Supplemental Information

In vivo hyperphosphorylation of tau is associated with synaptic loss and behavioral abnormalities in the absence of tau seeds

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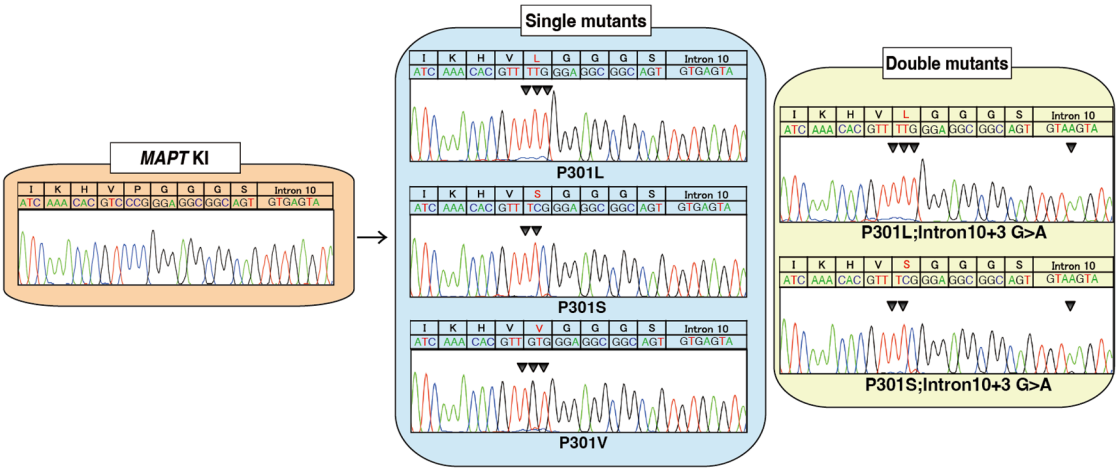
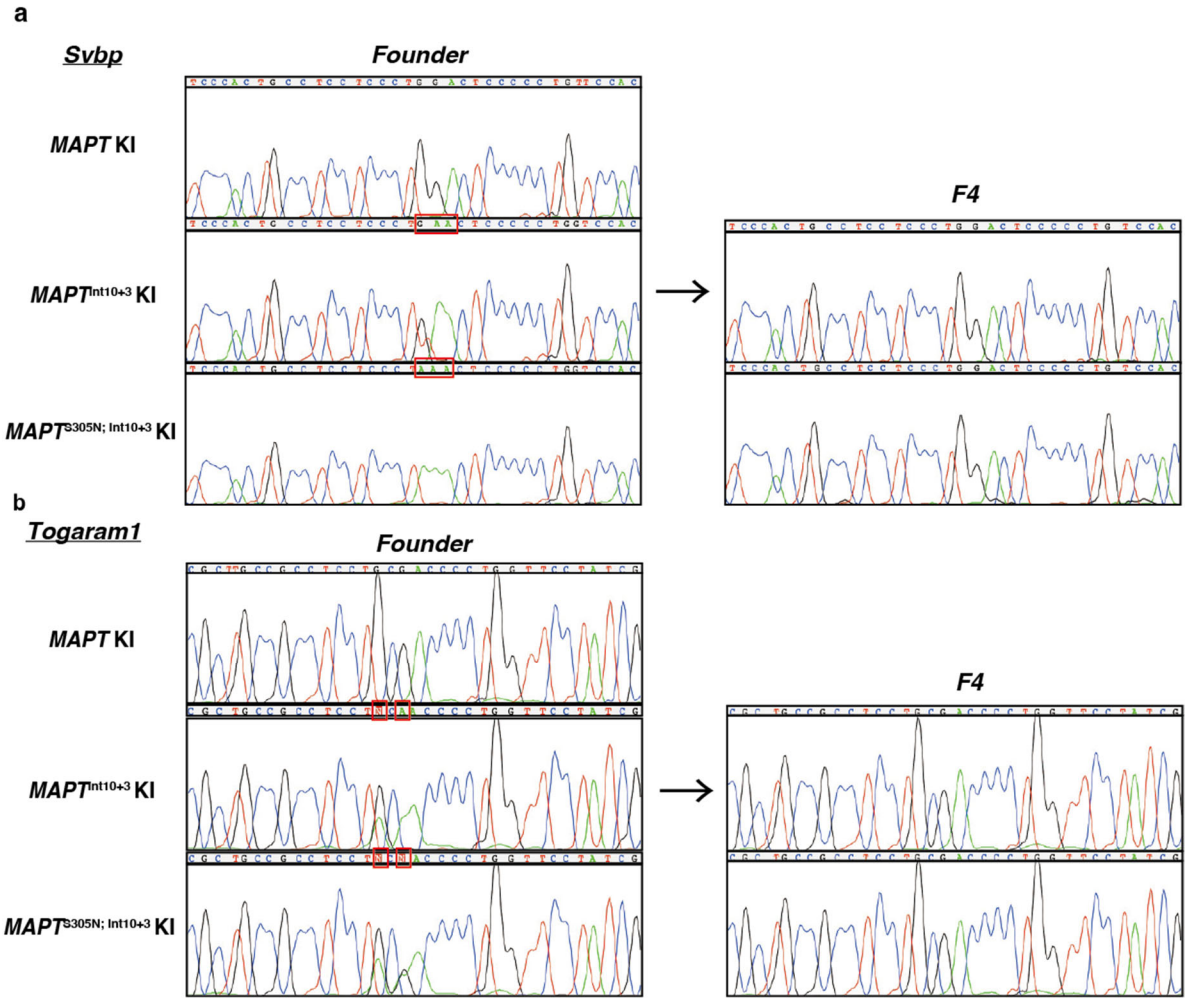
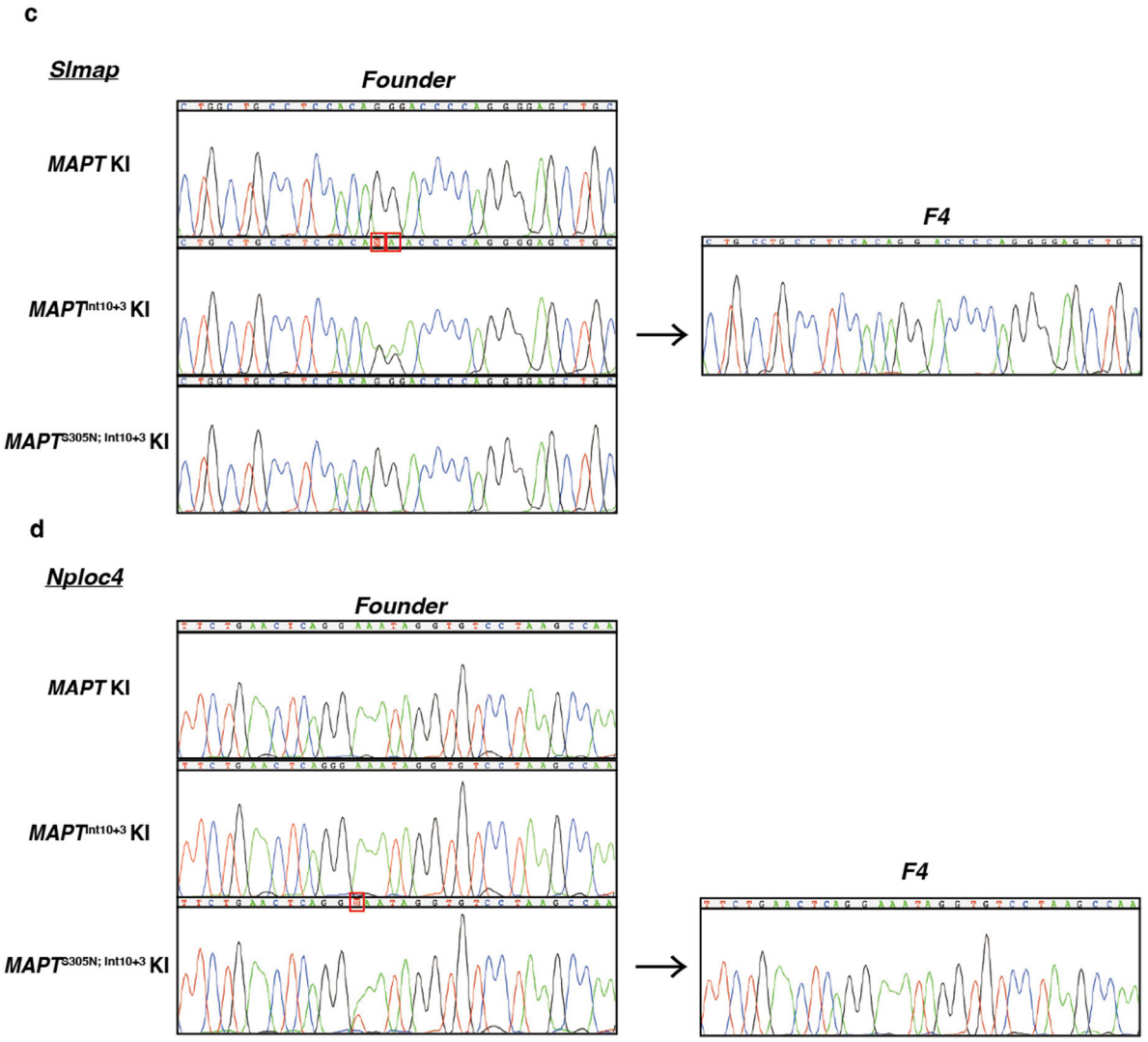


