Supplementary Information for

Truncated Tau caused by intron retention is enriched in Alzheimer disease cortex and exhibits altered biochemical properties

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Dataset S01

Materials and Methods

Cloning of Tau constructs

Full-length (FL) *Tau* gene was PCR amplified from VN-*Tau* plasmid (Addgene 87368) using *XbaI-Tau-F* and *SalI-Tau-R* primers and blunt-end cloned into pBlueScript II SK+ vector at *EcoRV* site to get PBS-Tau-FL. Following *XbaI*/*SalI* restriction of PBS-Tau-FL, full-length Tau fragment was cloned into pLVX-Flag-IRES-puro lentiviral vector at *XbaI*/*PspXI* sites to get pLVX-Flag-Tau-FL.

Three steps were taken to make Tau-11i construct. (i) Partial *Tau* exon and intron 11 region was PCR amplified from human genomic DNA using *Tau-11e-F* and *SalI-Tau-11i-R* primers and cloned into pGEMT vector (Promega) to get pGEMT-Tau-ex/in-11. (ii) *BstEII* cuts uniquely within exon 11. Fragment of *Tau* exon and intron 11, released by *BstEII*/*SalI* restriction of pGEMT-Tau-ex/in-11, was then used to replace 3'-end of full-length *Tau* in PBS-Tau-FL to get PBS-Tau-11i. (iii) Following *XbaI*/*SalI* restriction of PBS-Tau-11i, Tau-11i fragment was cloned into pLVX-Flag-IRES-puro vector at *XbaI*/*PspXI* sites to get pLVX-Flag-Tau-11i. DNA sequencing was performed at each step to verify sequence integrity.

XbaI-Tau-F	5'-CTATCTAGAGCTGAGCCCCGCCA-3'
SalI-Tau-R	5'-AACGTCGACTCACAAACCCTGCTTG-3'
Tau-11e-F	5'-GTGCAAATAGTCTACAAAC-3'
SalI-Tau-11i-R	5'-GTCGACTCACCAGGACTCCTCC-3'

PD and PSP datasets

MAYO temporal cortex (syn8612203) and cerebellum (syn8612213) PSP datasets[1] were downloaded from AD Knowledge Portal. BA9 brain tissue datasets of healthy control subjects and PD patients were downloaded from GEO (GSE68719)[2].

Cell Culture

HEK 293T cells (Clontech, 632180) were maintained in Dulbecco's modified Eagle's medium (DMEM) GlutaMAX™ (Gibco) supplemented with 10% FBS, 1x nonessential amino acids (NEAA) and 1.0 mM sodium pyruvate.

Human neural stem cells (NSC) (XCL-1, SC-001-1V, XCell Science Inc.) were seeded on matrigel (Corning, 354277) and cultured in neural expansion medium [1:1 ratio of DMEM GlutaMAX™ to Ham's F-12 Nutrient Mix (both Gibco), 1x NEAA, 1x N-2 supplement (Gibco, 17502048), 1x B-27 supplement minus vitamin A (Gibco, 12587010), 0.5X GlutaMAX (Gibco, 35050061), 20 ng/ml human FGF-basic (Gibco, PHG0261), 2 µg/ml Heparin Sulfate

(Sigma, H4784)]. Viral infected NSCs were differentiated according to manufacturer's protocols. Briefly, cells were seeded in NSC maintenance media at a density of 0.04×10^6 cells/cm^2 on poly-ornithine (20 μg/mL) & laminin (10 μg/mL)-coated plates (Day 0). Cells were then replenished with neuronal induction media on Day 1, 3 and 5. On Day 6, neuronal precursor cells formed (NPCs) were harvested by StemPro™ Accutase™ (Gibco, A1110501) and replated on poly-ornithine/laminin-coated wells at 0.04×10^6 cells/cm^2 in neuronal maturation medium. Neurons were cultured for designated duration with media change every alternate day.

Transient transfection and lentiviral transduction

Full-length Tau and Tau-11i expression constructs were transiently transfected into HEK 293T cells using Lipofectamine® 3000 reagent (Invitrogen, L3000008) according to manufacturer protocol. Cells were harvested 48 h post-transfection for analyses.

