanding of the role of Tau in disease, many transgenic or knockout mouse models have been developed and studied over the years. Notably, a classical gene knockout of Tau revealed no severe phenotype, indicating that a functional loss of Tau is tolerated and most likely compensated through other MAPs. Our data, showing severely reduced Tau binding to microtubules without major developmental of functional deficits in the Tuba4aPolyGlu mouse, support this view. They suggest that the Tau phosphorylation state, which functionally depends on Tuba4a polyglutamylation, represents a critical parameter in disease progression and therefore can be reversed in the hTau/Tuba4aPolyGlu mouse model. However, since Tuba4a expression in neurons is relatively late, these effects seem to be most critical at adult physiological and pathophysiological conditions. In contrast, the phosphorylation of Tau, during early development might be regulated by mechanisms independent of Tuba4a polyglutamylation.

One of the early signs of neurodegeneration is the activation of microglia, which together with other glial cells, leads to a pathological condition, known as gliosis. In the healthy brain, microglia display a basal motility of their processes without moving their cell bodies. Neuronal damage activates microglia, leading to their proliferation with an increased mobility and extension of their processes that rapidly phagocytose harmful material. Synapse loss and microglial activation are accompanied by tangle formation in Tau pathology and microglia remove synaptic compartments of neurons with Tau pathology.

In addition, microglia activation might promote the propagation of Tau aggregates, leading to negative feedback. Other studies that involved the hTau animal model and colleagues reported gliosis prior to neurodegeneration and in line with the data of the present study, 5XFAD mice, representing an alternative AD mouse model, are characterized by a specific loss of layer 5 neurons, thereby connecting Tau hyperphosphorylation/oligomerization and activated microglia within cortical layer 5. Furthermore, pyramidal cells in layer 5 are characterized by high levels of amyloid beta, supporting the view that this cortex layer might be highly vulnerable.

Remarkably, the number and activity state of microglia in the present study is normalized in hTau/Tuba4aPolyGlu mice, as compared to hTau mice. Although we cannot exclude parallel Tuba4a expression by microglia, this indicates at the in vivo level that the loss of Tuba4a C-terminal polyglutamylation not only protects against Tau hyper-phosphorylation and its oligomerization, but further normalizes microglia activation.

Methods

Animals. Animals were maintained in the animal facility of the ZMNH, Hamburg (Germany) under controlled environmental conditions. Mice (both sexes were used for experiments) were group-housed (2–5 mice per cage) under a 12-hour light/dark cycle. Temperature (22 ± 1 °C) and humidity (50 ± 5%) in the animal facility were kept constant, and the animals had ad libitum access to food and water. All animal experiments complied with all ethical regulations for animal testing and research in accordance with the European Communities Council Directive (2010/63/EU) and were approved by the ethics committee of the city-state of Hamburg (Behörde für Justiz und Verbraucherschutz, Fachbereich Lebensmittelsicherheit und Veterinärwesen (reference (ID number) 100/13) and the animal care committee of the University Medical Center Hamburg-Eppendorf.

Generation of Tuba4aPolyGlu mice. To generate the Tuba4a knock-in mouse, a Cre-activated allele approach was used. Based on Tuba4a cDNA sequence NM_009447 the exon/intron organization of the gene was established. The following mutations were introduced: E433D, E434D, E441D, E443D, E445D, E447D, and E449D. A targeting vector was generated and used for homologous recombination in embryonic stem (ES) cells to modify the endogenous tuba4a gene (constructed by GenOway, Lyon, France) (Supplementary Fig. 1C). ES cell clones were screened by Southern blot analysis. Chimeric males were obtained by injection of positive clones into C57BL/6 J-8-cell stage embryos and were mated with...
CS7B6/1] wild-type female mice to establish germline transmitted founders. Heterozygous offspring with a minimum of three directed backcrosses against the C57BL/6J background to eliminate mutations in Cgh (Rdh mutation), Cyp2k2, and Nnt, were intercrossed to obtain wild-type and homozygous knock-in animals. To remove the FRT-flanked neomycin selection cassette, these mice were mated with FLP-Deleter (ACTB-FLPe) mice (Jackson Laboratory, Bar Harbor, ME, USA, #005703) to obtain the floxed allele used in this study. Subsequent mating with CMV-Cre mice (Jackson Laboratory, Bar Harbor, ME, USA, #068491)37, an established mouse model for tauopathies, expressing all six isoforms of human Tau instead of mouse Tau.

