SRPK2 is abnormally activated in tauopathies including Alzheimer’s disease (AD). SRPK2 is known to play an important role in pre-mRNA splicing by phosphorylating SR-splicing factors. Dysregulation of tau exon 10 pre-mRNA splicing causes pathological imbalances in 3R- and 4R-tau, leading to neurodegeneration; however, the role of SRPK2 in these processes remains unclear. Here we show that delta-secretase (also known as asparagine endopeptidase; AEP), which is activated in AD, cleaves SRPK2 and increases its nuclear translocation as well as kinase activity, augmenting exon 10 inclusion. Conversely, AEP-uncleavable SRPK2 N342A mutant increases exon 10 exclusion. Lentiviral expression of truncated SRPK2 increases 4R-tau isoforms and accelerates cognitive decline in htau mice. Uncleavable SRPK2 N342A expression improves synaptic functions and prevents spatial memory deficits in tau intronic mutant FTDP-17 transgenic mice. Hence, AEP mediates tau-splicing imbalance in tauopathies via cleaving SRPK2.

Introduction

Alzheimer’s disease (AD) is a progressive neurodegenerative disorder with gradual cognitive function decline. The neuropathology of AD is characterized by both the accumulation of toxic, extracellular senile plaques with amyloid-β (Aβ) as a major component and neurofibrillary tangles (NFTs) resulting from the aggregation of hyperphosphorylated and truncated tau. The conformational changed tau alters the protein structures and forms paired helical filaments and NFTs, which are characterized in a wide range of neurodegenerative diseases, collectively called as tauopathies, including AD, frontotemporal dementia with parkinsonism-17 (FTDP-17), Pick disease (PiD), etc. (Wang and Mandelkow, 2016). Recently, we have reported that asparagine endopeptidase (AEP) is an age-dependent cysteine protease that is highly activated in AD patients and cleaves tau at both N255 and N368 residues in the brains and promotes its aggregation and neurotoxicities. Blockade of tau fragmentation by AEP prevents tau P301S mutant to trigger synaptic dysfunctions and cognitive deficits (Zhang et al., 2014). In addition, AEP also acts as delta-secretase by shredding amyloid precursor protein (APP) on N373 and N585 residues, generating C-terminal APP (aa 586–695) truncation that is a much more efficient substrate for BACE1 to produce Aβ than full-length (FL) APP. Consequently, inhibition of AEP cleavage of APP decreases Aβ production and diminishes senile plaque deposit, rescuing the learning and memory deficits (Zhang et al., 2015). Accordingly, inhibition of AEP by small molecular inhibitors in various AD mouse models abrogates AD pathologies, exerting the prominent therapeutic efficacy (Zhang et al., 2016, 2017b).

Serine-arginine protein kinase 2 (SRPK2) is a member of SRPKs, a group of cell cycle–regulated serine kinases. SRPKs recognize and phosphorylate protein substrates with serine-arginine dipeptide repeats (Gui et al., 1994). They are mainly involved in regulating pre-mRNA splicing via phosphorylating splicing factors, such as alternative splicing factor/splicing factor 2 (ASF/SF2) and SC35 (Ding et al., 2006). SRPKs also regulate the subcellular localization of splicing factors ASF/SF2 through phosphorylation (Koizumi et al., 1999). Unlike the ubiquitously expressed SRPK1, SRPK2 is highly enriched in the brain and plays an important role in controlling neuronal functions. Our previous study supports that SRPK2 participates in the neuronal survival, cell cycle progression, and memory determination in AD (Chan and Ye, 2013). For instance, we have shown that SRPK2 mediates cell cycle progression and cell death in mature neurons (Jang et al., 2008, 2009). Moreover, we found that SRPK2 is a caspase sub-
though there are no known tau mutations that have occurred in degenerative diseases (Hutton et al., 1998; Spillantini et al., 1998), been discovered and directly linked tau abnormalities to neurodegenerative diseases in 3R-enriched WT htau mice (MAPT mice) elevates 3R-tau and 4R-tau. Overexpressing AEP-truncated SRPK2 fragmenting the formation of tau into paired helical filaments (Goedert et al., 1989). Most recently, we showed that SRPK2 phosphorylates human MAPT gene encoding tau protein consists of 16 exons. Alternative splicing of exons 2, 3, and 10 generates six different isoforms in adult brain. Exon 10 encodes the second of four MT-binding repeat domains, and its alternative splicing produces tau isoforms with three or four MT-binding repeats, named 3R-tau or 4R-tau, that are under developmental and cell type–specific regulation (Goedert et al., 1989). Interestingly, only 3R is selectively expressed in the developing brain, whereas approximately equal levels of 3R-tau and 4R-tau are expressed in normal adult human brain (Goedert et al., 1989; Andreadis, 2005). Unbalanced ratios of 3R-tau/4R-tau trigger neurodegeneration in FTDP-17 and other neurodegenerative disorders, including PiD, corticobasal degeneration, and progressive supranuclear palsy (PSP; Neumann et al., 2001; Bronner et al., 2005; Yoshida, 2006). To date, >50 disease-associated mutations have been identified in MAPT, with one third of them affecting E10 splicing (Ghetti et al., 2015). Mutations in tau gene (MAPT) have been discovered and directly linked tau abnormalities to neurodegenerative diseases (Hutton et al., 1998; Spillantini et al., 1998), though there are no known tau mutations that have occurred in AD. In AD, there is a disproportionate level of the 3R-tau isoform compared with the 4R form, and this could be a key factor driving the formation of tau into paired helical filaments (Goedert and Jakes, 2005; Espinoza et al., 2008). In the current study, we report that AEP cleaves SRPK2 in human AD brains and escalates its kinase activity. The active SRPK2 phosphorylates serine/arginine (SR) proteins including SC35 and ASF/SF2–splicing factors, mediating tau exon 10 splicing and tilting the balance between 3R-tau and 4R-tau. Overexpressing AEP-truncated SRPK2 fragments in 3R-enriched WT htau mice (MAPT mice) elevates 4R-tau, accelerating tau pathology and cognitive dysfunctions, whereas overexpressing AEP-uncleavable SRPK2 N342A mutant in 4R abundant tau intronic mutant (10 +16C→T) FTDP-17 mouse model (tau609 mice) augments 3R-tau, decreasing tau pathology and cognitive deficits.

Results
AEP cleaves SRPK2 at N342 residue
Most recently, we reported that SRPK2 phosphorylates human AEP on S226 and enhances its auto-cleavage and enzymatic activities in AD (Wang et al., 2017). Proteomic analysis of lgmn WT and knockout brains indicates that SRPK2 might be a potential target of AEP. To explore whether AEP indeed cleaves SRPK2, we performed an in vitro cleavage assay using lgmn+/+ and lgmn−/− kidney lysates in the presence of purified recombinant glutathione S-transferase (GST)–SRPK2. Immunoblotting analysis showed that SRPK2 was selectively cut in WT, but not AEP-null lysates under pH 6.0, whereas it remained intact under neutral pH (Fig. 1A). To examine whether the fragmentation was executed by AEP in the lysates, we conducted truncation assay with AEP enzymatic–dead (C189S) or uncleavable inactive N323A mutant. Although WT AEP robustly cut SRPK2, both inactive mutants failed in the cotransfected HEK293 cells (Fig. 1B), indicating that AEP may mediate SRPK2 fragmentation. Moreover, titration assay with anti-AEP revealed that AEP antibody dose-dependently antagonized SRPK2 fragmentation, whereas anti-mouse IgG control was unable (Fig. 1C), suggesting that the active AEP in the acidic lysates might be responsible for SRPK2 fragmentation. Consequently, the peptidal inhibitor AENK but not inactive control AEQK for AEP selectively blocked SRPK2 proteolytic cleavage (Fig. S1A). Further, we observed the similar cleavage activity in lgmn+/+ but not lgmn−/− brain lysates under pH 6.0 with endogenous SRPK2. Notably, the 50-kD band was the major product identified in the brain, suggesting that it is the dominant form of truncated SRPK2 in the brain (Fig. 1D). Using purified SRPK2 recombinant protein and active AEP, we found that active AEP directly shredded SRPK2 into numerous fragments, supporting that AEP directly cleaves SRPK2 (Fig. S1B). Liquid chromatography with tandem mass spectrometry analysis showed that N342 residue was the major cutting site on SRPK2 by AEP (Fig. S1C). Site-directed mutagenesis demonstrated that N219, N342, and N435 were the potential cleaving residues on SRPK2 by AEP. Since N342A mutation completely blocked the main truncated band from SRPK2, thus N342 might be the predominant cutting site (Fig. 1E). Interestingly, SRPK2-phosphorylated AEP (S226D) displayed a much stronger protease activity in cleaving SRPK2 than WT AEP, whereas unphosphorylated mutant S226A barely cut SRPK2 (Fig. S1D), in alignment with our recent finding that SRPK2 phosphorylation of AEP escalates its protease activity (Wang et al., 2017).