Lenti-X lentiviral expression system (Clontech) was used to generate stable NSC lines that expressed either full-length (FL) Tau or Tau-11i proteins. Briefly, lentivirus was prepared by transfecting 293T cells with a mixture that contained 7 μg of lentiviral plasmids (pLVX-Flag-Tau-FL or pLVX-Flag-Tau-11i) and Lenti-X packaging single shot reagent (VSV-G) (Clontech, 631275). Media harvested 24 h and 72 h post transfection was combined and concentrated using Lenti-X concentrator (Clontech, 631231) to produce lentiviral supernatant. NSCs were infected with lentiviral supernatant in the presence of polybrene (12 μg/ml). After 48 h of viral transduction, stable clones were selected with 0.1 μg/ml of puromycin. Expression of Tau-FL and Tau-11i in stable lines were validated using western blot and immunofluorescence.

Protein stability assays

293T cells transfected with either full-length Tau441 or Tau11i cDNA (Day 0, herein D0) were split 24 h later (D1). Cells were treated with trypsin and resuspended into 1000 μl media. Two approaches were used to measure protein stability of Tau441 and Tau11i.

(i) For time-course experiment in Fig.4B, cells were seeded in a 24-well plate with the following density: 30% (300 μl for D2), 15% (D3), 7.5% (D4), 3.75% (D5), 2% (D6), 1% (D7) and harvested accordingly.

(ii) For cycloheximide (CHX) assay in Fig.4C, cells were seeded at 3.75% a day after transfection. Fresh media was replenished on D4 and 20 μg/ml of CHX was added on D5.

Cells were harvested immediately (0 h), 4 and 8 h later. The level of Tau protein was first normalized to actin and the ratio of 8 h to 0 h time-points was then calculated.

Sarkosyl extraction

Sarkosyl soluble and insoluble fractions were prepared according to published protocol[3]. Tau expressing 293T cells, NPC and neuronal stable lines were resuspended with 320 µl/3e6 cells of low salt buffer (50 mM HEPES pH 7.6, 250 mM sucrose, 1 mM EDTA pH 8.0, Roche protease inhibitors cocktail), dounced with pestle A and B for 10 times each, followed by addition of 40 µl of 5 M NaCl and 40 µl of 10 % Sarkosyl. Cell lysates were mixed thoroughly by pipetting, incubated on ice for 15 min with agitation, followed by brief sonication (10s ON / 30s OFF, high settings, 3 cycles, Bioruptor UCD-200TO). After complete resuspension, 40 µl of cell lysates were harvested as "total" lysate. Following maximum speed centrifugation at 4ºC for 1 h, supernatant was carefully collected as "soluble" extract. The insoluble pellet was gently washed with low salt buffer (with 0.5 M NaCl and 1% Sarkosyl) and centrifuged at maximum speed at 4ºC for 10 mins to remove wash buffer. Laemmli buffer was added to the "total" lysate, "soluble" fraction, and "insoluble" pellet, boiled for 10 min, and subjected to immunoblot assay. For control and AD hippocampus tissue samples, 12 µl of lysates was added to 100 µl of low salt buffer (50 mM HEPES pH 7.6, 250 mM sucrose, 1 mM EDTA pH 8.0, 0.5 M NaCl, 0.1% Sarkosyl, Roche protease inhibitors cocktail). After removing 11 µl as "total", the remaining mixture was centrifuged at 65000 rpm in a TLA120.2 rotor (Beckman) at 4ºC for 45 min. The supernatant was carefully removed as "soluble fraction" from the pellet. 1 x laemmli buffer was then added to the pellet to get "insoluble fraction".

Immunoblot and antibodies

Different Sarkosyl fractions, brain tissues and cellular lysates were boiled in 1x Laemmli buffer, resolved in SDS-PAGE gel and transferred to PVDF membrane by standard western protocol. For immunoblot, the antibodies used were as follow:

Flag (F1804, Sigma); Tau11i-A and Tau11i-B (GENSCRIPT) designed to target amino acids encoded by intron-11; Tau46 (4019S, CST) which recognizes amino acids 404 to 421; Tau4R (05-804, Millipore) which recognizes amino acids 275 to 291 or Tau 4-repeat isoform RD4; AT8 (MN1020, Invitrogen) which recognizes phosphor-Tau at S202/T205; pT181 (12885S, CST) which recognizes phosphor-Tau at T181; PHF1 (sc-32275, Santa Cruz) which recognizes phosphor-Tau at S396; and β-actin (A2228, Sigma).