**Protein modeling.** The protein structure of mouse Tubba4 (P68368) and mouse Tubb2a (Q7TMM9) (Fig. 1B–C) was modeled using the protein structure homology-modeling server SWISS-MODEL (swissmodel.expasy.org).

**Long range PCR.** To validate the correct targeting of the mutated tuba4a knock-in, long-range PCR (Supplementary Fig. 1F) was performed by using 2 oligonucleotides (00866-Neo ATGCTCGACAGTGCTGGTGGAAAAG (I), 70246sa: CAGAACACAGAGATGTCTGACACACC (II)). The resulting 383 base pair product was sub-cloned into a pGEM-T Easy Vector (Promega, Madison, WI, USA, #A1360) and was validated by Sanger sequencing analysis.

**Genotyping.** Genomic DNA was isolated from tail biopsies using the Quick Extract Buffer (Biozynm Scientific GmbH, Hessisch Oldendorf, Germany, #101098). For genotyping of mutated tuba4a knock-in mice, 2 oligonucleotides were used (70252GAG GAGGAGGGAGCTTTGGACTCTGTGC (VIII); 70253hom: AGCA

**Southern blotting.** Southern blot analysis was used to confirm correct 5′ and 3′ homologous recombination events. For the 5′-end, genomic DNA was digested using NsiI and an internal probe (346 bp) located within the 5′-end of the targeted gene. The following oligonucleotides were used to generate the 5′ probe: 70244PRO (GGGGAAGGGAGAGAGGTGTATGAAGG) (IV). Expected PCR product sizes: 1302 base pairs for the wild-type allele and 1440 base pairs for the knock-in allele. For genotyping of its Tau mice 3 oligonucleotides (dTau for: CAGGCTTGAAC CAGATGTGc; dTauGrev: TGAAGTTGCTGGCTTTAGGTc; mTau2rev: CTAAGGCTACTGTGAGACTG) were used to analyze the mouse Tau knockout-locus. Expected PCR product sizes: 383 base pairs for the wild-type allele and 170 base pairs for the knockout allele. The human Tau knock-in loci was analyzed using the following 4 oligonucleotides: hTau3c: GATGAAA TAAAGCGTTGGAGGAAGA; hTau: CGTGATGATGCTGTCACCTCCT; non_hTau: GAGGATGATGCTGTCACCTCCT; CATTGACCTT GGAGCAGCAG. Expected PCR product sizes: 273 base pairs for the wild-type allele and 353 base pairs for the knock-in allele.

**Ex vivo protein extracts.** To quantify the individual protein expression levels, the brains of adult mice were dissected in PBS on ice as previously described19. The tissue was immediately suspended in lysis buffer (PBS, containing 1% (v/v) Triton X-100, 100 mM NaCl, 50 mM Tris, pH 8.0, 1 mM PMSF, 5 mM DTT and 2 mM Mg-ATP) and homogenized using a Tissue Tek (SakuraTek, Ca, USA, #4583) and frozen. 30 μm coronal sections were cut using a cryostat (Cryostar, Thermo Fisher Scientific, Waltham, MA, USA) and stored at −80 °C. For Creyl violet staining, 30 μm sections were sequentially rinsed in 70% (v/v) ethanol, 95% ethanol (v/v), 2 x 100% ethanol. After dehydration, brains were immersed in three changes of Roti Histol (Carl Roth, Karlsruhe, Germany, #6640.1) for 30 min each at 30 °C, followed by three changes of Parafla