To interrogate the cleavage activity on SRPK2, we generated rabbit polyclonal SRPK2 N342 and C343 antibodies. The antigen affinity column–purified antibody specifically recognized the truncated SRPK2, but not SRPK2 FL in primary neuronal cultures treated with Aβ42, and the signal was totally stripped away by the preincubation with the free antigen (Fig. S2A). This indicates that anti-SRPK2 N342 and C343 are very specific toward the truncated products. Noticeably, both N342 and C343 antibodies specifically recognized the truncated SRPK2 by AEP, and they were inactive when AEP was knocked out (Fig. S2, B and C), underscoring that these two antibodies selectively detect SRPK2 fragments truncated at N342 residue by AEP. The specificity of anti-SRPK2 antibodies (N342 and C343) was further validated with SRPK2 knockdown or overexpression (Fig. S2, D–F).

AEP cuts SRPK2 in human tauopathy brains
Our recent study shows that AEP is up-regulated and active in the brain in an age-dependent manner (Zhang et al., 2014, 2015, 2017a). As expected, we found that SRPK2 was gradually truncated in mouse brain during aging. Accordingly, SRPK2 N342

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Figure 1. AEP cleaves SRPK2. (A) SRPK2 cleavage assay. Western blot showing the cleavage of GST-SRPK2 after transfected HEK293 cell lysates were incubated with kidney lysates from Lgmn+/+ or Lgmn−/− mice at pH 7.4 or pH 6.0. Bar graph shows data as mean ± SEM; n = 3 independent experiments; **, P < 0.01; one-way ANOVA. (B) Western blot showing the cleavage of SRPK2 by WT and enzymatic-dead mutated AEP constructs. Bar graph shows data as mean ± SEM; n = 3 independent experiments; **, P < 0.01; one-way ANOVA. (C) Antibody titration assay. Mouse IgG was used as negative control. AEP antibody
fragment temporally escalated in the mouse brains (Fig. 2 A, first and second panels from the top). Our previous finding showed that SRPK2 T492 phosphorylation was also age-dependently augmented; so was the kinase activity (Hong et al., 2012). Consequently, its biological substrate tau S214 phosphorylation increased in a similar manner (Fig. 2 A, third and fourth panels from the top). As positive control, both AEP auto-cleavage and its well-characterized substrate tau N368 were elevated with the age increase (Fig. 2 A, seventh and eighth panels from the top). Strikingly, 3R-tau and 4R-tau appeared increased in a similar manner (Fig. 2 A, third and fourth panels from the top). Once more, SRPK2 was strongly phosphorylated on T492 and activated in AD mouse model with 5XFAD stronger than 5XFAD/AEP−/− mice. Consequently, tau S214 and acinus S422, another SR-enriched SRPK2 substrate that we identified previously (Jang et al., 2008), were potently phosphorylated by active SRPK2 (Fig. 2 B, third through sixth panels from the top). We made the similar observation in human AD brains, which exhibited prominent AEP, tau N368, and SRPK2 N342 cleavage. Again, p-SRPK2 T492, a marker for its kinase activation, tightly coupled with p-tau S214 in patient brains (Fig. 2 C). Collectively, these data strongly suggest that SRPK2 displays a much higher kinase activity in human AD brains, when it is cleaved by AEP.

To assess whether SRPK2 is also cleaved in other tauopathies, we performed immunoblotting with the brain samples from human FTDP-17 (tau P301L or G389R) and PSP patients and their age-matched controls. Evidence for the presence or absence of silver-positive neuronal tau inclusions was shown for AD, FTDP-17, and PSP brains as well as for the healthy control of similar age (Fig. 3 A). Notably, SRPK2 was prominently cut in both FTDP-17 and PSP patients, which was validated by both SRPK2 N342 and C343 antibodies (Fig. 2, D and E, first through fourth panels from the top). Surprisingly, SRPK2 was not cleaved in tau G389R mutant FTDP-17 patient (Fig. 2 D, last lane, first through fourth panels from the top). Strikingly, 3R-tau and 4R-tau appeared inversely correlated in these patient brains. SRPK2 truncation activities tightly linked to 4R-tau abundance in both tauopathies (Fig. 2, D and E, fourth through sixth panels from the top; Fig. S3, B and C), indicating that SRPK2 might implicate in tau exon 10 alternative splicing. Subcellular fractionation supported that truncated SRPK2 fragments were preferentially distributed in the nuclear fraction versus cytosolic fraction. Moreover, C-terminal SRPK2 fragment (C343–688) was allocated more in the nuclear than N-terminal fragment (1–342), but they barely resided in the lysosomal fraction (Fig. 2 F). Immunofluorescent staining showed that AEP was augmented in both FTDP-17 and PSP brains, colocalizing with pronounced SRPK2 N342 or SRPK2 C343 fragment as compared with these of healthy controls (Fig. S3, D and E). Together, these findings support that AEP is activated in human patient brains with tauopathies and cleaves SRPK2 at N342, associating with augmented kinase activity.

**AEP-cleaved SRPK2 displays higher kinase activity and translocates into the nucleus**

SRPK2 contains bipartite kinase domains on both N and C termini with a cytoplasm tethering spacer between them (Ding et al., 2006). To further assess the biological consequence of AEP cleavage on SRPK2 kinase activity, we monitored tau S214 phosphorylation in the presence of SRPK2 FL and mixed fragments and purified recombinant GST-tau proteins. Using AEP WT and enzymatic-dead C189S, we found that AEP-cleaved SRPK2 possessed much higher kinase activity toward tau than uncleaved SRPK2. Quantitative analysis revealed that p-tau S214 was increased time-dependently with fragments much stronger than SRPK2 FL, indicating that AEP cleavage escalates SRPK2 kinase activity (Fig. S4, A and B). To determine which kinase domain possesses more robust enzymatic activity, we conducted an in vitro phosphorylation assay with purified fragments and SRPK2 FL in the presence of acinus and tau recombinant proteins, respectively. Immunoblotting revealed that both fragments exhibited more robust kinase activities than SRPK2 FL toward p-acinus S422 and p-tau S214 (Fig. 3 A). Immunofluorescent staining with GFP-SRPK2 constructs showed that WT SRPK2 has its two truncated fragments localized in both the cytoplasm and the nucleus, whereas uncleavable mutant N342A exclusively resided in the cytoplasm (Fig. 3 B), indicating that AEP cleavage is required for SRPK2 nuclear translocation. Infection of SRPK2 fragments in neurons significantly redistributed SC35 from the nuclear speckles into the nucleoplasm, whereas N342A failed (Fig. 3 C), confirming a previous report that SRPK2 dissembles the nuclear speckle (Kuroyanagi et al., 1998). Interestingly, the insoluble tau from tau P301S mouse brains elevated AEP expression in primary neurons, leading to the up-regulation of AEP, which subsequently truncated SRPK2 that was positive for N342 staining. The elevated N-terminal 1–342 fragment distributed in both the cytoplasm and the nucleus, whereas C-terminal SRPK2 (C343–688) predominately resided in the nucleus, resulting in SC35 nuclear relocation (Fig. S4, C and D). Subcellular fractionation demonstrated that insoluble tau but not soluble tau fraction from tau P301S mouse brains provoked AEP activation in the cytosolic fraction, triggering SRPK2 cleavage. Fitting with immunofluorescent staining, C-terminal SRPK2 C343 fragment was relatively more abundant than N-terminal N342 fragment in the nuclear fraction in SH-SYSY cells treated with insoluble tau. Noticeably, insoluble tau elevated 4R-tau and decreased 3R-tau in the cytosolic fraction of neuronal SH-SYSY cells. It is worth noting that splicing factors ASF and SC35 expression levels were augmented in the nuclear dose-dependently neutralized SRPK2 cleavage in the lysates. (D) Western blot showing the cleavage of endogenous SRPK2 by AEP. Bar graph shows data as mean ± SEM; n = 3 independent experiments; *P < 0.05, one-way ANOVA. (E) Cleavage of mutant SRPK2 by AEP. SRPK2 cleavage was analyzed by Western blot after lysates of HEK293 cell expressing GST-SRPK2 WT, N219A, N343A, or N415A mutants were incubated with active mouse kidney lysates. Western blot data in A–E are representative of three independent experiments. AFU, arbitrary fluorescence units; MW, molecular weight; WB, Western blot.
Figure 2. AEP cleaves SRPK2 during aging and in tauopathies. (A) Western blot analysis of SRPK2 and AEP in mouse brain during the aging process. SRPK2 activation (p-T492) and its cleavage (SRPK2 N342 and Tau N368) by active AEP were escalated as the age escalation and so were SRPK2 kinase activities (p-tau S214). (B) Western blot detection of SRPK2 fragments in 15-mo-old WT, 5XFAD/Lgmn−/− and 5XFAD/Lgmn+/+ mouse brain. Knockout of AEP in 5XFAD mice abolished both SRPK2 N342 and tau N368 cleavage by AEP, diminishing SRPK2 kinase activities on phosphorylation of acinus S422 and tau S214. (C–E) Western
Truncated SRPK2 promotes and uncleavable mutant reduces tau exon 10 inclusion