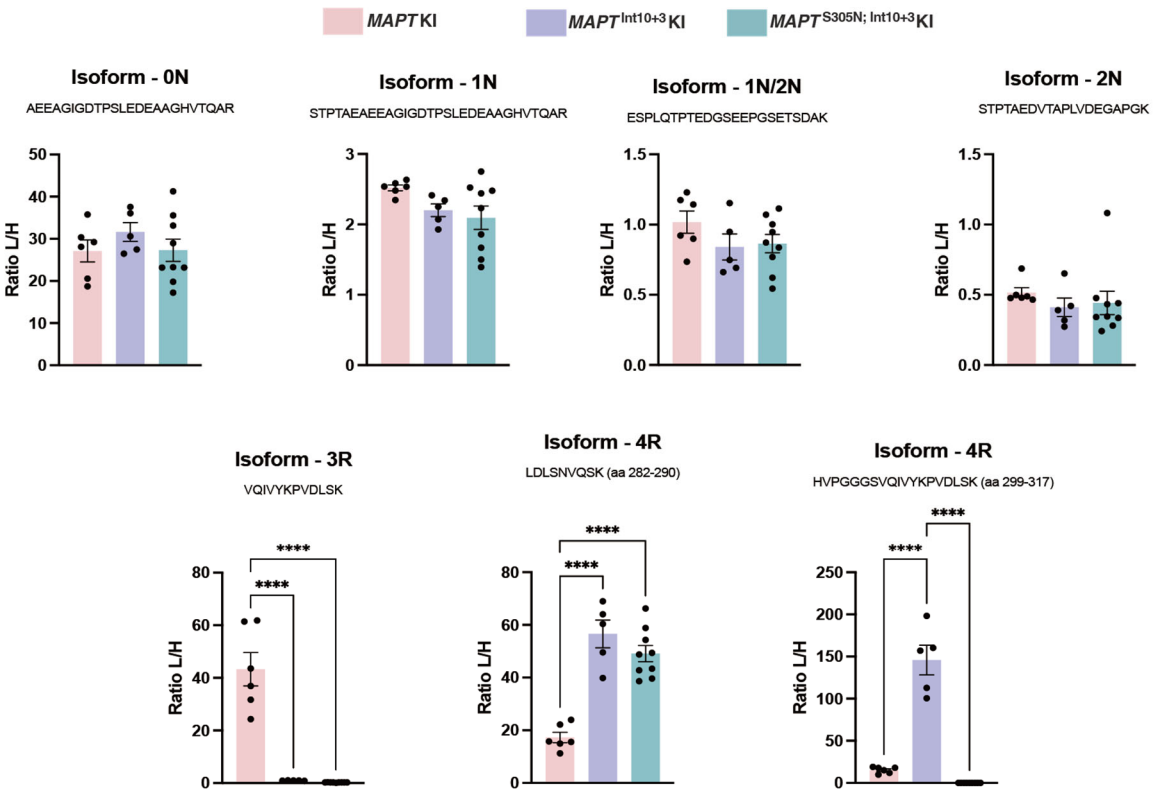
Supplemental Figure 1. Sanger sequencing of other mutant *MAPT* KI mice. Sanger sequencing results determined *MAPT*^{P301L} KI, *MAPT*^{P301S} KI, *MAPT*^{P301V} KI, *MAPT*$^{P301L;\ Int10+3}$ KI, and *MAPT*$^{P301S;\ Int10+3}$ KI mice. Mutation loci are indicated by arrowheads in black and the substituted amino acids are highlighted in red.