**Histology and immunohistochemistry.** For Nissl staining adult mice were euthanized with CO2 and perfused using 4% PFA/PBS (w/v). Brains were harvested and post-fixed for 6 h in 4% PFA/PBS. A 30% Sucrose/PBS (w/v) solution was used for dehydration. Brains were then embedded in Tissue Tek (SakuraTek, Ca, USA, #4583) and frozen. 30 μm coronal sections were cut using a cryostat (Cryostar, Thermo Fisher Scientific, Waltham, MA, USA) and stored at −80 °C. For Creyl violet staining, 30 μm sections were sequentially rinsed in 70% (v/v), 95% (v/v), 100% ethanol and xylene. Slides were dehydrated by performing the washing row backwards with a final washing step in water and were subsequently incubated for 3 min in Creyl violet. Following a 1 min wash in water, sequential incubations in 70% ethanol (v/v), 95% acid ethanol (acetic acid), 95% ethanol (v/v), 100% ethanol and Xylene were performed. Sections were then mounted with Entellan (Sigma-Aldrich, St. Louis, MO, USA, #107906).

For immunohistochemistry, brains were harvested and post-fixed in 4% PFA/PBS (w/v) for 48 h followed by a wash in PBS for 24 h. Brains were subsequently dehydrated by incubation in the following solutions for 1 h each at 30 °C: 50% (v/v) ethanol, 70% (v/v) ethanol, 95% ethanol (v/v), 100% ethanol and Xylene were performed. Sections were then mounted with Entellan (Sigma-Aldrich, St. Louis, MO, USA, #107906).

**Western blotting.** For detection of relative immunoblot signal intensities, images were acquired using a Chemo-Cam Imager ECL HR 16-3200 (Intas). Alternatively, images were acquired using an Odyssey CLx (LI-COR) imaging system. Signal intensities were analyzed using Fiji (ImageJ, version 2.0.0, NIH, USA) or ImageJ (version 5.2, LI-COR). Total protein staining was performed using Revert 700 Total Protein Stain (LI-COR, NE, USA, #926-11021), according to the manufacturer’s instructions.

**Immunoprecipitation.** For co-immunoprecipitation experiments, hippocampi of adult Tuba4a (+/+ wild type) and (−/−) knock-in mice were dissected in ice cold PBS, homogenized in IM-Ac buffer (20 mM HEPES, 100 mM KAc, 5 mM MgCl2, 5 mM EGTA, 5 mM Mg-ATP and 2 mM Mg-ATP) and incubated for 30 min on ice. All following steps were performed at 4 °C. The
homogenate was clarified by centrifugation at 1000 × g for 10 min and the resulting supernatant (S1) was precollected by incubation with magnetic Protein G Dynabeads (Invitrogen, Carlsbad, CA, Invitrogen, #1004D) for 20 min and washed with 1 ml anti-alpha-tubulin antibodies to magnetic Protein G Dynabeads, precollected extracts from S1 were incubated with beads for 2 h, followed by extensive washing steps with IP-buffer (150 mM NaCl, 50 mM Tris, pH 6.75, 5 mM MgCl2). Bound proteins were eluted in SDS sample buffer, subjected to SDS-PAGE and subsequently analyzed by western blotting.