To investigate whether SRPK2 regulates tau exon 10 pre-mRNA splicing, we transfected HEK293 cells with tau exon 10 containing mini-gene (pCI-S19-S10) in the presence of WT SRPK2 or truncated SRPK2 1–342 or C343–688 or uncleavable N342A mutant. Quantitative inclusion/exclusion of exon 10 revealed SRPK2 WT almost doubled the inclusion compared to control. The N-terminal SRPK2 1–342 fragment elevated the ratio more than sixfold, and the C-terminal 343–688 truncate augmented approximately two to three times. In contrast, N342A barely increased the inclusion (Fig. 4A). TaqMan Real-Time PCR Assay for the specific detection and quantification (Connell et al., 2005) of 3R- and 4R-tau confirmed that cleaved SRPK2 regulated tau exon 10 pre-mRNA splicing (Fig. 4B). As expected, depletion of SRPK2 reduced the inclusion (Fig. 4, C and D). We made the similar observations with endogenous tau in dopaminergic cell line SH-SY5Y and primary neurons (Fig. 4, E–G). In alignment with the splicing results, immunoblotting demonstrated that both 1–342 and 343–688 fragments increased 4R-tau and repressed 3R-tau, fitting with the exon 10 inclusion/exclusion data (Fig. 4H). As expected, both SC35 and ASF were strongly phosphorylated by the fragmentated SRPK2 truncates with N-terminal 1–342 stronger than C-terminal 343–688. It is worth noting that uncleavable N342A mutant decreased splicing factor phosphorylation (Fig. 4I). To test whether SR-splicing factors SC35 and ASF are accountable for SRPK2’s tau exon 10 splicing activity or not, we knocked down SC35 or ASF or their combination with the specific siRNAs in SY5Y cells, respectively, in the presence of SRPK2 FL or AEP-truncated fragments. Quantitative analysis of exon 10 inclusion/exclusion showed that depletion of SC35 or ASF reduced the ratios, and the maximal effect occurred when both factors were depleted. These effects were most prominent in the presence of 1–342 fragment, followed by 343–688, and WT SRPK2 exhibited the modest effect (Fig. 5, A–C). Immunoblotting analysis with the cell lysates revealed the similar results. N-terminal 1–342 strongly increased 4R-tau, which was decreased when either SC35 or ASF was depleted, and the weakest 4R-tau was detected when both factors were knocked down. The similar pattern applied to C-terminal 343–688 fragment and SRPK2 WT. Conversely, 3R-tau was the minimum in the presence of 1–342, in agreement with its strongest activity in promoting exon 10 inclusion. As the splicing factor was knocked down, 3R-tau were elevated and climaxed when both were depleted. The same effect occurred to SRPK2 C-terminal 343–688 fragment as well (Fig. 5D). Thus, these observations are consistent with the previous report that active SRPK2 translocates into the nucleus, phosphorylating SR-splicing factors including SC35 and ASF (Jang et al., 2009). Together, these data strongly suggest that AEP-cleaved SRPK2 escalates tau exon 10 inclusion via phosphorylating SC35 and ASF pre-mRNA-splicing factors.

AEP-cleaved SRPK2 increases 4R-tau and decreases cognitive functions in htau mice

MAPT (WT htau) transgenic mice lack endogenous mouse tau and express mostly 3R-human tau (Andorfer et al., 2003). The htau mice develop tau pathology in a time course and distribution that is comparable to that occurring in the early stages of human AD. Hyperphosphorylated, conformational altered tau accumulates in the cell bodies and dendrites of neurons in htau mice as they age (Andorfer et al., 2003). To explore whether up-regulating 4R-tau in htau mice via increasing exon 10 inclusion would affect tau pathology and cognitive behaviors or not, we injected lentivirus (LV)-expressing GFP-tagged SRPK2 FL, N-terminal 1–342, or C-terminal 343–688 into the hippocampus of 2-mo-old htau mice, respectively. In 3 mo, we conducted immunoblotting to examine 3R- and 4R-tau expression in the hippocampus. Consequently, SRPK2 FL stimulated 4R-tau expression as compared with GFP control. As expected, the kinase fragments greatly elevated 4R-tau with N-terminal 1–342 stronger than C-terminal 343–688 domain. Inversely, 3R-tau was reduced when the truncated kinase domains were expressed with N-terminal 1–342 more robust than C-terminal 343–688 (Fig. 6A, first through fifth panels from the top). To test whether different tau isoforms could aggregate into insoluble inclusion, we conducted protein fractionation with Sarkosyl detergent buffer. Notably, 4R-tau was highly distributed in the soluble fraction from truncated kinase domain–injected samples with 343–688 stronger than 1–342. However, 1–342–induced 4R-tau was more prominent in Sarkosyl-insoluble fraction compared with 343–688 (Fig. 6A, sixth through eleventh panels from the top). RT-PCR analysis of exon 10 inclusion/exclusion ratios showed that 1–342 fragment largely increased the inclusion and decreased exclusion, followed by 343–688 truncation, in alignment with 4R-tau protein level augmentation by these two truncated kinase domains (Fig. 6, B and C). Immunohistochemistry (IHC) staining with AT8 and AT100 indicated that tau was highly phosphorylated in 1–342–injected hippocampus, followed by 343–688, whereas AT8 and AT100 signals were modest in GFP control or WT-injected brains. This was validated with silver staining as well (Fig. 6D and Fig. S5, A and B). Golgi staining revealed that the dendritic spines in the hippocam-
Figure 3. **Cleavage of SRPK2 by AEP promotes its kinase activity and nuclear localization.** (A) Analysis of SRPK2 kinase activity. SRPK2 activity was shown by Western blot after purified GFP-SRPK2 FL, 1–342, or 343–688 was incubated with purified acinus or tau at different time points. Both N-terminal and C-terminal kinase domains from truncated SRPK2 revealed higher kinase activities than FL counterpart. **(B)** SRPK2 truncation mediates its subcellular residency.
tions as compared with GFP or SRPK2 WT. Remarkably, N342A mutant strongly elevated 3R-tau and decreased 4R-tau; accordingly, 4R-tau was evidently reduced in both Sarkosyl soluble and insoluble fractions as compared with GFP or SRPK2 WT (Fig. 7 A). RT-PCR analysis of the hippocampal mRNA also demonstrated the exclusion augmentation by uncleavable SRPK2 N342A mutant (Fig. 7 B). Thus, the blockade of SRPK2 cleavage by AEP increases tau exon 10 exclusion, up-regulating 3R-tau expression. Consequently, IHC staining with AT8 and AT100 revealed that tau pathological phosphorylation was significantly alleviated in N342A-expressing brains compared to GFP or SRPK2 WT–infected brains (Fig. 7 C). In contrast, lentiviral expression of SRPK2 N1–342 promoted 4R expression and tau pathological phosphorylation in 609 mice (Fig. 7, A–D). Golgi staining supported that the dendritic spines were more prominently escalated in SRPK2 N342A–infected brains than GFP or SRPK2 WT–expressing samples (Fig. 7 E). EM analysis indicated that SRPK2 N342A notably increased the synapses in the hippocampus compared with GFP or SRPK2 WT (Fig. S5 F). As a result, electrophysiology assay demonstrated that overexpression of uncleavable SRPK2 N342A significantly augmented the LTP compared to mice expressing GFP or SRPK2 WT (Fig. 7 F). Accordingly, MWM cognitive behavioral test revealed that SRPK2 N342A more substantially improved the cognitive functions in 609 mice than those expressing GFP or SRPK2 WT (Fig. 7 G), though the swim speed rates were comparable among the groups, suggesting that the motor functions were not altered by these proteins overexpressed in the hippocampus (Fig. S5 G). In alignment with the improved learning and memory performances by N342A group in water maze test, contextual fear-conditioning assay showed the similar findings (Fig. S5 H), though there was no discernable difference in the nest-building capability among these groups (Fig. 7 H). Conversely, lentiviral expression of SRPK2 N1–342 exacerbated cognitive impairment in 609 mice (Fig. 7 G). Together, our data support that AEP-uncleavable SRPK2 N342A represses tau exon 10 inclusion splicing and elevates 3R-tau expression, resulting in tau NFT pathology alleviation and cognitive functions improvement in tau intronic 10 +16C→T mutation FTDP-17 mice.