Immunofluorescence

Immunostaining of neurons and image quantification were performed as previously described[4]. Briefly, mature neurons cultured in 12-well plates were fixed with 4% paraformaldehyde in PBS for 20 min, followed by two rounds of PBS washes and 1 h of blocking (10% normal goat serum, 0.1% triton-X 100 in PBS) at room temperature. Neurons were then stained with anti-MAP2 (Millipore, MAB3418), anti-α-tubulin (Santa Cruz, sc-53029) and anti-Flag (Sigma, F1804) antibodies (5 μg/ml in blocking buffer) overnight at 4°C. After three rounds of PBS washes the following day, neurons were incubated with anti-mouse 594 and anti-rabbit FITC secondary antibodies (1:500, Invitrogen) for 2 h at room temperature. Cells were washed thrice with PBS and mounted in VECTASHIELD with DAPI (Vector labs).

Frozen temporal lobe sections were thawed at room temperature for 20 min followed by three rounds of 5 min washes with TBST (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.05% Triton). The sections were blocked in TBS containing 1% BSA and 10% NGS for 2 hr at room temperature and stained with primary antibodies (Tau4R at 1:100, α-tubulin at 1:25 or Tau11i-B at 1:500 in blocking buffer) overnight at 4°C. After three rounds of 5 min TBST washes the following day, sections were incubated with secondary antibodies at 1:500 dilution in blocking buffer for 2 hr at room temperature in dark. The secondary antibodies used are anti-mouse 488 (Tau4R), anti-rat 488 (α-tubulin) and anti-rabbit-594 (Tau11i-B) (Invitrogen). Sections were subjected to 3 x 5 min of TBS washes and stained with DAPI (2 μg/ml in TBS) for 10 min. Following three additional rounds of TBS washes, the sections were mounted in VECTASHIELD.

Images captured with either Olympus (IX71, 20× objective) or confocal microscopes (FV3000, 10-60× objective) were analysed using Imaris software (version 9.2.0, Bitplane). The signal threshold was adjusted to obtain optimal coverage of the different antibody staining. The relative fluorescence intensity of FLAG/α-tubulin for human cortical neurons and Tau11i/DAPI+ cells count for temporal lobe section was then quantified. The statistical significance was tabulated using the relative density of all the fields from each condition.

Validation of IR by quantitative PCR (qPCR)

Total RNA of different brain tissues from healthy control subjects and AD patients (Amsbio) were purchased from Biochain. High-capacity cDNA reverse transcription kit (Applied Biosystems) was used to generate cDNA libraries from 1 μg of total RNA pre-treated with DNase I (Thermo Scientific). Real-time quantitative PCR was carried out in triplicates (6

µl/reaction) with 0.2 µM of primers and 2X SYBR green master mix (Thermo Scientific) using 7900HT Fast Real-Time PCR machine (Applied Biosystems). Relative level of Tau-11i (*Tau ex-in11-F/R)* was calculated using $2^{-\Delta CT}$ method where threshold cycle (CT) values were normalized to either *Tau* exon1/exon2 (*Tau ex-1-F*/*ex-2-R*) or *Gapdh* expression.

Tau ex-in11-F	5'-ACCAGGTAGCCCTGTGGAAG-3'
Tau ex-in11-R	5'-CCGCAAGTTTCACACTCAAC-3'
Tau ex-1-F	5'-AAGACCAAGAGGGTGACACG-3'
Tau ex-2-R	5'-ACATCTTCCGCTGTTGGAGT-3'
Tau ex-5-F	5'-CGCATGGTCAGTAAAAGCAAAG-3'
Tau ex-6-R	5'-CTGGAATCCTGGTGGCGTT -3'
Gapdh-F	5'-CAATGACCCCTTCATTGACC-3'
Gapdh-R	5'-GACAAGCTTCCCGTTCTCAG-3'

Human brain samples and tissue sections.

"C" denotes Control and "A" denotes Alzheimer disease.

Total protein lysates from different brain regions were purchased from:

(A) MYBIOSOURCE

1) **C1:** Adult Normal, Brain, Temporal Lobe Lysate (MBS657128) - male, 77 years old.
2) **A1:** Adult AD, Brain, Temporal Lobe Lysate (MBS657032) - male, 87 years old.
3) **C4:** Adult Normal, Brain, Frontal Lobe Lysate (MBS657031) - male, 60 years old.
4) **A4:** Adult AD, Brain, Frontal Lobe Lysate (MBS657343) - female, 85 years old.
5) **C5:** Adult Normal, Brain, Hippocampus Lysate (MBS657489) - male, 71 years old.
6) **A5:** Adult AD, Brain, Hippocampus Lysate (MBS657034) – female, 93 years old.