Cell culture, transfection, tissue extraction, and immunocytochemistry. Primary hippocampal neurons were prepared as previously described. Briefly, one day prior to the preparation, 12 mm sterile glass coverslips were placed into 24-well plates and coated with 0.1% (w/v) gelatin in PBS overnight. Neurons were dissociated from embryonic day 18 (E18) rat brains in PBS lysis buffer, containing 1% (v/v) Triton X-100 (LB-1000, Thermo Fisher Scientific), 50 μM DTT, 10 mM NaF, 10 mM Na3VO4, 1 μM E64 and 1 μM PMSF. Neurons were transferred into tissue culture plates containing 1% (v/v) FBS in DMEM/F12 containing serum (Thermo Fisher Scientific, #1320074) and kept in HBSS medium (Thermo Fisher Scientific, #14065056). Hippocampi from both sexes were gently dissociated into cells, using fire polished Pasteur pipettes. Cell density was calculated using a Neubauer Counting Chamber (Marienfeld, Lauda-Könighofen). 60,000 neurons were plated in 24-well plates, maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO2 in a cell incubator. Cells cultured for 7 days on glass coverslips were used for live-cell imaging.

For stimulated emission depletion (STED) super-resolution microscopy, imaging was carried out as previously described. Brieﬂy, for multichannel fluorescent imaging, images were sequentially recorded with 488-nm and 561-nm argon lasers. For the analysis of microtubules and STED imaging, an optimized protocol was used: following ﬁxation with 4% paraformaldehyde (w/v) and 4% sucrose (w/v) in PBS for 12 min. Cells were permeabilized for 4 min in PBS containing 0.25% Triton-X-100 (v/v), and blocked in PBS containing 1% (w/v) BSA. Neurons were incubated for one hour in primary antibodies diluted in blocking buffer at RT or overnight at 4 °C. After a wash step, secondary antibodies and optionally DAPI were incubated for one hour at RT. Coverslips were mounted in Aqua Poly/Mount.

For the analysis of microtubules and STED imaging, an optimized protocol was used as previously described. Briefly, cells were rinsed in pre-warmed HEPES buffer (10 mM HEPES (pH 7.4), 135 mM NaCl, 5 mM KCl, 2 mM CaCl2,2 mM MgCl2, 5 mM glucose) pre-extracted and homogenized using a 200 μl-pipette (in-soluble cytoskeletal fraction). Afterward, the homogenate was clarified by centrifugation at 1000 × g for 10 min and the resulting supernatant (S1) was precollected by incubation with magnetic Protein G Dynabeads, precollected extracts from S1 were incubated with beads for 2 h, followed by extensive washing steps with IP-buffer (150 mM NaCl, 50 mM Tris, pH 6.75, 5 mM MgCl2). Bound proteins were eluted in SDS sample buffer, subjected to SDS-PAGE and subsequently analyzed by western blotting.

Cell culture, transfection, tissue extraction, and immunocytochemistry. Primary hippocampal neurons were prepared as previously described. Briefly, one day prior to the preparation, 12 mm sterile glass coverslips were placed into 24-well plates and coated with 0.1% (w/v) gelatin in PBS overnight. Neurons were dissociated from embryonic day 18 (E18) rat brains in PBS lysis buffer, containing 1% (v/v) Triton X-100 (LB-1000, Thermo Fisher Scientific), 50 μM DTT, 10 mM NaF, 10 mM Na3VO4, 1 μM E64 and 1 μM PMSF. Neurons were transferred into tissue culture plates containing 1% (v/v) FBS in DMEM/F12 containing serum (Thermo Fisher Scientific, #1320074) and kept in HBSS medium (Thermo Fisher Scientific, #14065056). Hippocampi from both sexes were gently dissociated into cells, using fire polished Pasteur pipettes. Cell density was calculated using a Neubauer Counting Chamber (Marienfeld, Lauda-Könighofen). 60,000 neurons were plated in 24-well plates, maintained at 37 °C in a humidified atmosphere of 95% air and 5% CO2 in a cell incubator. Cells cultured for 7 days on glass coverslips were used for live-cell imaging.

For live-cell imaging, coverslips with the transfected neurons were mounted into a live-cell imaging chamber containing a conditioned medium and kept at 37 °C and 5% CO2 levels in an incubator coupled to the spinning disk confocal microscope. The observations were performed with a Zeiss LSM 880 laser scanning microscope at 25 kV (LVEM25, Delong Instruments, Brno, Czech Republic).