a
Svbp
Founder
F4
MAPT KI
MAPTInt10+3 KI
MAPTS305N; Int10+3 KI
b
Togaram1
Founder
F4
MAPT KI
MAPTInt10+3 KI
MAPTS305N; Int10+3 KI



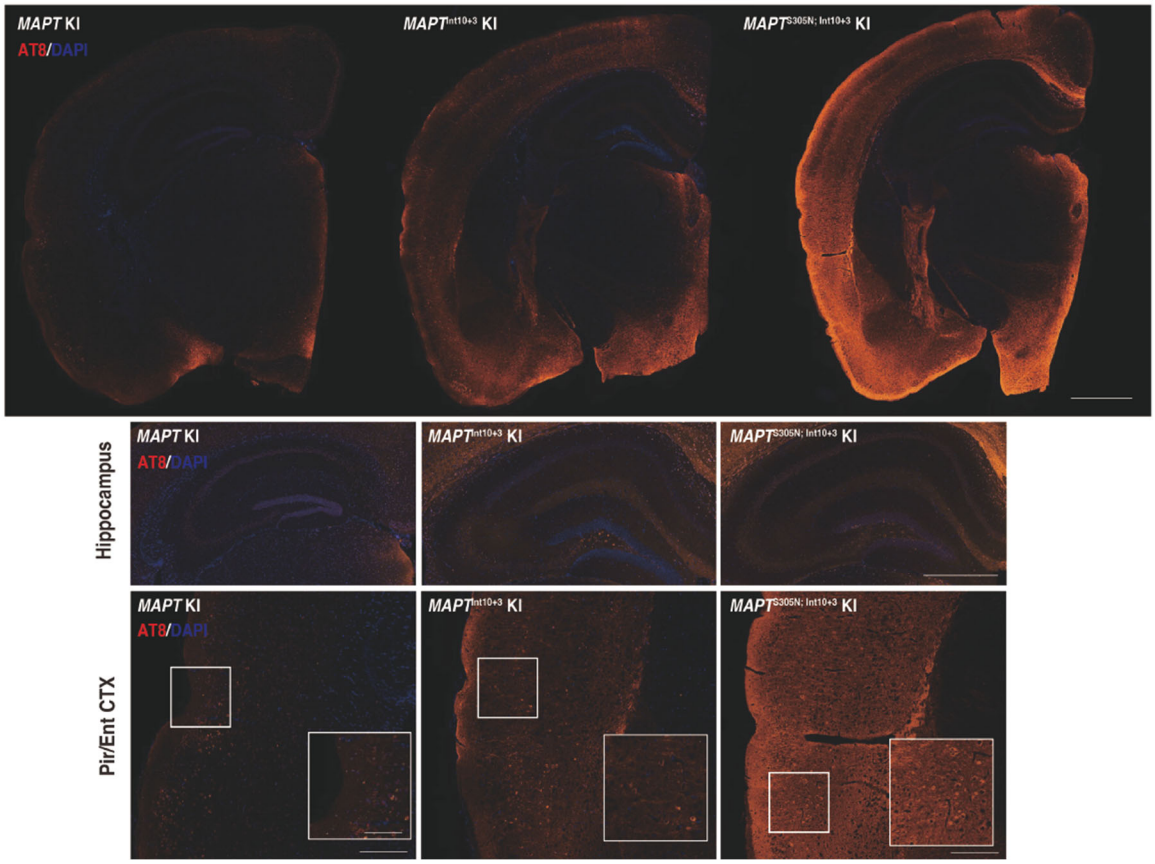
Supplemental Figure 2. Removal of off-target mutations in *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N; Int10+3}$ KI mice by backcrossing with C57B6/J mice.

a-d, Off-target mutations identified in founders (highlighted in red boxes) were removed by backcrossing with C57B6/J mice more than four times.