### Nuclear/cytosolic localization of FL or truncated GFP-tagged SRPK2

Nuclear/cytosolic localization of FL or truncated GFP-tagged SRPK2. Rat primary neurons were infected with various LV expressing GFP-SRPK2 fusion proteins. Left, localization of SRPK2 was visualized by GFP under fluorescent microscopy. Bars, 5 μm. Right, percentage of cells exhibiting predominantly nuclear (Nuc > Cyto), predominantly cytoplasmic (Cyto > Nuc), or equivalent nuclear and cytoplasmic (Cyto = Nuc) GFP signal. Three individual experiments were performed, and at least 100 total cells were counted for each condition. Pairwise comparisons between WT protein and each mutant were performed using the χ² test (*, P < 0.001). (C) Immunofluorescent analysis of SC35 distribution in rat primary neurons infected with various LV expressing GFP-SRPK2 fusion protein. Bar, 5 μm. Bar graph shows data as mean ± SEM; n = 15–18 neurons; *, P < 0.05; one-way ANOVA. (D) Western blot showing Sarkosyl-insoluble tau induced AEP activation, accompanied with increased SRPK2 truncation, and expression of 4R-tau in SH-SY5Y cells. (E) RT-PCR analysis of human MAPT mRNA in 4R to 3R MAPT splicing in SH-SY5Y cells upon soluble and insoluble tau treatment. *, P < 0.01; one-way ANOVA (F) TaqMan probe–based quantification of 4R and 3R human MAPT mRNA in SH-SY5Y cells upon soluble and insoluble tau treatment. Data represent mean ± SEM of three independent experiments; *, P < 0.05, **, P < 0.01, one-way ANOVA. (G) AEP activity assay. The enzymatic activities of AEP in SH-SY5Y cells treated with the indicated conditions were analyzed. Data represent mean ± SEM of three independent experiments; *, P < 0.05; one-way ANOVA. Western blot data in A and D are representative of three independent experiments. MW, molecular weight; WB, Western blot.
**Discussion**

In the current study, we show that AEP cleaves SRPK2 at multiple locations with N342 residue the major site. The bipartite kinase catalytic core in SRPK2 is separated by a unique spacer sequence (Ding et al., 2006). Cleavage of SRPK2 by AEP separates its two kinase domains and escalates the kinase activities in tauopathy brains. Notably, N-terminal SRPK2 1–342 elevates tau exon 10 inclusion and up-regulates 4R-tau expression levels in MAPT mice.

Figure 4. **SRPK2 cleavage by AEP promotes tau exon 10 inclusion.** (A) AEP-truncated SRPK2 promoted tau exon 10 inclusion. The pCI/SI9-SI10 mini-gene of MAPT was transfected with into HEK293 cells infected with virus expressing various indicated SRPK2 constructs. Total RNA was subjected to RT-PCR for measurement of tau exon 10 splicing 36 h after transfection. Both N-terminal and C-terminal kinase domains of SRPK2 displayed elevated effect in augmenting exon 10 inclusion compared with SRPK2 FL counterpart. (B) TaqMan probe–based quantification of tau exon 10 inclusion and exclusion in HEK293 cells coexpressing pCI/SI9-SI10 mini-gene and SRPK2. Data represent mean ± SEM of three independent experiments; *, P < 0.05; **, P < 0.01; one-way ANOVA. (C and D) Knockdown of SRPK2 by its shRNA-suppressed tau exon 10 inclusion in HeLa cells. Data in D represent mean ± SEM of three independent experiments; *, P < 0.05; two-tailed Student’s t test. (E–G) RT-PCR analysis of 4R to 3R MAPT splicing in SH-SY5Y cells (E and F) and neurons (G) infected with LV expressing WT, uncleavable (N342A), or cleaved SRPK2 fragments (1–342 and 343–688). Data in F represent mean ± SEM of three independent experiments; *, P < 0.05, **, P < 0.01; one-way ANOVA. (H and I) Western blot showing SRPK2 cleavage increased ratio of 4R-/3R-tau protein expression levels (H) and phosphorylation of splicing factors ASF and SC35 (I) in neurons. Data in A, C, E, G, and H are representative of three independent experiments. IP, immunoprecipitation; WB, Western blot.
accelerating tau pathologies and cognitive impairments in young htau mice. On the other hand, uncleavable SRPK2 N342A mutant facilitates tau exon 10 exclusion and augments 3R-tau expression levels in FTDP-17 mice, reversing tau pathologies and ameliorating cognitive dysfunctions. In addition to the major function as an SR protein kinase, SRPK2 is involved in the cell cycle regulation, cell proliferation, and neuronal apoptosis (Jang et al., 2009; Giannakouros et al., 2011). Our previous study demonstrates that SRPK2 directly phosphorylates tau on S214 residue and suppresses its MT-binding affinity in AD brains (Hong et al., 2012). Here, we provide extensive evidence supporting that SRPK2 also regulates tau exon 10 alternative splicing in tauopathies via phosphorylating SR-splicing factors including SC35 and ASF/SF2 proteins. Clearly, AEP-cleaved SRPK2 fragments translocate into the nucleus, and
we found that the nuclear translocation of both fragments is comparable as shown by subcellular fractionation in Fig. 3 D, though SRPK2 C343–688 appears a little bit more than SRPK2 N1–342 (Fig. 3 B). However, the nucleus is not the only location where SRPK2 phosphorylates SC35/ASF. In the cytoplasm, phosphorylation of SC35/ASF by active SRPKs leads to nuclear translocation of SC35/ASF, promoting alternative splicing (Liu and Gong, 2008; Zhong et al., 2009). Noticeably, the phosphorylation of SC35 by the cytoplasmic SRPK2 N1–342 elicits much more SC35 to the nucleus (Fig. 3 C), leading to a stronger increase of 4R-tau than SRPK2 C343–688. Further, as shown in Fig. 3 A, SRPK2 N1–342 fragment exhibits higher kinase activity than SRPK2 C343–688, which may also contribute to a more robust phosphorylation of splicing factors SC35/ASF and more abundant 4R-tau expression (Fig. 4 and Fig. 5). Though both fragments triggered AT-8-positive and NFT pathologies in young htau mice, N-terminal 1–342 mice display significantly worse synaptic plasticity and cognitive dysfunctions as compared with C-terminal SRPK2 343–688 fragment (Fig. 5), consistent with its higher activity in phosphorylating both ASF- and SC35-splicing factors and more abundant 4R-tau.

Dysregulation of tau exon 10 splicing causes neurodegenerative diseases (Yen et al., 1999; Ingram and Spillantini, 2002; Umeda et al., 2013). Tau isoforms are balanced with approximately equal ratio of 3R/4R in normal adult human brain; imbalances in 3R/4R ratio have been tightly associated with the pathogenesis of several neurodegenerative diseases, yet the underlying molecular mechanisms remain elusive (Lacovich et al., 2017). Alternative splicing of tau exon 10 is regulated by splicing factors acting on the cis-elements located mainly on exon 10 and intron 10 (Hartmann et al., 2001; Qian et al., 2011). These splicing factors include serine- and arginine-rich (SR) proteins and heterogeneous nuclear ribonucleoproteins as well as tissue-specific factors (Manley and Krainer, 2010). Numerous SR proteins, including SrP55, 9G8, SC35, and ASF/SF2, mediate the alternative splicing of tau exon 10 (Qian et al., 2011; Shi et al., 2011; Ding et al., 2012; Gu et al., 2012). In this regard, one such SR protein is SC35, which promotes exon 10 inclusion by acting on a SC35-like enhancer at the 5′ end of the tau RNA transcript (Qian et al., 2011). Interestingly, phosphorylation of SC35 by protein kinase A enhances inclusion of exon 10, resulting in increased expression of the 3R-tau isoform (Chen et al., 2014). Inclusion of exon 10 generating 4R-tau isoform can lead to enhanced NFTs and tau aggregation (Goedert et al., 1989). In addition to protein kinase A, dual-specificity tyrosine-phosphorylated and regulated kinase 1A (DYRK1) also mediates tau exon 10 splicing via phosphorylating various SR proteins (Shi et al., 2008; Qian et al., 2011; Ding et al., 2012). Here, we show that AEP-truncated SRPK2 translocates into the nucleus, where it strongly phosphorylates SR-splicing factors (e.g., SC35 and ASF/SF2) and regulates tau exon 10 splicing (Fig. 4). Recently, we reported that SRPK2 phosphorylates tau and suppresses tau-dependent MT polymerization, inhibiting axonal elongation in neurons. Depletion of SRPK2 in dentate gyrus inhibits tau phosphorylation in APP/PS1 mouse and improves the defective LTP, alleviating the impairments in cognition. Moreover, active SRPK2 is increased in APP/PS1 mice and human AD brains (Hong et al., 2012). Plausibly, abnormal activation of SRPK2 may phosphorylate numerous substrates including AEP (Wang et al., 2017), tau (Hong et al., 2012), and SR domain-containing splicing factors including acinus (Jang et al., 2008), coordinating the aberrant AD pathologies.