(B) BIOCHAIN

1) **C8:** Adult Normal, Brain, Temporal Lobe Lysate (P1234078) - female, 70 years old.
2) **A1:** Adult AD, Brain, Temporal Lobe Lysate (P1236978Alz) - male, 87 years old.
3) **C9:** Adult Normal, Brain, Frontal Lobe Lysate (P1234051) - male, 82 years old.
4) **C2:** Adult Normal, Brain, Amygdala Lysate (P1234036) - male, 22 years old.
5) **A2:** Adult AD, Brain, Amygdala Lysate (P1236036Alz) - male, 65 years old.
6) **C3:** Adult Normal, Brain, Parietal lobe Lysate (P1234066) - male, 66 years old.
7) **A3:** Adult AD, Brain, Parietal lobe Lysate (P1236066Alz) - male, 73 years old.

The following samples were obtained from Newcastle Brain Tissue Resource, part of the Brains for Dementia Research network[5].

1) **C6:** Adult Normal, Brain, Parietal Lobe Lysate (283/96) - male, 77 years old.
2) **A6:** Adult AD, Brain, Parietal Lobe Lysate (120/09) - female, 85 years old.
3) **C7:** Adult Normal, Brain, Parietal lobe Lysate (308/09) - male, 66 years old.
4) **A7:** Adult AD, Brain, Parietal lobe Lysate (350/09) - female, 98 years old.

Frozen tissue sections were purchased from BIOCHAIN.

1) **C10:** Adult Normal, Brain, Temporal Lobe ((T1234078) - male, 26 years old.
2) **A10:** Adult AD, Brain, Temporal Lobe (T1236078Alz) – male, 73 years old.

Total RNA from different brain regions (AMSBIO) were purchased from BIOCHAIN.

1. AD Brain, R1236035Alz-50 (Lot#: A507294) - male, 87 years old.
2. Normal Brain R1234035-P (Lot#: C404081) - 5 males, pooled, 21-29 years old.
3. AD Frontal Lobe, R1236051Alz-10 (Lot#: B204064) - male, 87 years old.
4. Normal Frontal Lobe, R1234051-P (Lot#: B804011) - 5 males, pooled, 22-29 years old.
5. AD Temporal Lobe, R1236078Alz-10 (Lot#: B408032) - male, 80 years old.
6. Normal Temporal Lobe, R1234078-P (Lot#: B411025) - 5 males, pooled, 23-27 years old.

Legend for Dataset S01

Individual normalized IR ratio of *Tau* intron 11 from (i) female and (ii) male dorsal lateral prefrontal cortex (DLPFC) AD cohorts. Related to Figure 1C.

(iii) Individual normalized read counts of *Tau* mRNA from female and male DLPFC AD cohorts. Related to Figure 1C.

Individual normalized IR ratio of *Tau* intron 11 from (iv) Brodmann area 9 (BA9) brain tissue for Parkinson Disease (PD); (v) cerebellum and (vi) temporal cortex for Progressive Supranuclear Palsy (PSP). Related to Figure S1A.