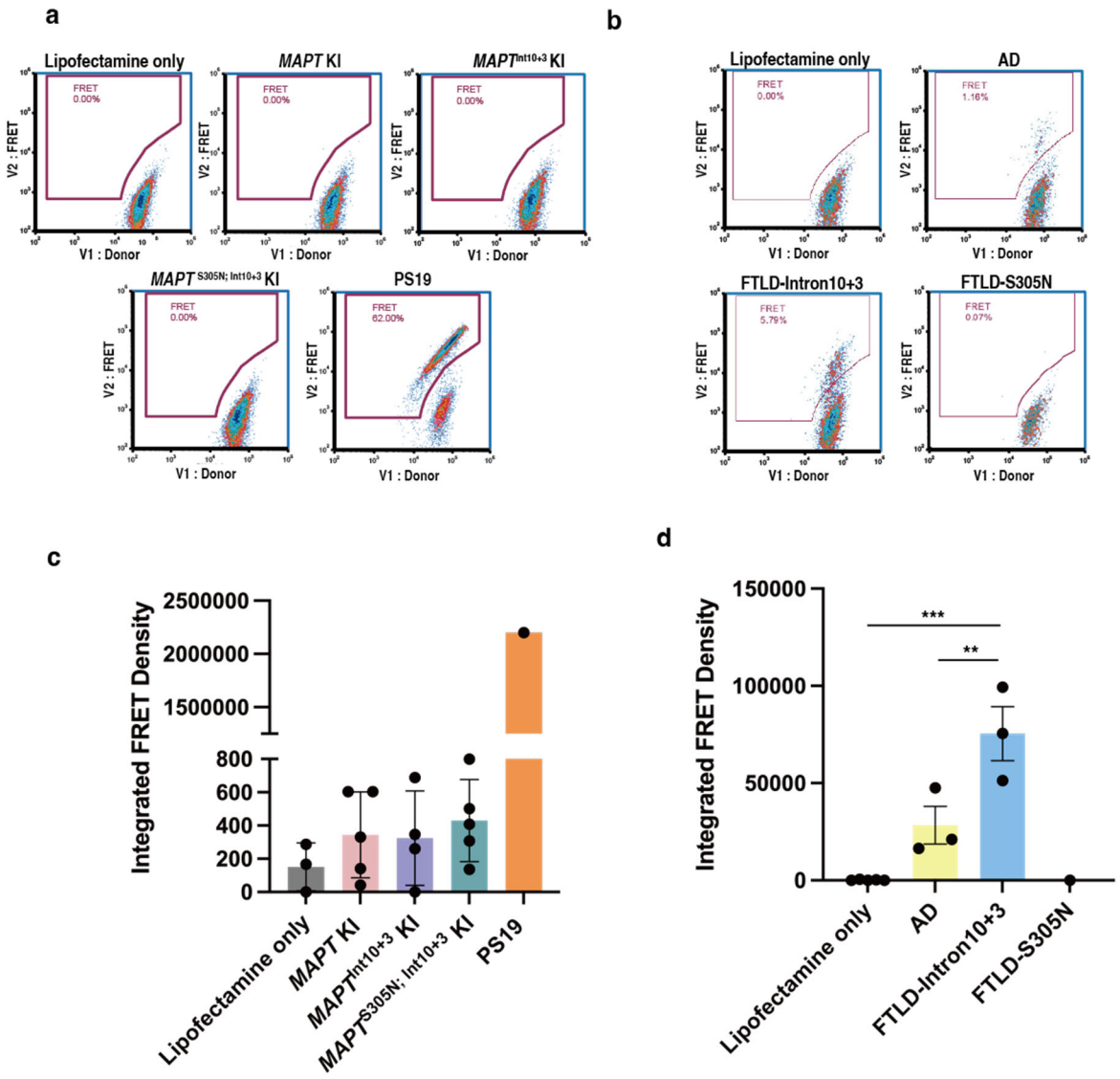


Supplemental Figure 3. LC-MS/MS analysis of tau in *MAPT* KI, *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N; Int10+3}$ KI mice.

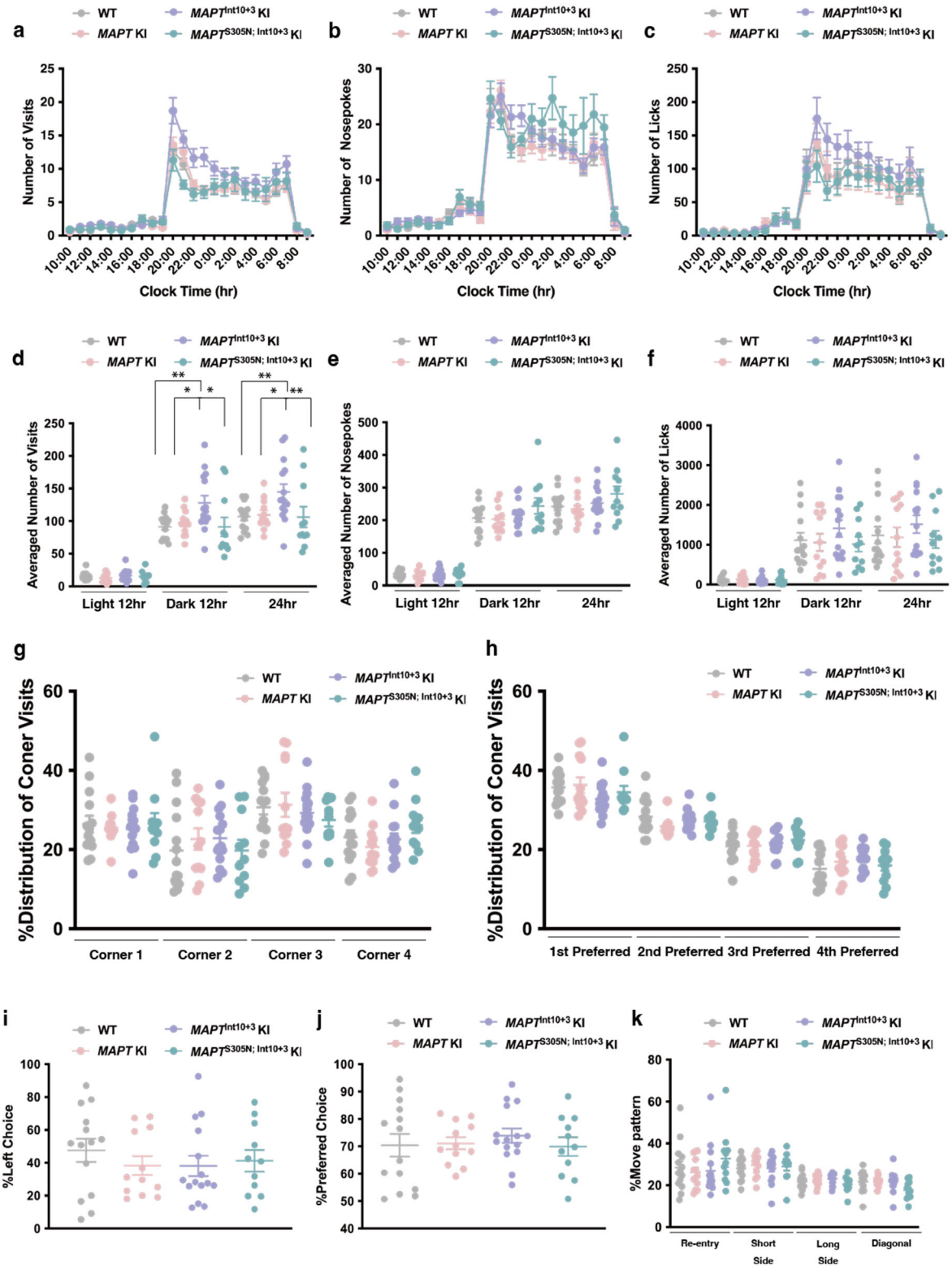
Amount of tau peptides based on the isoform resulting from alternative splicing by LC-MS/MS analysis in the brains of 12-15-month-old *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N; Int10+3}$ KI mice. 4R-tau (299-317 aa) in *MAPT*$^{S305N; Int10+3}$ KI line was not detected because serine (S) at position 305 was converted to asparagine (N). Data represents the mean ± SEM. ****P<0.0001 (One-way ANOVA followed by Holm-Sidak's multiple comparison test).