The RNA splicing is performed by the massive protein complex, the spliceosome, which consists of small nuclear ribonucleoproteins (snRNP) and a range of associated splicing regulatory factors (Kornblitt et al., 2013). A comprehensive study of the human brain–insoluble proteome in AD by mass spectrometry has recently been reported. Among thousands of proteins identified, 36 proteins accumulate in AD brains, including U1-70K and other U1 snRNP spliceosome components. Similar accumulation in mild cognitive impairment (cases indicates that spliceosome changes occur in early stages of AD). Multiple U1 snRNP subunits including U1-70K and U1A form cytoplasmic tangle-like structures in AD but not in other neurodegenerative disorders (Bai et al., 2013; Hales et al., 2014). Consequently, evidence of widespread alterations in RNA processing in human AD brains has been observed, suggesting that disruption of neuronal RNA processing may play a key role in AD pathogenesis (Bai et al., 2014). Phosphorylation of a SR protein (ASF/SF2) by either SRPK1 or SRPK2 enhances its interaction with U1-70K, and overexpression of either kinase induces specific redistribution of splicing factors in the nucleus. These observations indicate a function of SRPK family of kinases.
Figure 7. Blocking of SRPK2 cleavage by AEP prevents 4R-tau-induced synaptic dysfunction and cognitive impairment. (A) AEP-uncleavable SRPK2 mutant (N342A) represses 4R-tau expression and SRPK2 1–342 promotes 4R-tau in MAPT intronic mutated FTDP-17 mouse model. Western blot analysis of ratio of 4R-/3R-tau expression and Sarkosyl-insoluble or soluble tau levels in tau609 mice CA1-infected with LV expressing SRPK2 WT, N342A or 1–342. (B) RT-PCR analysis of tau exon 10 splicing in SRPK2 WT/N342A/1-342–expressed tau609 mice CA1. (C) TaqMan probe–based quantification of tau exon 10 splicing. Data represent mean ± SEM of three mice in each group. *, P < 0.05; one-way ANOVA. (D) IHC staining of phosphorylated tau in tau609 mice CA1 expressing...
in spliceosome assembly and in mediating the trafficking of splicing factors in mammalian cells (Wang et al., 1998). Conceivably, Aβ-activated SRPK2 may phosphorylate SR proteins and elicit their association with U1-70K, leading to the cytoplasmic accumulation in AD brains. The abnormal distribution may trigger global RNA processing defects in AD etiology. Remarkably, U1-70K knockdown or antisense oligonucleotide inhibition of U1 snRNP increases the protein level of APP (Bai et al., 2013). Imaginably, SRPK2 may also influence APP metabolism via mediating U1-70K trafficking (Chan and Ye, 2013), in addition to affecting the enzymatic activity of AEP via phosphorylation (Wang et al., 2017).

Tau intronic mutant mice (609 line) display the pathological abnormalities induced just by changing the 3R-/4R-tau ratio, even in the absence of a missense mutation. Patients with this mutation have been reported to present prominent frontotemporal lobar atrophy with neuronal and glial tau inclusions in the form of NFT and pretangles (Janssen et al., 2002; Lantos et al., 2002; Doran et al., 2007). Using WT httau MAPT and tau intronic 10 +16C→T mutation FTDP-17 (609 line) animal models, we manipulated tau exon 10 alternative splicing by administrating AEP-cleaved SRPK2 fragments or uncleavable SRPK2 N342A mutant into the mouse brains, and established that the event of AEP cleavage of SRPK2 plays a critical role in dictating tau exon 10 splicing. Interestingly, AEP-cleaved SRPK2 possesses higher kinase activities and promotes exon 10 inclusion and 4R-tau expression, resulting in neurotoxicity in young MAPT mice. Nonetheless, preventing SRPK2 cleavage by AEP in 609 mice decreases 4R-tau and alleviates tauopathies, improving the cognitive functions (Fig. 5 and Fig. 6). Conceivably, active AEP in tauopathies may regulate tau splicing via proteolytically truncating SRPK2, which feeds back and phosphorylates AEP on S226, decreasing 4R-tau and alleviating tau pathologies, improving the nesting building ability in htau mice (Fig. 5G). Accordingly, augmentation of 4R-tau by overexpressing SRPK2 in 609 mice that exhibit evidently reduced 4R-tau in Sarkosyl-insoluble fraction, bars, 50 µm. Data represent mean ± SEM of 9–12 sections from three mice in each group (*, P < 0.05; **, P < 0.01; one-way ANOVA). (E) Blocking of SRPK2 cleavage by AEP increases the dendritic spine density. Left, Golgi staining was conducted on brain sections from LV-GFP/SRPK2 SRPK2 WT, N342A or 1–342. Treated apical dendritic layer of the CA1 region. Bar, 5 µm. Right, quantitative of spine density represents mean ± SEM of 9–12 sections from three mice in each group (*, P < 0.05; one-way ANOVA). (F) Electrophysiology analysis. Uncleavable SRPK2 (N342A) mutant expression in CA1 rescued the LTP defects in tau609 mice. The ratio of paired pulses in different groups (mean ± SEM; n = 6 in each group); *, P < 0.05 tau609-SRPK2 N342A compared with tau609-GFP; **, P < 0.01 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; left). LTP of EPSPs (mean ± SEM; n = 6 in each group); *, P < 0.05 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; right). The traces are representative EPSPs recorded before (black) and 60 min after (red) TBS. (G) MWM analysis of cognitive functions. Uncleavable SRPK2 (N342A) mutant expression in the CA1 reversed the learning dysfunctions in tau609 mice (mean ± SEM; n = 8–9 mice per group); **, P < 0.01 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; left). SRPK2 N342A rescued the impaired memory, while SRPK2 1–342 exacerbated memory impairment in 609 mice (right). Data represent mean ± SEM of eight to nine mice in each group (*, P < 0.05; one-way ANOVA). (H) Nesting activity analysis. There is no significant difference in the three groups. MW, molecular weight; WB, Western blot.

It is known that tau exon 10 splicing is developmentally regulated; in fetal brains, 3R-tau is exclusively expressed in rodents and humans; however, in adult brains, 4R-tau expression becomes exclusive in rodents, whereas 3R- and 4R-tau are comparably expressed at a ratio of almost 1:1 in humans (Goedert et al., 1989; Kosik et al., 1989). Thus, mutation-induced alteration of tau exon 10 splicing presumably causes pathological imbalances in 3R- and 4R-tau, potentially leading to neurodegeneration. Although there is no consensus in the field about the imbalance ratio of 3R/4R in AD (Murray et al., 2014), it is clear that unbalanced ratios of 3R/4R occur in diseases such as PSP and corticobasal degeneration. It remains unclear of how imbalance of tau isoforms causes neurodegenerative diseases. The neurodegeneration occurs regardless of which way of the exon 10 tilts. Hence, the balance between 3R and 4R isoforms must remain within a narrow window to ensure normal neuronal functions. Perhaps the 1:1 3R/4R ratio is critical for MT dynamics in specific contexts within various cell types of the human brain, a balancing act between fluidity and stability (Andreadis, 2012). Collectively, our current finding provides innovative insight into the molecular mechanism of how AEP and SRPK2 crosstalk may provoke tau isoform imbalance in various tauopathies.

Materials and methods

Mice, primary cultured neurons, cell lines, and human tissue samples

5XFAD mice, WT C57BL/6J mice, and MAPT mice were ordered from the Jackson Laboratory (stock nos. 006554, 000664, and 005491, respectively). Tau intronic 10 +16C→T mutation FTDP-17 mice (609 line) were a gift from H. Mori (Osaka City University, Osaka, Japan). The Lgmn−/− mice on a mixed 129/Ola and C57BL/6 background were generated as previously reported (Shirahama-Noda et al., 2003). Lgmn−/− mice were crossed with 5XFAD mice to generate 5XFAD/Lgmn−/− mice. In animal experiments related to Fig. 1 (A and D), Fig. 2 B, Fig. 6, Fig. 7, Fig. S2, and Fig. S5, WT SRPK2 WT, N342A or 1–342. AT8 and AT100 signals were reduced in uncleavable SRPK2 mutant–infected hippocampus and increased in 1–342 group. Bars, 50 µm. Data represent mean ± SEM of 9–12 sections from three mice in each group (*, P < 0.05; **, P < 0.01; one-way ANOVA). (E) Blocking of SRPK2 cleavage by AEP increases the dendritic spine density. Left, Golgi staining was conducted on brain sections from LV-GFP/SRPK2 SRPK2 WT, N342A or 1–342. Treated apical dendritic layer of the CA1 region. Bar, 5 µm. Right, quantitative of spine density represents mean ± SEM of 9–12 sections from three mice in each group (*, P < 0.05; one-way ANOVA). (F) Electrophysiology analysis. Uncleavable SRPK2 (N342A) mutant expression in CA1 rescued the LTP defects in tau609 mice. The ratio of paired pulses in different groups (mean ± SEM; n = 6 in each group); *, P < 0.05 tau609-SRPK2 N342A compared with tau609-GFP; **, P < 0.01 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; left). LTP of EPSPs (mean ± SEM; n = 6 in each group); *, P < 0.05 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; right). The traces are representative EPSPs recorded before (black) and 60 min after (red) TBS. (G) MWM analysis of cognitive functions. Uncleavable SRPK2 (N342A) mutant expression in the CA1 reversed the learning dysfunctions in tau609 mice (mean ± SEM; n = 8–9 mice per group); **, P < 0.01 tau609-SRPK2 N342A compared with tau609-GFP; one-way ANOVA; left). SRPK2 N342A rescued the impaired memory, while SRPK2 1–342 exacerbated memory impairment in 609 mice (right). Data represent mean ± SEM of eight to nine mice in each group (*, P < 0.05; one-way ANOVA). (H) Nesting activity analysis. There is no significant difference in the three groups. MW, molecular weight; WB, Western blot.
littermate mice were used as controls. Animal care and handling was performed according to National Institutes of Health (NIH) animal care guidelines and Emory Medical School guidelines. The protocol was reviewed and approved by the Emory Institutional Animal Care and Use Committee. Primary cortical neurons were cultured as previously described (Zhang et al., 2014). All rats were purchased from the Jackson Laboratory. The study was approved by the Biospecimen Committee. AD was diagnosed according to the criteria of the Consortium to Establish a Registry for AD and the National Institute on Aging. Diagnoses were confirmed by the presence of amyloid plaques and neurofibrillary tangles in formalin-fixed tissue. Informed consent was obtained from the subjects. Diagnosis criteria used for FTDP-17 and PSP brains were assigned using current criteria (Hauw et al., 1994; Foster et al., 1997). Evidence for the presence or absence of silver-positive neuronal tau inclusions are shown in Fig. S3 A. Detailed reagents and resources are provided in Table S1.