Live cell imaging. For time-lapse imaging, coverslips with the transfected neurons were mounted into a live-cell imaging chamber containing a conditioned medium and kept at 37 °C and 5% CO2 levels in an incubator coupled to the spinning disk confocal microscope. The observations were performed with a Zeiss LSM 880 laser scanning microscope at 25 kV (LVEM25, Delong Instruments, Brno, Czech Republic).

Confocal laser-scanning and STED imaging. For confocal microscopy, imaging was carried out as described before. Briefly, for imaging of fluorescent signals, a Laser-Scanning Confocal Microscope FluoView FY1000 (Olympus Hamburg, Germany) equipped with a 60× objective and Fluoview software version 2.1b was used. For almost all experiments, images were stitched from a large number of overlapping images captured with the “stitch region” function. Brightness was adjusted with the “inclusive thresholding” function to define image thresholds. For measurements of fluorescence intensity, the integrated “morphometry analysis” function was used to assess total cell area and average signal intensity in multiple frames. Overlay files were separated using the “color separate” function within the software package. Identical ROIs were selected using the “transfer region” function. Brightness was adjusted with the “inclusive thresholding” function to define image thresholds. For measurements of fluorescence intensity, the integrated “morphometry analysis” function was used to assess total cell area and average signal intensity in multiple frames. Overlay files were separated using the “color separate” function within the software package.

For stimulated emission depletion (STED) super-resolution microscopy, imaging was carried out as previously described. Briefly, STEM images were acquired in gating mode by mean of an Abberior expert line laser scanning STED microscope. As excitation, two pulsed laser sources operating at 561 and 640 nm were used while as depletion beam a short-pulsed 775 laser was employed. For detection, a 60X NA = 1.4 P-Apo Oil objective from Nikon was used. Images were acquired at different scan speeds. Images were acquired at constant scanning speed (pixel dwell 3 s/μm) and accumulated multiple times. Line scan analysis. Line scans were applied using the “Plot profile” tool of Fiji (ImageJ, version 2.0, NIH, USA), to measure signal intensities and identify signal peaks of individual STED channels along a defined region of interest. To assess localization, the distance between signal peaks was analyzed.

Immunoglobulin labeling and transmission electron microscopy analysis. Adult male mice were terminally anesthetized with sodium pentobarbital (1.6 g/kg body weight). Brains were removed and post-fixed in 1% (v/v) Triton X-100 (soluble cytosolic fraction). The remaining core of tissue was post-fixed in extraction buffer containing 1% (v/v) Triton X-100 (remaining cytoskeleton fraction). After two days, tissues were rinsed in several washes of 1% (v/v) Triton X-100, 4% PFA (w/v) and 4% (w/v) sucrose in PBS with 4% PFA (w/v) and 4% (w/v) sucrose for 10 min at 4 °C. After fixation, tissues were washed in several washes of 1% (v/v) Triton X-100, 4% PFA (w/v) and 4% (w/v) sucrose in PBS with 4% PFA (w/v) and 4% (w/v) sucrose for 10 min at 37 °C, and then incubated in propylene oxide for 1 h prior to embedding in propylene oxide (1:1, 2 h at room temperature) and Epon. Sections containing 2% accelerator were cut and subjected to low-voltage electron microscopy at 25 kV (LVM25, Delong Instruments, Brno, Czech Republic).