Supplemental Figure 4. Immunostaining of phospho-tau detected by AT8 antibody in *MAPT* KI, *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N;\ Int10+3}$ KI mice.
Immunostaining of phosphorylated tau detected by AT8 antibody in the brains of 15-month-old *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. Scale bar represents 1 mm in upper panel (hemibrain), 500 µm in the middle panel (hippocampus), 100 µm in the lower panel (piriform/entorhinal (Pir/Ent) cortex) and 50 µm in the insert.



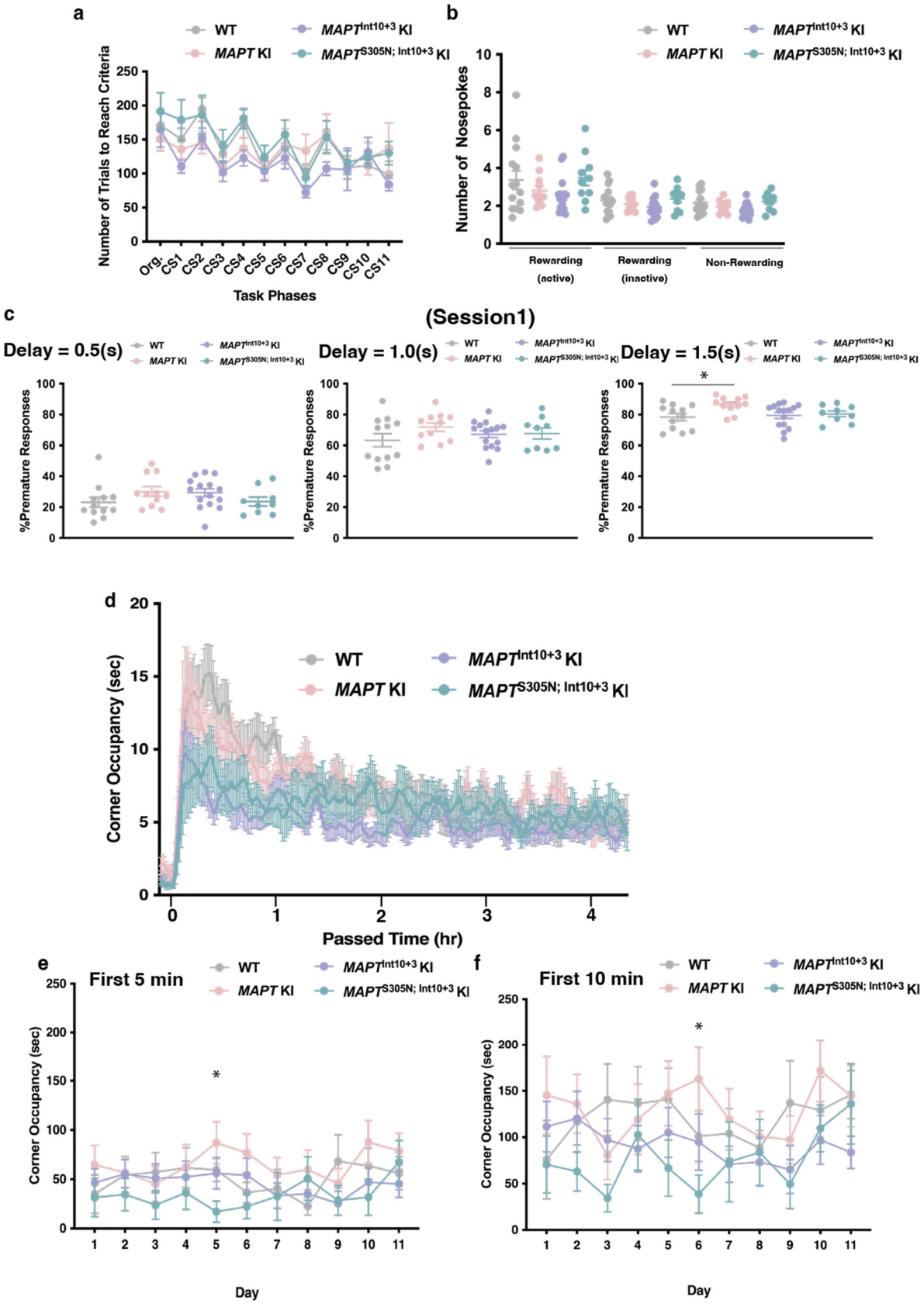
Supplemental Figure 5. Seeding assays of tau with P301S biosensor cell line. **a,** FRET *versus* donor (CFP) bivariate plots showing FRET-positive and FRET-negative P301S biosensor cells incubated with brain lysates from *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N; Int10+3}$ KI mice at 15 months and from PS19 mice at 9 months. **b,** FRET *versus* donor (CFP) bivariate plots showing the FRET-positive and FRET-negative P301S biosensor cells incubated with extracts from FFPE-embedded AD and FTLD patients with Intron10+3 and S305N mutations in the *MAPT* gene. **c,** Integrated FRET density from brain lysates from *MAPT* KI, *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N; Int10+3}$ KI mice at 15 months and PS19 mice at 9 months. **d,** Integrated FRET density from AD and FTLD patients with IVS10+3 and S305N mutations on the *MAPT* gene. Data represents the mean ± SEM. ***P*<0.01, ****P*<0.001 (one-way ANOVA with Tukey's multiple comparison test).