Plasmids

FL, truncated, and site-mutated SRPK2 were inserted into pEGFP-C2 between BglII and SalI cutting sites, or into pFCGW-N1 between BamHI and EcoRI cutting sites for LV package. The shRNA sequences targeting SRPK2 were fused into the lentiviral vector pFHUGW. The sites indicated for tau protein represent the amino acids in the longest form of human tau protein (Tau441). The target sequences recognized by shSRPK2 (5′-GCA GAGAGTGATTACAGGTAT-3′) were acquired from Sigma-Aldrich MISSION shRNA library. pCI/SI9–SI1 was a gift from F. Liu (New York State Institute for Basic Research in Developmental Disabilities, New York, NY).

LV packaging

LV packaging system (pFCGW-N1) was acquired from the Viral Core Facility of Emory University. Transfection of the lentiviral vectors were performed in HEK293FT cells (50–60% confluent) with calcium phosphate reagents. After 16 h, the transfection media were discarded, and the cells were washed with sterile PBS and incubated with prewarmed fresh culture media. After 48 h incubation, the cell culture media were collected, and the lentiviral particles were concentrated by ultracentrifugation at 22,000 RPM (SW28) for 2 h at 4°C. All the LV-containing materials were handled according to the Biosafety Regulation of Emory University. Viral titer was ∼1–5 × 10⁴ IU/ml.

Transfection and infection of the cells

HEK293 transfection was performed using Lipofectamine 2000 (Invitrogen). SH-SY5Y transfection was performed using Lipo-fectamine 3000 (Invitrogen). The LVs used in neuronal infection were packaged in Viral Vector Core of Emory University.

Quantitation of tau exon 10 splicing by RT-PCR

For cell experiment, total cellular RNA was isolated from cultured cells by using a RNeasy mini kit (Qiagen GmbH). 600 ng of total RNA was used for first-strand Cdna synthesis with oligo (dT)18 by using an Omniscript reverse transcription kit (Qiagen GmbH). PCR was performed by using Prime-STARTM HS DNA Polymerase (Takara Bio Inc.) with primers (forward, 5′-GGGTGTC CACTCCCCCCGTCAA-3′; and reverse, 5′-CCCTGGTTATATGGGAT GTGGCCTAAGG-3′) for human tau in HEK293 cells transfected with pCI/SI9–SI1, and with primers (forward, 5′-CAGGAAAAT CCGGAGGGAGA-3′; and reverse, 5′-CTTTGTCAGGTCTACCCGGC-3′) for mouse tau to measure alternative splicing of tau exon 10 under the following conditions: denaturation for 5 min at 98°C was followed by 30 cycles with denaturation for 10 s at 98°C, annealing for 15 s at 55°C, polymerization for 30 s at 72°C, and a final extension for 10 min at 72°C. The PCR products were resolved on 1.5% agarose gels and quantitated using the Molecular Imager system (Bio-Rad).

For SH-SY5Y cells and MAPT mice mRNA, the sequences of the primers were as follows: forward, 5′-CTGAAAAATCAGGG ATGCC-3′; and reverse, 5′-CTTTGTCAGGTCTACCTGGT-3′. RT was omitted in control reactions. RT-PCR products were separated in 2% (wt/vol) agarose gels and stained with ethidium bromide. Isoforms were identified based on their expected sizes. Products containing exon 10 (4R) were 390 bp, while those without exon 10 (3R) were 253 bp.

For tau 609 mice, primer sequences used were as follows: human exon 9F, 5′-CTGAAAAATCAGGG ATGCC-3′; and human exon 11R, 5′-CCCTGGTCAAGGTCTACCTGGT-3′. PCR was performed as 30 cycles at 94°C for 30 s, at 62°C for 30 s, and at 72°C for 45 s, with a final 72°C extension for 10 min. The expected sizes of PCR products were 390 bp for exon 10 mRNA and 297 bp for exon 10 mRNA. Relative amounts of 4R- and 3R-tau RNA were estimated by densitometric analysis of the gels using the Image J software (NIH).

TaqMan probe–based quantification

The levels of mRNA were analyzed by real-time, quantitative PCR. RNA was isolated by Trizol (Life Technologies). RT was performed with SuperScriptIII reverse transcription (Life Technologies). Gene-specific primers and probes were designed and bought from TaqMan (Life Technologies).

Changes in MAPT expression were detected quantitative real-time PCR was performed using a ABI 7500-Fast Real-Time PCR System with TaqMan Fast Universal PCR Master Mix and Applied Biosystems TaqMan Gene Expression Assay Mix, ID no. Hs00902194_m1. This probe binds the junction of MAPT exons 12–13; constitutive exons present in all isoforms and therefore this assay helps determine total levels of all MAPT transcripts.

Primers and probes for 3R and 4R human MAPT mRNA were designed as reported (Connell et al., 2005). For 3R isoform detection, the sequences used were as follows: forward, 5′-GAGCGGGGAGGTGCAAAT-3′; and reverse, 5′-GGTGGTTG CTAATGAGCCAC-3′; probe, 5′-AACAACAGTTGACCTAGCCA GGTGACCTCG-3′.
homogenates were spun for 15 min at 80,000 g, and the supernatant was saved as the cytosolic fraction. The 20,000-g pellet was collected. After protein quantification, the samples were centrifuged at 105,000 g for 1 h, and the resulting supernatants were discarded, and the pellets were resuspended and layered over 36 ml of 27% (vol/vol) Percoll and centrifuged at 20,000 g for 90 min. The lysosomes were used for Western blots.

Generation of antibodies that specifically recognize the AEP-generated SRPK2 fragment (anti-SRPK2 N342 and anti-SRPK2 C343)
The anti-SRPK2 N342 and anti-SRPK2 C343 antibodies were generated by immunizing rabbits with the following peptides: Ac-CAEAETAKDN-OH (anti-SRPK2 N342) and H2N-GEAEDQ EEKC-amide (anti-SRPK2 C343), respectively. The antiserum was pooled, and the titers against the immunizing peptide were determined by ELISA. The maximal dilution giving a positive response using chromogenic substrate for HRP was >1:30,000. The immunoreactivity of the antisera was further confirmed by Western blot and IHC.

Subcellular fractionation
The protocol was as previously described (Basurto-Islas et al., 2013). Brain tissue and SH-SY5Y cell lysates were prepared in three volumes of cold homogenizing buffer (0.32 M sucrose, 1 mM EDTA disodium, and 10 mM Hepes, pH 7.4). Lysates were centrifuged at 14,000 g at 4°C. The supernatants were then incubated at 37°C for 0.5 h, followed by the incubation with primary antibody at 4°C overnight, and with the secondary antibody at room temperature for 1 h, followed by the incubation with primary antibody at 4°C overnight, and with the secondary antibody at room temperature for 1 h. After washing with TBS, the membrane was developed using the ECL detection system.

IHC
IHC was performed by using the peroxidase protocol. In brief, tissue sections were deparaffinized in xylene, hydrated through descending ethanol, and endogenous peroxidase activity was eliminated by incubation in 3% hydrogen peroxide in methanol for 5 min. After antigen-retrieval in boiling sodium citrate buffer (100 µl reaction buffer (20 mM citric acid, 60 mM Na2HPO4, 1 mM EDTA, 0.1% CHAPS, and 1 mM DTT, pH 5.5) containing 20 µM AEP substrate Z-Ala-Ala-Asn-AMC (Bachem). AMC released by substrate cleavage was quantified by measuring at 460 nm in a fluorescence plate reader at 37°C in kinetic mode.