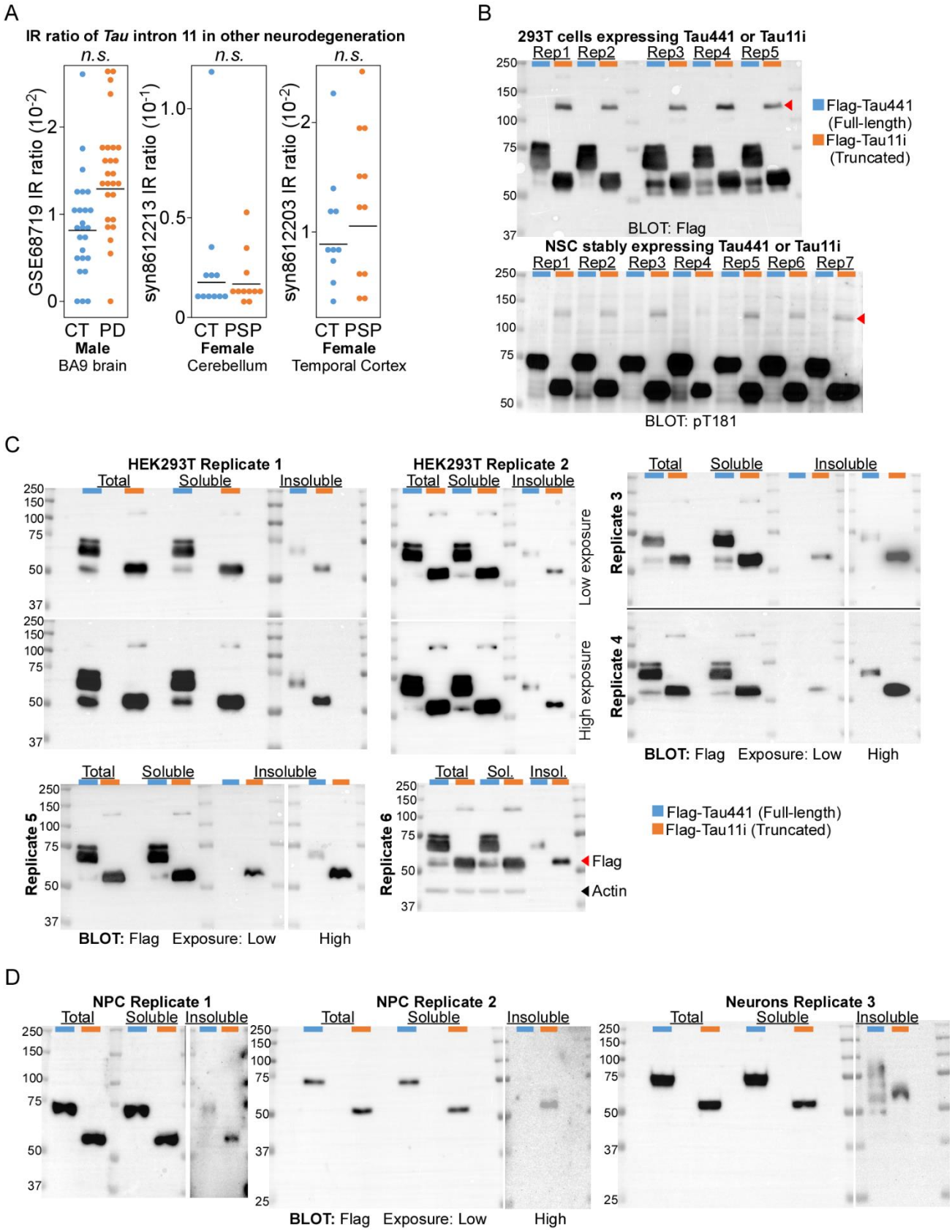
A
IR ratio of Tau intron 11 in other neurodegeneration
n.s.
GSE68719 IR ratio (10-2)
CT PD
Male
BA9 brain
syn8612213 IR ratio (10-1)
CT PSP
Female
Cerebellum
syn8612203 IR ratio (10-2)
CT PSP
Female
Temporal Cortex
B
293T cells expressing Tau441 or Tau11i
Rep1 Rep2 Rep3 Rep4 Rep5
Flag-Tau441 (Full-length)
Flag-Tau11i (Truncated)
BLOT: Flag
NSC stably expressing Tau441 or Tau11i
Rep1 Rep2 Rep3 Rep4 Rep5 Rep6 Rep7
BLOT: pT181
C
HEK293T Replicate 1
HEK293T Replicate 2
Total Soluble Insoluble
Low exposure
High exposure
Replicate 3
Replicate 4
BLOT: Flag Exposure: Low High
Replicate 5
Replicate 6
Total Sol. Insol.
Flag
Actin
Flag-Tau441 (Full-length)
Flag-Tau11i (Truncated)
BLOT: Flag Exposure: Low High
D
NPC Replicate 1
NPC Replicate 2
Neurons Replicate 3
Total Soluble Insoluble
BLOT: Flag Exposure: Low High

Figure S1. Retention of intron 11 of the *Tau* gene in other neurodegeneration and characterization of Tau11i protein. (*A*) Dot plot of normalized intron retention (IR) ratio from PD and PSP cohorts. Each dot represents individual value. Blue is healthy control and orange labels diseased patient. Related to Figure 1. (*B*) Immunoblot of 293T cells (top) and NSC (bottom) expressing either Flag-Tau441 or Flag-Tau11i. HMW species is marked by red arrowhead. "Rep" stands for replicate. (*C*) Immunoblot of total lysates, soluble and insoluble fractions following Sarkosyl extraction of 293T cells which expressed either Flag-Tau441 or Flag-Tau11i. Replicate 1 was also presented in Figure 2D. (*D*) Immunoblot of total lysates and different fractions following Sarkosyl extraction of NPC and neurons expressing either Flag-Tau441 or Flag-Tau11i. Related to Figure 2.