Supplemental Figure 6. Basal activity parameters calculated from 2 weeks of data in IntelliCage.

a-c, Circadian variations in number of visits (**a**), nosepokes (**b**), and licks (**c**) in WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N;\ Int10+3}$ KI mice. **d-f,** Average number of visits (**d**), nosepokes (**e**), and licks (**f**) in a day in WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N;}$

$^{Int10+3}$ KI mice. **g,** Percentages of corner visit distribution for each corner displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. **h,** Percentages of corner visit distribution in sequence of preferred corners displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. **i,** Percentages of left bottle choice exhibited by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. **j,** Percentages of preferred choice exhibited by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. **k,** Percentages of movement pattern (Re-entry, Short side, Long side, Diagonal) displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice. All experiments were performed with 14-16-month-old female mice (WT: n = 14, *MAPT* KI: n = 12, *MAPT*$^{Int10+3}$ KI: n = 15, *MAPT*$^{S305N;\ Int10+3}$ KI: n = 11). Data represent the mean ± SEM. **P*<0.05, ***P*<0.01 (two-way ANOVA with Tukey's multiple comparison test).



Supplemental Figure 7. Results from SP-FLEX (behavioral sequence learning and behavioral flexibility task), behavioral inhibition and competitive dominance tests

a, b, Number of trials to reach criteria and nosepokes of 14-16-month old WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice in the CS (complete shift)-only session of Self-paced Learning and Behavioral Flexibility Test (SP-FLEX) (WT: n = 14, *MAPT* KI: n = 11, *MAPT*$^{Int10+3}$ KI: n = 15, *MAPT*$^{S305N;\ Int10+3}$ KI: n = 10). **c,** Percentage of premature responses displayed by 14-16-month-old WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice in Session 1 of the behavioral inhibition test (WT: n = 12, *MAPT* KI: n = 11, *MAPT*$^{Int10+3}$ KI: n = 15, *MAPT*$^{S305N;\ Int10+3}$ KI: n = 9). **d,** Average corner occupancy time displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice at 0-4h over 11 days. (WT: n = 10, *MAPT* KI: n = 9, *MAPT*$^{Int10+3}$ KI: n = 14, *MAPT*$^{S305N;\ Int10+3}$ KI: n = 9) **e,** Average corner occupancy time displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice in the first 5 min over 11 days. **f,** Average corner occupancy time displayed by WT, *MAPT* KI, *MAPT*$^{Int10+3}$ KI, and *MAPT*$^{S305N;\ Int10+3}$ KI mice in the first 10 min over 11 days. In (**c**), (**e**), and (**f**), data represent the mean ± SEM. **P*<0.05 (two-way ANOVA with Tukey's multiple comparison test).

Supplemental Tables

Mutations	Substitution Efficien (%)
No substitution	7/166 (4.2)
Indel	10/166 (6.0)
P301L	79/166 (47.6)
P301S	19/166 (11.4)
P301V	1/166 (0.6)
Int10+3 G>A	36/166 (21.7)
P301L; Int10+3 G>A	9/166 (5.4)
P301S; Int10+3 G>A	4/166 (2.4)
S305N; Int10+3 G>A	1/166 (0.6)

Supplemental Table 1. Summary of substitution efficiency and generation of tau mutants after BE microinjection targeting *MAPT*-P301 and *MAPT*-Intron10+3.