Soluble-tubulin extraction assay (TX-100 extraction). To separate soluble tubulin, dissociated microtubule (MT), and dissociated proteins (e.g., MAPs) from the intact/membrane-associated MT, dissociated hippocampal neurons at DIV 15 were extracted using a detergent-containing buffer. Therefore, 80,000 cells were seeded in a 24-well size vessel and cultured for the indicated time. All following steps were performed at 37 °C. Cells were briefly washed with HEPES buffer (10 mM HEPES (pH 7.4), 135 mM NaCl, 5 mM KCl, 2 mM CaCl2,2 mM MgCl2, 5 mM glucose) and then incubated for 90 s in 300 μl microtubule-extraction buffer (MT-extraction buffer; 80 mM PIPES, 7 mM MgCl2, 1 mM EGTA, 0.3% (v/v) Triton X-100, 150 mM NaCl, 5 mM glucose, pH 6.9 (KOH)). The extract was entirely removed and supplemented with a final concentration of 1% (v/v) Triton X-100 (soluble cytosolic fraction). The remaining tissue in the tubes was transferred to fresh tubes. Overlays were harvested in 300 μl MT-extraction buffer supplemented with a final concentration of 1% (v/v) Triton X-100 using a cell-scraping and homogenized using a 200 μl-pipette (in-soluble cytoskeletal fraction). After incubation for 30 min on ice, both extracts were centrifuged at 1000 × g for 10 min at 4 °C. Resulting supernatants were boiled in SDS sample buffer after adjustment of protein concentration. Ponceau stained protein per sample were subjected to SDS-PAGE and subsequently analyzed by western blotting.
Endogenous microtubule preparation from adult mouse brain. Protocol according to the Mitchinson lab, Harvard University (http://mitchinson.med.harvard.edu/Protocols.htm). To polymerize microtubules from mouse brain and to analyze the fraction of attached MAPs, adult mice were sacrificed and the hippocampus was dissected in ice-cold PBS. The tissue was homogenized in 1.3 ml BRBB80 buffer (80 mM PIPES, pH 6.8 (KOH), 1 mM MgCl2, 1 mM EGTA, 1 mM DTT) by vigorous shearing of the tissue with five strokes using a 1 ml syringe equipped with 26 gage needle followed by 8 strokes at 900 rpm using a Teflon-pestle (Barbantius AG, Göttingen, Germany). The homogenate was centrifuged at 70,000 × g for 20 min at 4 °C (rotor: TLA100.3, Beckman Coulter GmbH, Krefeld, Germany). Afterwards, the supernatant was removed, supplemented with 1 mM GTP and 2 mM taxol and incubated for 10 min at RT. Subsequently, the homogenate was divided into three parts supplemented with: (i) 10 % (v/v) DMSO kept at 37 °C for 30 min, (ii) 2 mM Taxol (Sigma-Aldrich) 5 mM Taxol kept at RT for 3 min, followed by 30 min at 37 °C and (iii) 5 mM CaCl2 stored on ice for 30 min. In addition, all parts were supplemented with a complete protease inhibitor. Afterwards, the samples were transferred on top of a pre-warmed BRBB80 cushion containing 40 % (v/v) glycerol and centrifuged at 200,000 × g at 37 °C for 30 min. The resulting supernatant (containing soluble tubulin dimers and detached MAPs) was removed and kept on ice. The resulting pellet (containing polymerized microtubules and attached and co-pelleted MAPs) was resuspended in 8 M urea over 30 min at RT. Finally, fractions were boiled in SDS sample buffer and a fixed volume of total protein per sample were subjected to SDS-PAGE and subsequently analyzed by western blotting.

Analysis of mRNA expression levels. RNA extraction and quality control: RNA extraction was performed using Trizol (Invitrogen, #15960626), with one hippocampus from wild-type or transgenic animals aged 12 or 18 weeks, respectively, as starting material. The samples were homogenized by vigorous shearing of the tissue in 1000 µl Trizol with 10 strokes of a 1 ml syringe equipped with a 20 and 23 gauge needle, successively. Total RNA was photometrically quantified by NanoDrop 2000 (Thermo Fisher Scientific) measurement. Moreover, ~500–1000 ng RNA were analyzed on a 1.2% agarose gel (w/v) with 1-Kb marker for overall RNA quality control. Reverse transcription was performed using 5 µg total RNA, oligo dT primers, and hexamer primers of the SuperScript Reverse Transcriptase Kit (Thermo Fisher Scientific, #18060711).