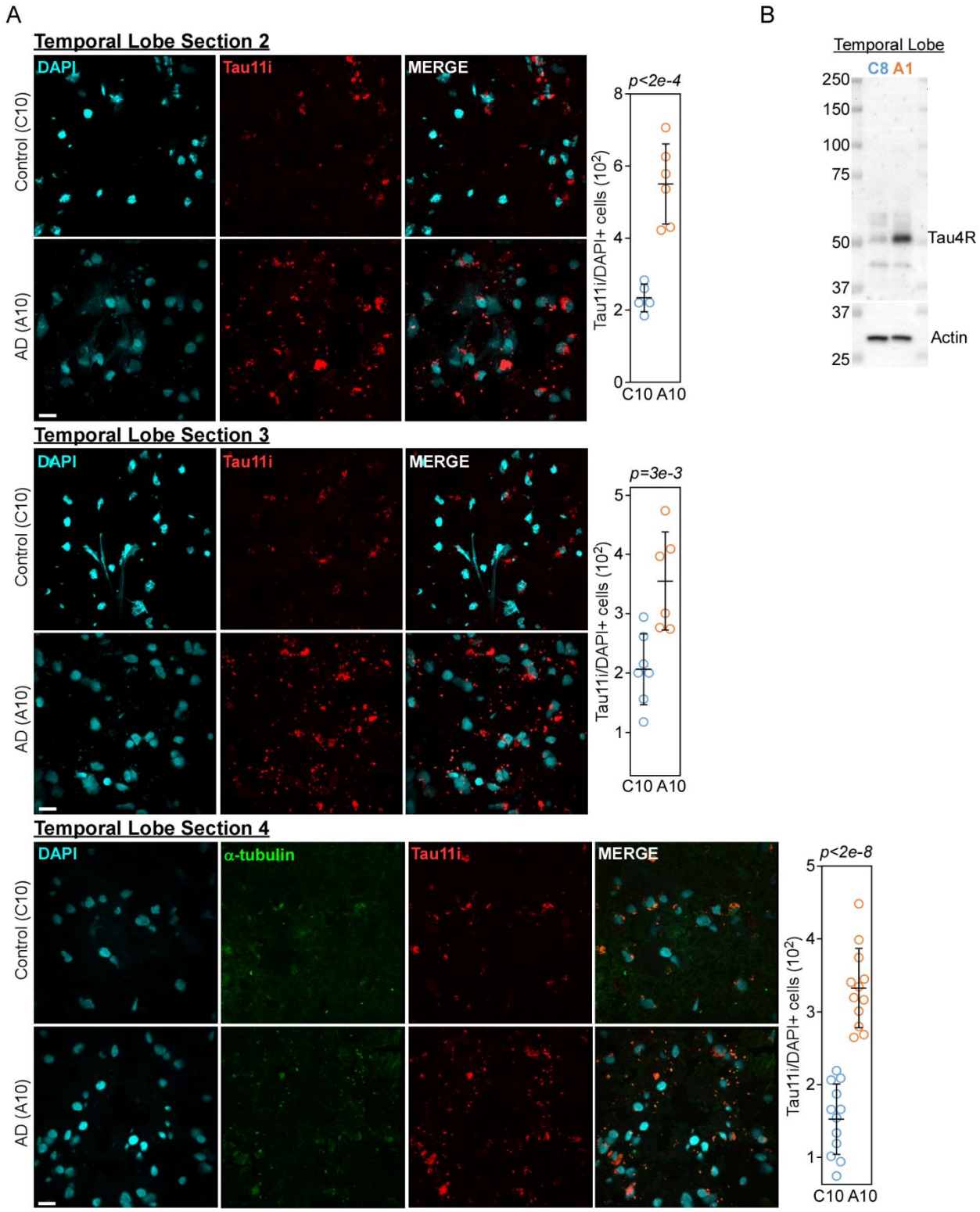

Figure S2. Immunofluorescence showed higher level of Tau11i in AD temporal lobe.

(A) Image and quantification of Tau11i over DAPI positive cells is presented as mean ± SD where each circle represents one field. *P*-value was calculated by 2-tailed *t*-test. *(B)* Immunoblot of control "C8" and AD "A1" temporal lobe. Related to Figure 3C.