AEP activity assay
Tissue homogenates or cell lysates (10 µg) were incubated in 200 µl reaction buffer (20 mM citric acid, 60 mM Na2HPO4, 1 mM EDTA, 0.1% CHAPS, and 1 mM DTT, pH 5.5) containing 20 µM AEP substrate Z-Ala-Ala-Asn-AMC (Bachem). AMC released by substrate cleavage was quantified by measuring at 460 nm in a fluorescence plate reader at 37°C in kinetic mode.

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titors >1 × 10⁹ IU/ml) were delivered at a rate of 0.3 µl/min (an-

Mice were anesthetized with isoflurane, and LV (3 µl with similar 

Stereotaxic injection of LV in mouse CA1 

post-synaptic densities. 

Synapses were identified by the presence of synaptic vesicles and 

sections (90 nm) were stained with uranyl acetate and lead ace-

prepared and examined using standard procedures. Ultrathin 

CX-31) and perfused at a rate of 3 ml/min with a-CSF (containing 

Instruments) on the stage of an upright microscope (Olympus 

was used for the intensity and colocalization analysis.

Gallyas silver staining 

Silver staining was performed using the Gallyas method. A semi-

quantitative subjective scoring system was developed based on rel-

ative numbers of silver-positive cells per section, analyzing four 

sections per animal. Silver stain scoring was based on the numbers 

of positive neurons, not intensity, which can vary between sec-

After deparaffinization, 5-µm sections were incubated in 

5% periodic acid for 5 min, washed in water, and then placed in 

alkaline silver iodide solution (containing 1% silver nitrate) for 1 

min. The sections were then washed in 0.5% acetic acid for 10 min, 

placed in developer solution for 15 min, before washing with 0.5% 

acetic acid, then water. The sections were then treated with 0.1% 

gold chloride for 5 min before washing in water, and incubation in 1% sodium thiosulphate (hypo) for 5 min, before a final wash.

EM 

After deep anesthesia, mice were perfused transcardially with 

2% glutaraldehyde and 3% paraformaldehyde in PBS. Hippocam-

pal slices were post-fixed in cold 1% OsO₄ for 1 h. Samples were 

prepared and examined using standard procedures. Ultrathin 

sections (90 nm) were stained with uranyl acetate and lead ace-

tate and viewed at 100 kV in a JEOL 2000CX electron microscope. 

Synapses were identified by the presence of synaptic vesicles and 

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Stereoaxic injection of LV in mouse CA1 

Mice were anesthetized with isoflurane, and LV (3 µl with similar 
titers >1 × 10⁹ IU/ml) were delivered at a rate of 0.3 µl/min (an-
teroposterior, −2.2 mm; mediolateral, ±1.7 mm; dorsoventral, −1.6 

mm). Mice were assigned into gender- and age-matched treat-

ment groups using a randomized block design.

Golgi staining 

Mouse brains were fixed in 10% formalin for 24 h, and then 

immersed in 3% potassium bichromate for 3 d in the dark. The 
solution was changed each day. Then the brains were transferred 

into 2% silver nitrate solution and incubated for 24 h in the dark. 

Vibratome sections were cut at 60 µm, air dried for 10 min, de-

hydrated through 95% and 100% ethanol, cleared in xylene, and 
coverslipped.

Electrophysiology 

Acute hippocampal transversal slices were prepared from mice 

injected with LV as previously described (Zhang et al., 2014, 

2015). In brief, mice were anesthetized with isoflurane, de-
capitated, and their brains were dropped in ice-cold artificial 
cerebrospinal fluid (a-CSF) containing 124 mM NaCl, 3 mM KCl, 
1.25 mM NaH₂PO₄, 6.0 mM MgCl₂, 26 mM NaHCO₃, 2.0 mM 
CaCl₂, and 10 mM glucose. Hippocampi were dissected and cut 

into 400-µm-thick transverse slices with a vibratome. After 

incubation at room temperature (23–24°C) in a-CSF for 60–90 

min, slices were placed in a recording chamber (RC-22C; Warner 

Instruments) on the stage of an upright microscope (Olympus 

CX-31) and perfused at a rate of 3 ml/min with a-CSF (containing 

1 mM MgCl₂) at 23–24°C. A 0.1 MΩ tungsten monopolar electrode 

was used to stimulate the Schaffer collaterals. The fEPSPs were 

recorded in CA1 stratum radiatum by a glass microelectrode filled 

with a-CSF with a resistance of 3–4 MΩ. The stimulation output 

(Master-8; AMPI) was controlled by the trigger function of an 
EPC9 amplifier (HEKA Elektronik). fEPSPs were recorded under 
current-clamp mode. Data were filtered at 3 kHz and digitized at 
sampling rates of 20 kHz using Pulse software (HEKA Elek-
tronik). The stimulus intensity (0.1-ms duration, 10–30 µA) was 
set to evoke 40% of the maximum fEPSP, and the test pulse was 

applied at a rate of 0.033 Hz. LTP of fEPSPs was induced by three 
TBs (four pulses at 100 Hz, repeated three times with a 200-ms 
interval). The magnitudes of LTP are expressed as the mean per-
centage of baseline fEPSP initial slope.

MWM 

Mice injected with the various LVs were trained in a round, wa-
ter-filled tub (52-inch diameter) in an environment rich with 
extra maze cues as described previously (Zhang et al., 2014). Each 
subject was given four trials a day for five consecutive days with 
a 15-min intertrial interval. The maximum trial length was 60 s, 
and if subjects did not reach the platform in the allotted time, 
they were manually guided to it. Following the 5 d of task acquisi-
tion, a probe trial was presented, during which time the platform 
was removed, and the percentage of time spent in the quadrant 
that previously contained the escape platform during task ac-
quision was measured over 60 s. All trials were analyzed for 
latency and swim speed by means of MazeScan (Clever Sys, Inc.).

Contextual fear conditioning 

The ability to form and retain an association between an aversive 
experience and environmental cues was tested with a standard fear 
conditioning paradigm that occurred over a period of 3 d. Mice were 
placed in the fear conditioning apparatus (width, 7 inches; depth, 7 

inches; height, 12 inches; Coulbourn) composed of Plexiglas with 
a metal shock grid floor and allowed to explore the enclosure for 3 
min. Following this habituation period, three conditioned stimulus 
(CS)—unconditioned stimulus (US) pairings were presented with a 
1-min intertrial interval. The CS was composed of a 20-s, 85-dB 
tone, and US was composed of 2 s of a 0.5-mA foot shock, which 
was coterminate with each CS presentation. 1 min following the last 
CS–US presentation, mice were returned to their home cage. On 
day 2, the mice were presented with a context test, during which 
subjects were placed in the same chamber used during conditioning 
on day 1, and the amount of freezing was recorded via a camera and 
the software provided by Coulbourn. No shocks were given during 
the context test. On day 3, a tone test was presented, during which 
time subjects were exposed to the CS in a novel compartment. Ini-
tially, animals were allowed to explore the novel context for 2 min. 
Then the 85-db tone was presented for 6 min and the amount of 
freezing behavior was recorded.

Nesting activity 

Mice were individually housed and previous nesting material 
was removed. An intact 3.0-g nestlet was placed within the cages. 
Approximately 14 h later, following the nocturnal period, untorn 
nestlet was weighed.
Quantification and statistical analysis
All data are expressed as mean ± SEM from three or more independent experiments, and the level of significance between two groups was assessed with Student’s t test. For more than two groups, one-way ANOVA followed by least-significant difference post hoc test was applied. A value of P < 0.05 was considered to be statistically significant.

Online supplemental material
Fig. S1, related to Fig. 1, presents additional evidence showing that AEP cleaves SRPK2 in vitro. Fig. S2, related to Fig. 2, presents validation of the specificity of anti-cleaved SRPK2 antibodies. Fig. S3, related to Fig. 2, presents additional information of AEP-cleaved SRPK2 in human 4R-tauopathy brains. Fig. S4, related to Fig. 3, additionally shows that cleavage of SRPK2 by AEP enhances its kinase activity. Fig. S5, related to Fig. 6 and Fig. 7, shows that AEP-truncated SRPK2 in hippocampal CA1 accelerates the synapse loss and exacerbates cognitive impairment in mouse models of tauopathy. Table S1 provides information on reagents and resources of this study.