Quantitative PCR (qPCR) analysis: qPCR reactions were performed as duplex assays with the Taqman gene expression master mix (Thermo Fisher Scientific, #4369542). Primers were used with purification grade desalted. qPCR reactions were performed on Applied Biosystems 7900 HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in a 96-well format. Normalization was performed on 18S rRNA, with 1 µg total RNA, oligo dT primers, 1X qPCR reagents and 20 µl, 100 ng of cDNA in a 96-well plate. Reactions were performed in triplicate. The cycle threshold (Ct) values were normalized to the housekeeping gene, and the fold-change was calculated using the 2−∆ΔCt method. The expression levels were calculated using the relative quantification method (2−∆∆Ct).

De-phosphorylation of protein extracts. To analyze proteins independent of posttranslational phosphorylation, the tissue was immediately suspended in lysis buffer (PBS, containing 1 % (v/v) Triton X-100, Complete protease inhibitor) and homogenized by vigorous shearing of the tissue with five strokes of a 1 ml syringe equipped with a 21 (first stroke) and 26 (remaining strokes) gauge canula. The homogenate was incubated for 30 min on ice and subsequently centrifuged at 1000 x g for 10 min at 4 °C. After adjusting to 100 µg protein per lane (using a BCA assay, the homogenate was treated with Lambda Protein Phosphatase (Lambda PP; Tuba4a, TU-Munich, Germany). The samples were incubated for 30 min on ice and subsequently centrifuged at 100,000 × g for 1 h at RT with rotation. After washing for another 3 × 5 min in TBST at RT, signal intensities were detected using the Odyssey Clx (LI-COR) imaging system.

Sholl analysis. For a manual Sholl analysis on each individual cell, existing images were converted to grayscale using Adobe Photoshop. An initial circle of 12 µm diameter was drawn to exclude the soma, followed by concentric circles with increasing radii of 6 µm per circle. Intersections between Iba1-positive microglial branches and each increasing circle were counted.

Statistics and reproducibility. At least three biologically independent repeats were conducted for each experiment. Statistical analyses were performed with either SPSS (Chicago, IL, USA) or Prism (GraphPad Software Inc., CA, USA). Briefly, after an exploratory data analysis to identify outliers, data were checked for normality using Kolmogorov-Smirnov or Shapiro-Wilk tests. To separate the means of normally distributed data, either a two-tailed unpaired Student t-test or one-way or two-way ANOVA was used, while Mann-Whitney or Kruskal-Wallis tests were used to analyze nonparametrically distributed data. Graphs were constructed using Excel (Microsoft, Redmond, WA, USA) or Prism. Nonparametric data are shown as box plots. Normally distributed data are shown as bar diagrams and individual data points are shown as dots, if n < 10. Significance was defined as follows: *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001. Further statistical details for individual experiments are outlined in the respective figure legends.

Data availability. All data supporting the findings of this study are provided within the paper and its Supplementary Information. Any data are available from the authors upon request. Source data are provided with this paper.
Sun, W. et al. Glycogen synthase kinase-3beta is complexed with tau protein in brain microtubules. J. Biol. Chem. 277, 11933–11940 (2002).

Martin, L. et al. Tau protein kinases: involvement in Alzheimer’s disease. Ageing Res. Rev. 12, 289–309 (2013).

Materia, D. & Mandelkow, E. M. The tau of MARK: a polarized view of the cytoskeleton. Trends Biochem. Sci. 34, 332–342 (2009).

Timm, T. et al. Glycogen synthase kinase (GSK) 3beta directly phosphorylates Serine 212 in the regulatory loop and inhibits microtubule affinity-regulating kinase (MARK) 2. J. Biol. Chem. 283, 18873–18882 (2008).

Denk, F. & Wade-Martins, R. Knock-out and transgenic mouse models of tauopathies. Neurobiol. Aging 30, 1–13 (2009).