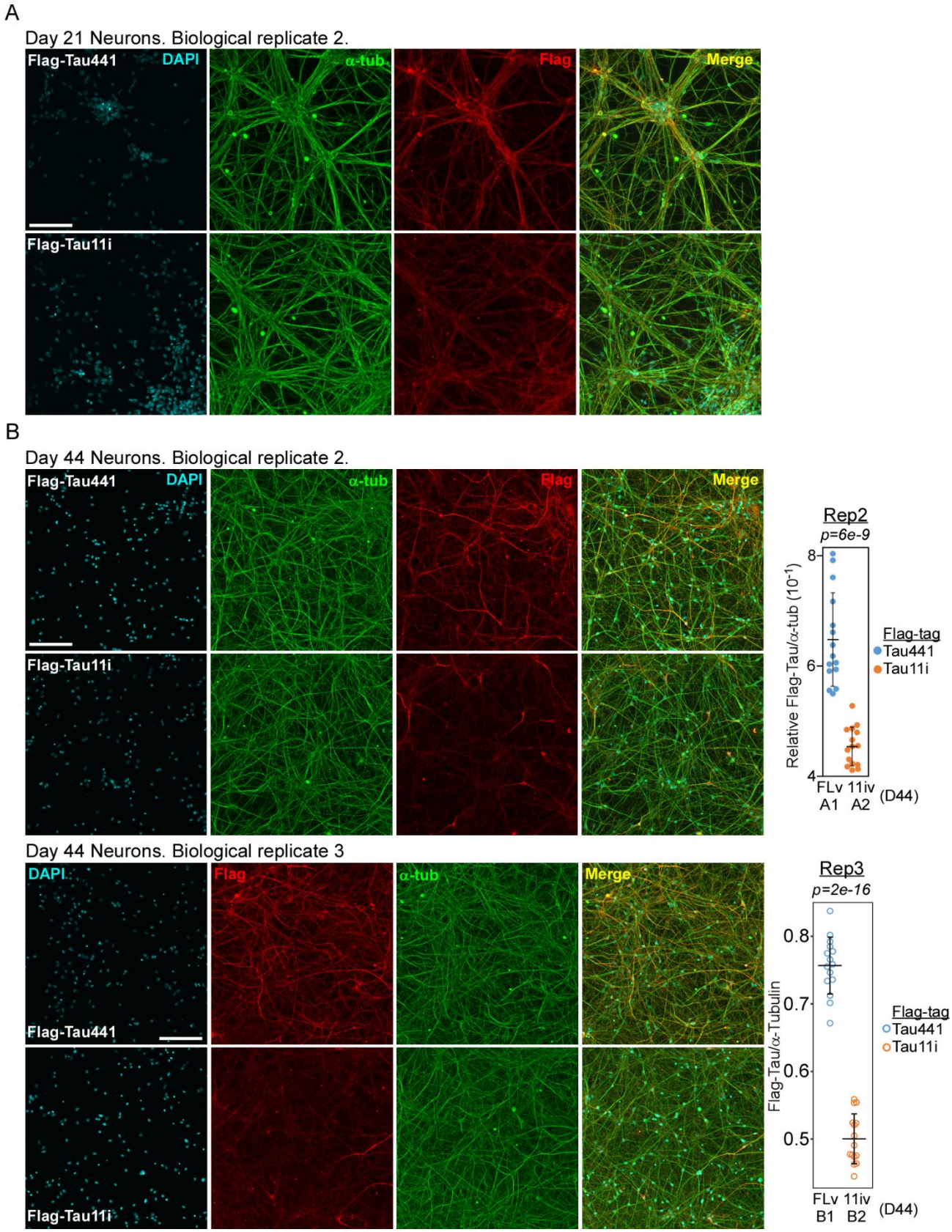

Figure S3. Lower co-localization of Tau11i with α-tubulin in mature neurons.

Representative image from different biological replicate of (*A*) Day 21 and (*B*) Day 44 neurons. Scale bar is 100 μm. Quantification of Flag-Tau over α-tubulin is presented as mean ± SD where each circle represents one field. *P*-value was calculated by 2-tailed *t*-test. "Rep" stands for replicate. Related to Figure 4A.