Off-target candidate	Query type	Mismatch	Chr Position	Gene	Annotation	Off-target	
						MAPT KI$^{Inton10+3\ G>A}$	*MAPT* KI$^{S305N;\ Inton10+3\ G>}$
MAPT-P301-1	No indel	3	Chr11:70351826-70351849	*Alox15*	Intron	-	-
MAPT-P301-2	No indel	3	Chr4:119200276-119200299	*Svbp*	Intron	○	○
MAPT-P301-3	No indel	3	Chr9:64060473-64060496		Intergenic	-	-
MAPT-P301-4	No indel	3	Chr6:95725509-95725532		Intergenic	-	-
MAPT-P301-5	No indel	3	Chr12:64966002-64966025	*Togaram1*	Exon	○	○
MAPT-P301-6	No indel	3	Chr14:78141609-78141632		Intergenic	-	-
MAPT-P301-7	No indel	3	Chr2:126701111-126701134		Intergenic	-	-
MAPT-P301-8	No indel	3	Chr12:72964402-72964425		Intergenic	-	-
MAPT-P301-9	No indel	3	Chr1:180813179-180813202	*H3f3a*	Intron	-	-
MAPT-P301-10	No indel	3	Chr6:146888630-146888653	*Ppfibp1*	Non-coding	-	-
MAPT-P301-11	Del 8	2	Chr9:121021200-121021222	*Ulk4*	Intron	-	-
MAPT-P301-12	Del 3	2	ChrX:87776462-87776484	*Il1rapl1*	Intron	-	-
MAPT-P301-13	Del 2	2	Chr16:18687059-18687081		Intergenic	-	-
MAPT-P301-14	Del PAM 2	2	Chr2:167736542-167736564		Intergenic	-	-
MAPT-P301-15	Ins 17	2	Chr10:118675703-118675727		Intergenic	-	-
MAPT-P301-16	Ins 16	2	Chr2:133528883-133528907		Intergenic	-	-
MAPT-P301-17	Ins 13	2	Chr14:26429735-26429759	*Slmap*	Intron	○	-
MAPT-P301-18	Ins 7	2	Chr6:146527101-146527125		Intergenic	-	-
MAPT-P301-19	Ins 5	2	Chr2:131262710-131262734	*Pank2*	Intron	-	-
MAPT-intron10+3 G>A-1	No indel	3	Chr14:31275639-31275661	*Dnah1*	Intron	-	-
MAPT-intron10+3 G>A-2	No indel	3	Chr10:67285234-67285256	*Nrbf2*	Non-coding	-	-
MAPT-intron10+3 G>A-3	No indel	3	Chr3:108148064-108148086		Intergenic	-	-
MAPT-intron10+3 G>A-4	No indel	3	Chr5:137062275-137062297	*Serpine1*	Non-coding	-	-
MAPT-intron10+3 G>A-5	No indel	3	Chr6:72577777-72577799	*Elmod3*	Intron	-	-
MAPT-intron10+3 G>A-6	No indel	3	Chr5:64508703-64508725		Intergenic	-	-
MAPT-intron10+3 G>A-7	Del 18	2	Chr6:59413774-59413795	*Gprin3*	Intron	-	-
MAPT-intron10+3 G>A-8	Del 18	2	Chr7:96207591-96207612	*Tenm4*	Intron	-	-
MAPT-intron10+3 G>A-9	Del 17	2	Chr2:168700314-168700335	*Atp9a*	Intron	-	-
MAPT-intron10+3 G>A-10	Del 16	2	Chr2:146621362-146621383		Intergenic	-	-
MAPT-intron10+3 G>A-11	Del 16	2	Chr8:69915135-69915156	*Gatad2a*	Intron	-	-
MAPT-intron10+3 G>A-12	Del 16	2	Chr11:99056068-99056089		Intergenic	-	-
MAPT-intron10+3 G>A-13	Del 15	2	Chr2:167736538-167736559		Intergenic	-	-
MAPT-intron10+3 G>A-14	Del 14	2	Chr1:82288807-82288828	*Irs1*	Exon	-	-
MAPT-intron10+3 G>A-15	Del 14	2	Chr4:150626514-150626535		Intergenic	-	-
MAPT-intron10+3 G>A-16	Del 14	2	Chr17:30516288-30516309	*Btbd9*	Intron	-	-
MAPT-intron10+3 G>A-17	Del 14	2	Chr8:112093736-112093757		Intergenic	-	-
MAPT-intron10+3 G>A-18	Del 14	2	Chr12:84648519-84648540	*Vrtn*	Exon	-	-
MAPT-intron10+3 G>A-19	Del 14	2	Chr11:87664708-87664729	*Rnf43*	Exon	-	-
MAPT-intron10+3 G>A-20	Del 13	2	Chr4:139161305-139161326	*Gm33304*	Non-coding	-	-
MAPT-intron10+3 G>A-21	Del 12	2	Chr18:80778335-80778356	*Atp9b*	Intron	-	-
MAPT-intron10+3 G>A-22	Del 12	2	Chr2:119770882-119770903	*Rpap1*	Intron	-	-
MAPT-intron10+3 G>A-23	Del 12	2	Chr6:109857978-109857999		Intergenic	-	-
MAPT-intron10+3 G>A-24	Del 12	2	Chr5:142468117-142468138	*Ap5z1*	Exon	-	-
MAPT-intron10+3 G>A-25	Del 11	2	Chr17:25386575-25386596	*Cacna1h*	Intron	-	-
MAPT-intron10+3 G>A-26	Del 11	2	Chr9:119555524-119555545	*Scn5a*	Intron	-	-
MAPT-intron10+3 G>A-27	Del 9	2	Chr9:43536132-43536153		Intergenic	-	-
MAPT-intron10+3 G>A-28	Del 9	2	Chr7:125974764-125974785	*Gsg1l*	Intron	-	-
MAPT-intron10+3 G>A-29	Del 6	2	Chr12:91965237-91965258		Intergenic	-	-
MAPT-intron10+3 G>A-30	Del 4, or Del 5	2	Chr11:118367373-118367394		Intergenic	-	-
MAPT-intron10+3 G>A-31	Del 4, or Del 5	2	Chr4:43394834-43394855	*Rusc2*	Intron	-	-
MAPT-intron10+3 G>A-32	Del 4, or Del 5	2	Chr5:121818513-121818534	*Sh2b3*	Exon	-	-
MAPT-intron10+3 G>A-33	Del 3	2	Chr12:21295846-21295867	*Cpsf3*	Intron	-	-
MAPT-intron10+3 G>A-34	Del 3	2	Chr9:113870747-113870768	*Clasp2*	Intron	-	-
MAPT-intron10+3 G>A-35	Del 3	2	Chr6:125633288-125633309	*Vwf*	Intron	-	-
MAPT-intron10+3 G>A-36	Del 3	2	Chr2:130840193-130840214	*9304O2H24Rik*	Non-coding	-	-
MAPT-intron10+3 G>A-37	Del 1, or Del 2	2	Chr17:49049099-49049120	*Lrfn2*	Intron	-	-
MAPT-intron10+3 G>A-38	Ins 17	2	Chr16:22558979-22559002	*Dgkg*	Intron	-	-
MAPT-intron10+3 G>A-39	Ins 16	2	Chr15:74631843-74631866	*Mroh4*	Intron	-	-
MAPT-intron10+3 G>A-40	Ins 15	2	Chr17:54077434-54077457		Intergenic	-	-
MAPT-intron10+3 G>A-41	Ins 13	2	Chr7:139648287-139648310	*Cfap46*	Intron	-	-
MAPT-intron10+3 G>A-42	Ins 13	2	Chr2:30194586-30194609	*Kyat1*	Intron	-	-
MAPT-intron10+3 G>A-43	Ins 6	2	Chr8:71290281-71290304	*Myo9b*	Non-coding	-	-
MAPT-intron10+3 G>A-44	Ins 6	2	Chr5:138859529-138859552		Intergenic	-	-
MAPT-intron10+3 G>A-45	Ins 6	2	Chr16:93903368-93903391	*Chaf1b*	Intron	-	-
MAPT-intron10+3 G>A-46	Ins 1	2	Chr13:63111073-63111096	*Aopep*	Intron	-	-