Dawson, H. N. et al. Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice. J. Cell Sci. 114, 1179–1187 (2001).

Harada, A. et al. Altered microtubule organization in small-calibre axons of Tau protein kinases. J. Neurochem. 108, 1480–1494 (2009).

Leys, C. T., Holtzman, D. M. Glial contributions to neurodegeneration in tauopathies. Mol. Neurodegeneration 12, 50 (2017).

Das, R., Balmik, A. A. & Chinnathambi, S. Phagocytosis of full-length Tau isoforms in this article are included in the article

Yu, Y. et al. Developmental regulation of tau phosphorylation, tau kinases, and tau phosphatases. J. Neurochem. 105, 1179–1187 (2009).

Vogels, T., Murgoci, A. N. & Hromadka, T. Intersection of pathological tau and microtubula at the synapse. Acta Neurobiologica Commun. 7, 109 (2019).

Ising, C. et al. NLRP3 inflammasome activation drives tau pathology. Nature 575, 669–673 (2019).

Andorfer, C. et al. Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. J. Neurosci. 25, 5446–5454 (2005).

Jawhar, S., Trawicka, A., Jenneckens, C., Bayer, T. A. & Wirths, O. Motor deficits, neuron loss and microtubule loss of the 5xFAD mouse model of Alzheimer’s disease. Neurobiol. Aging 33, 196.e129–140 (2012).

Ohno, M. et al. BACE1 gene deletion prevents neuron loss and memory deficits in 5xFAD APP/PS1 transgenic mice. Neurobiol. Dis. 36, 134–145 (2007).

Murphy, M. P. & LeVine, H. 3rd Alzheimer’s disease and the amyloid-beta peptide. J. Alzheimers Dis. 19, 311–323 (2010).

Berenzin, I. et al. Cytosolic carboxypeptidase I is involved in processing alpha- and beta-tubulin. J. Biol. Chem. 287, 6503–6517 (2012).

Mathys, H. et al. Single-cell transcriptomic analysis of Alzheimer’s disease. Nature 570, 332–337 (2019).

Sheffer, R. et al. Biallelic variants in AGTPBP1, involved in tubulin deglutamylation, are associated with cerebellar degeneration and motor neuropathy. Eur. J. Hum. Genet. 27, 1419–1426 (2019).

Rogez, P. et al. High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nat. Genet. 25, 139–140 (2000).

Schwenk, F., Baron, U. & Rajewsky, K. A. cre-transgenic mouse strain for the conditional deletion of floxed loxP-flanked gene segments in vitro. Nucleic Acids Res. 23, 5080–5081 (1995).

Chen, C. & Okayama, H. High-efficiency transformation of mammalian cells by plasmid DNA. Mol. Cell. Biol. 7, 2745–2752 (1987).

Mikhaylova, M. et al. Resolving bundled microtubules using anti-tubulin antibodies. Nat. Commun. 6, 7933 (2015).

Heisler, F. F. et al. Muscle proteins coordinates PrP(Sc) lysosoma versus exosome degradation and impacts prion disease progression. Neuron 99, 1155–1169.e9 (2018).

Diercks, B. P. et al. ORAI1, STIM1/2, and RYR1 shape subsecond Ca(2+)-mobilizing microtubules in the distal region of growing axons. Neuron 53, 338–351 (2007).

Black, M. M., Slaughter, T., Moshiah, S., Obrocka, M. & Fischer, I. Tau is enriched on dynamic microtubules in the distal region of growing axons. J. Neurosci. 16, 3601–3619 (1996).

Funding
Open Access funding enabled and organized by Projekt DEAL.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information
The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-31776-5.

Correspondence and requests for materials should be addressed to Torben Johann Haurat or Matthias Kneussel.

Peer review information Nature Communications thanks Susanne Wegmann, Hans Zempel and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Reprints and permission information
Available at http://www.nature.com/reprints.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

© The Author(s) 2022