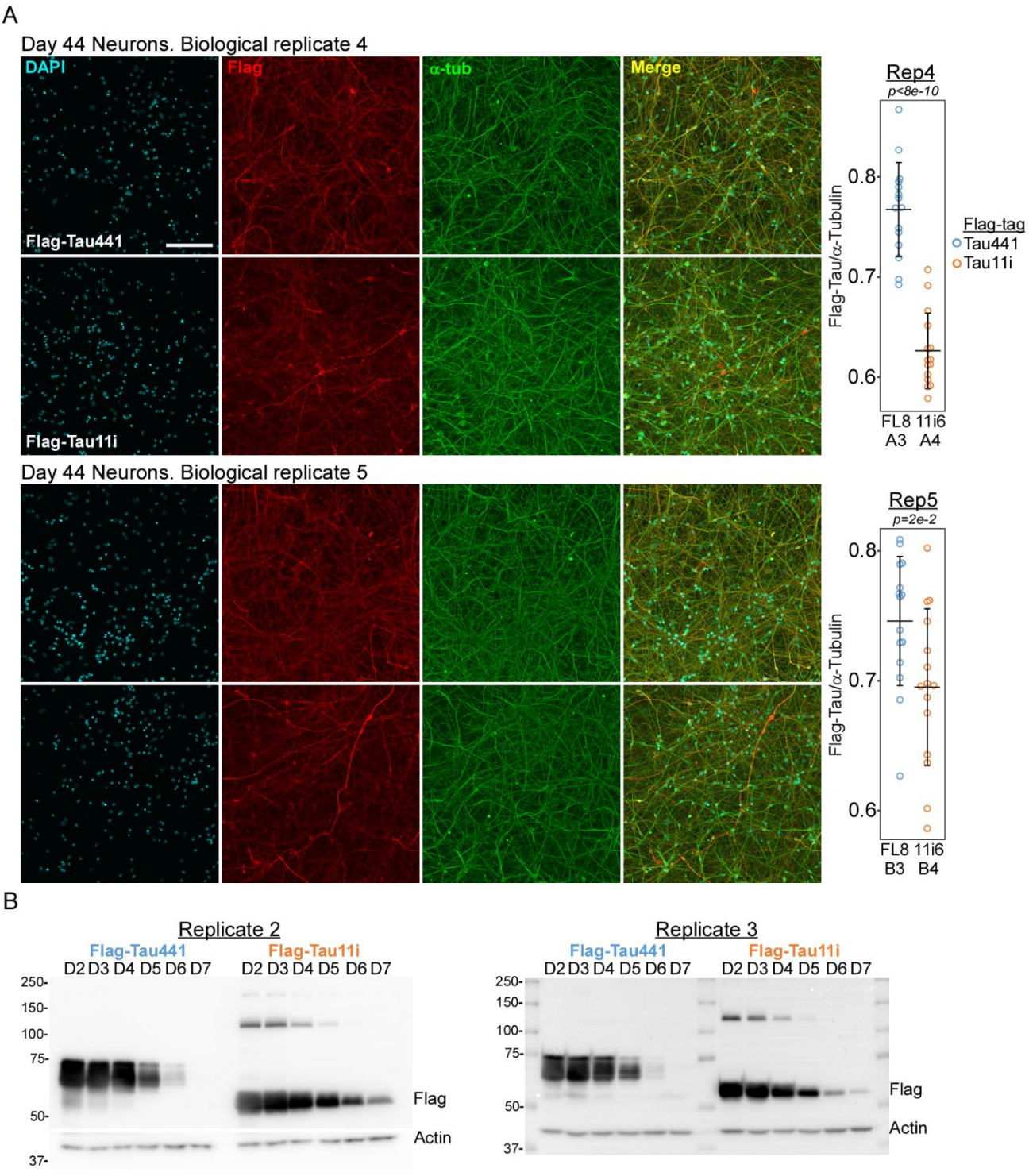

Figure S4. Tau11i has weaker co-localization signal with α-tubulin in mature neurons and higher protein stability.

(A) Representative images from two biological replicates of Day 44 neurons expressing either Flag-Tau441 or Flag-Tau11i. Scale bar is 100 μm. Quantification of Flag-Tau over α-tubulin is presented as mean ± SD where each circle represents one field. *P*-value was calculated by 2-tailed *t*-test. Related to Figure 4A. "Rep" stands for replicate. *(B)* Representative immunoblots

of 293T cells harvested on different day "D" after transfection with Flag and actin antibodies. Related to Figure 4B.

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