Supplemental Table 2. Summary of off-target candidate sites predicted by COSMID for *MAPT*$^{Int10+3}$ KI and *MAPT*$^{S305N;\ Int10+3}$ KI mice.

antibody / Sample	AT8	CP13	PHF-1	AT180	AT270	TOC1	MC1
WT	-	-	-	-	-	-	-
MAPT KI	+	+	-	-	-	-	-
MAPT KI$^{Int10+3}$	++	++	++	++	++	-	-
MAPT KI$^{S305N; Int10+3}$	+++	+++	+++	++	++	-	-
FTLD-Int10+3	+++	+++	+++	+++	+	++	+
FTLD-S305N	+++	+++	+++	+++	+++	++	+

Supplemental Table 3. Summary of histological visual quantification analyses of tau phosphorylation detected by several phospho-tau antibodies.

The following antibodies were used at the dilutions described below.					
Antibody				Dilution	
				WB	IHC
CP-13	Kindly provided by Cristina D'Abramo			1:500	1:500
AT8	Inoggenetics #90206 (anti-PHF-TAU) and ThermoFisher MN1020b			1:2000	1:500
PHF-1	Kindly provided by Cristina D'Abramo			1:2000	1:1000
AT180	Thermo #MN-1040			-	1:100
AT270	Thermo #1050			-	1:100
TOC1	Kindly provided by Lester I Binder			-	1:1500
MC1	Kindly provided by Peter Davis			-	1:500
Tau13	Santa Cruz #sc-21796			1:2000	-
Tau5	Thermo #AHB0042			1:2000	-
HT7	Thermo #MN-1000			1:2000	-
K9JA	Dako # A0024			1:10000	-
RD3	Merk Millipore #05-803			-	1:100
RD4	Merk Millipore #05-804			-	1:100
Synaptotagmin	Synaptic System 105-002			-	1:500
Homer	Synaptic System 160-011			-	1:500
VGLUT1	Synaptic System 135-303			-	1:500
β-actin	Sigma A5441			1:5000	-

Supplemental Table 4. Antibodies used for immunoblotting and immunohistochemical (IHC) analyses.

***In vitro* transcription for BE3**	
T7-BE3-F	GCCGCTAATACGACTCACTATAG
T7-BE3-R	GCGGGTTTAAACTCAATGGT
***In vitro* transcription for sgRNAs**	
sgRNA-MAPT-P301	taatacgactcactataGGTCCCGGGAGGCGGCAGTGgttttagagctagaa
sgRNA-MAPT-Intron10+3 G>A	taatacgactcactataGGACTCACACTGCCGCCTCCgttttagagctagaa
CRISPRscan tail primer	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTA
RT-PCR for tau	
3R-Tau F	GTCCGTACTCCACCCAAGTC
3R-Tau R	TTTGTAGACTATTTGCACCTTCC
4R-Tau F	GAAGCTGGATCTTAGCAACG
4R-Tau R	GACGTGTTTGATATTATCCT
Total Tau F	AGCCAAGACATCCACACGTT
Total Tau R	ATCAGAGGGTCTGAGCTACCA
G3PDH F	CCATGGCACCGTCAAGGCTGA
G3PDH R	GCCAGTAGAGGCAGGGATGAT
Sangar-sequencing for off-target sites	
Svbp F	GGGGGTCAGAGTGTTTTCAA
Svbp R	GGAGCTGGGGAGCTAAAAAG
Togaram1 F	AATGGTTCCCTCCAGCTCTC
Togaram1 R	TTTTTCTCGCCTCTCATGGT
Slmap F	CACTGGTTGTTGGAGCATCT
Slmap R	GCCAAACAAGACATTCAGCA
Nploc4 F	CAAAATGACCATCGGGAGAG
Nploc4 R	AGTCATCCTGCTGGCTCAAC

Supplemental Table 5. Primer lists for *in vitro* transcription of BEs and sgRNAs

Supplemental Table 6. Human sample information

Sample ID	Gender	Age	PMI	ApoE genotype	Braak Tau Stage	Region	Import source	Sample
P6/20	F	86	94.05	N/A	6	Frontal Cortex	from QSBB	AD
P14/21	F	72	59.1	N/A	6	Frontal Cortex	from QSBB	AD
P3/21	F	61	36.55	N/A	6	Frontal Cortex	from QSBB	AD
HR09-5902	M	41.9	N/A	33	0	Frontal Cortex	from Mayo Clinic	FTLD-Intron10+3
HR10-25426	M	54.7	N/A	33	0	Frontal Cortex	from Mayo Clinic	FTLD-Intron10+3
HR10-64047	M	57	N/A	34	0	Frontal Cortex	from Mayo Clinic	FTLD-Intron10+3
H024	M	46	N/A	N/A	N/A	Frontal Cortex	from Iou National Hospital	FTLD-S305N