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Author contributions: K. Ye conceived the project, designed the experiments, analyzed the data, and wrote the manuscript. Z.-H. Wang designed and performed most of the experiments. P. Liu and X. Liu prepared primary neurons and assisted with animal experiments. S.-P. Yi and J.-Z. Wang assisted with data and resources. S. Liu prepared primary neurons and assisted with animal experiments. P. Liu and X. Liu prepared primary neurons and assisted with animal experiments. J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Basurto-Islas, G., I. Grundke-Iqbal, Y.C. Tung, F. Liu, and K. Iqbal. 2013. Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. J. Biol. Chem. 288:17495–17507. https://doi.org/10.1074/jbc.M112.446070

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Basurto-Islas, G., I. Grundke-Iqbal, Y.C. Tung, F. Liu, and K. Iqbal. 2013. Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. J. Biol. Chem. 288:17495–17507. https://doi.org/10.1074/jbc.M112.446070

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Basurto-Islas, G., I. Grundke-Iqbal, Y.C. Tung, F. Liu, and K. Iqbal. 2013. Activation of asparaginyl endopeptidase leads to Tau hyperphosphorylation in Alzheimer disease. J. Biol. Chem. 288:17495–17507. https://doi.org/10.1074/jbc.M112.446070

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

https://doi.org/10.1074/jbc.M112.446070

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

https://doi.org/10.1074/jbc.M112.446070

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

Bai, B., P.C. Chen, C.M. Hales, Z. Wu, V. Pagala, A.A. High, A.I. Levey, J.J. Lah, and J. Peng. 2014. Integrated approaches for analyzing U1-70K cleavage in Alzheimer’s disease. J. Proteome Res. 13:4526–4534. https://doi.org/10.1021/pr5003593

https://doi.org/10.1074/jbc.M112.446070
Inherited frontotemporal dementia in nine British families associated with a mutational defect in SC35 and Dyrk1A. Neur. Cell. 18:80–90. https://doi.org/10.1016/j.neuron.2001.1000

Qian, W., H. Liang, J. Shi, N. Jin, I. Grundke-Iqbal, K. Iqbal, C.X. Gong, and F. Liu. 2011. Regulation of the alternative splicing of tau exon 10 by SC35 and Dyrk1A. Nucleic Acids Res. 39:6161–6171. https://doi.org/10.1093/nar/gkr195

Schoch, K.M., S.L. DeVos, R.L. Miller, S.J. Chun, M. Norrbom, D.F. Wozniak, H.N. Dawson, C.F. Bennett, F. Rigo, and T.M. Miller. 2016. Increased 4R-Tau Induces Pathological Changes in a Human-Tau Mouse Model. Neuron. 90:941–947. https://doi.org/10.1016/j.neuron.2016.04.042

Shi, J., T. Zhang, C. Zhou, M.O. Chohan, X. Gu, J. Wegiel, J. Zhou, Y.W. Hwang, K. Iqbal, I. Grundke-Iqbal, et al. 2008. Increased dosage of Dyrk1A alters alternative splicing factor (ASF)-regulated alternative splicing of tau in Down syndrome. J. Biol. Chem. 283:28660–28669. https://doi.org/10.1074/jbc.M802645200

Shi, J., W. Qian, X. Yin, K. Iqbal, I. Grundke-Iqbal, X. Gu, F. Ding, C.X. Gong, and F. Liu. 2011. Cyclic AMP-dependent protein kinase regulates the alternative splicing of tau exon 10: a mechanism involved in tau pathology of Alzheimer’s disease. J. Biol. Chem. 286:14639–14648. https://doi.org/10.1074/jbc.M111.245436

Shirahama–Noda, K., A. Yamamoto, K. Sugihara, N. Hashimoto, M. Asano, M. Nishimura, and I. Hara-Nishimura. 2003. Biosynthetic processing of cathepsins and lysosomal degradation are abolished in asparaginyl endopeptidase-deficient mice. J. Biol. Chem. 278:33194–33199. https://doi.org/10.1074/jbc.M302742200

Spillantini, M.G., J.R. Murrell, M. Goedert, M.R. Farlow, A. Klug, and B. Ghetti. 1998. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc. Natl. Acad. Sci. USA. 95:7377–7381. https://doi.org/10.1073/pnas.95.13.7737

Umeda, T., T. Yamashita, T. Kimura, K. Oshimi, H. Takuma, T. Ozeki, A. Takashima, T. Tomiyama, and H. Morii. 2013. Neurodegenerative disorder FTD-17-related tau intron 10 +16→ + T mutation increases tau exon 10 splicing and causes tauopathy in transgenic mice. Am. J. Pathol. 183:211–225. https://doi.org/10.1016/j.ajpath.2013.03.015

Wang, X., and E. Mandelkow. 2016. Tau in physiology and pathology. Nat. Rev. Neurosci. 17:5–21. https://doi.org/10.1038/nrn.2015.1

Wang, H.Y., W. Lin, J.A. Dyck, J.M. Yeakly, Z. Songyang, L.C. Cantley, and X.D. Yen. 1999. Fibrillogenic activity of the amyloid precursor protein and regulates the pathogenesis in Alzheimer’s disease. J. Biol. Chem. 268:4559–4570. https://doi.org/10.1074/jbc.68.125–126.2001

Shi, J., W. Qian, X. Yin, K. Iqbal, I. Grundke-Iqbal, X. Gu, F. Ding, C.X. Gong, and F. Liu. 2011. Cyclic AMP-dependent protein kinase regulates the alternative splicing of tau exon 10: a mechanism involved in tau pathology of Alzheimer’s disease. J. Biol. Chem. 286:14639–14648. https://doi.org/10.1074/jbc.M111.245436

Yoshida, M. 2006. Cellular tau pathology and immunohistochemical study of tau isoforms in sporadic tauopathies. Neuropathology. 26:457–470. https://doi.org/10.1007/s00093-006-00743-x

Yoshida, M. 2006. Cellular tau pathology and immunohistochemical study of tau isoforms in sporadic tauopathies. Neuropathology. 26:457–470. https://doi.org/10.1007/s00093-006-00743-x

Zhang, Z., M. Song, X. Liu, S.S. Kang, I.S. Kwon, D.M. Duong, N.T. Seyfried, W.T. Hu, Z. Jiang, Z.W. Wang, et al. 2014. Cleavage of tau by asparagine endopeptidase mediates the neurofibrillary pathology in Alzheimer’s disease. J. Biol. Chem. 289:1245–1256. https://doi.org/10.1074/jbc.M114.57077-9

Zhang, Z., M. Song, X. Liu, S.S. Kang, I.S. Kwon, D.M. Duong, N.T. Seyfried, X. Cao, L. Cheng, Y.E. Sun, S. Ping Yu, et al. 2015. Delta-secretase cleaves amyloid precursor protein and regulates the pathogenesis in Alzheimer’s disease. Nat. Commun. 6:6786. https://doi.org/10.1038/ncomms7972

Zhang, Z., M. Xie, and K. Ye. 2016. Asparaginyl endopeptidase is an innovative therapeutic target for neurodegenerative diseases. Expert Opin. Ther. Targets. 20:1254–1245. https://doi.org/10.1517/147282016.1182990

Zhang, Z., S.S. Kang, X. Liu, E.H. Ahn, Z. Zhang, L. He, P.M. Iuvone, D.M. Duong, N.T. Seyfried, M.J. Benksey, et al. 2017a. Asparagine endopeptidase cleaves α-synuclein and mediates pathologic activities in Parkinson’s disease. Expert Opin. Ther. Targets. 8:555–562. https://doi.org/10.1523/JNEUROSCI.3300-12.2012

Kornblihtt, A.R., I.E. Schor, M. Allo, G. Dujardin, E. Petrillo, and M.J. Munoz. 2013. Alternative splicing: a pivotal step between euukaryotic transcription and translation. Nat. Rev. Mol. Cell. Biol. 14:153–165. https://doi.org/10.1038/nrm3525

Kosik, K.S., L.D. Orcchio, S. Bakalis, and R.L. Neve. 1989. Developmentally regulated expression of specific tau sequences. Neuron. 2:1389–1397. https://doi.org/10.1016/0896-6273(89)90079-3

Kuroyanagi, N., O. Negishi, T. Okuno, and M. Hagiwara. 1998. Novel SR-proline-serine/arginine protein-specific kinase2 (SRPK2) translocates into the nucleus and promotes apoptosis. J. Biol. Chem. 273:777–786. https://doi.org/10.1074/jbc.19979913

Liu, F., and C.-X. Gong. 2008. Tau exon 10 alternative splicing and tauopathies. Mol. Neurodegener. 3:8. https://doi.org/10.1186/1750-1328-3-8

Manley, J.L., and A.R. Krainer. 2010. A rational nomenclature for serine/arginine-rich protein splicing factors (SR proteins). Genes Dev. 24:1073–1074. https://doi.org/10.1101/gad.193490

Murray, M.E., N. Kouri, W.-L. Lin, C.R. Jack Jr., D.W. Dickson, and P. Vemuri. 2014. Clinicopathologic assessment and imaging of tauopathies in neurodegenerative dementias. Alzheimers Res. Ther. 6:1. https://doi.org/10.1186/alzhiemersres ther.6.1

Neumann, M., W. Schutz-Schaeffer, R.A. Crowther, M.J. Smith, M.G. Spillantini, M. Goedert, and H.A. Kretzschmar. 2001. Pick’s disease associated with the novel Tau gene mutation K369I. Ann. Neurol. 50:503–513. https://doi.org/10.1002/ana.1223

Pickering-Brown, S.M., A.M. Richardson, J.S. Snowden, A.M. McDonagh, A. Burns, W. Braude, M. Baker, W.K. Liu, S.H. Yen, J. Hardy, et al. 2002. AEP-cleaved SRPK2 promotes tau exon 10 inclusion

Wang et al. https://doi.org/10.1038/jem.20180539

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