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Supplementary Data

Characteristics of TBS-Extractable Hyperphosphorylated Tau Species: Aggregation Intermediates in rTg4510 Mouse Brain

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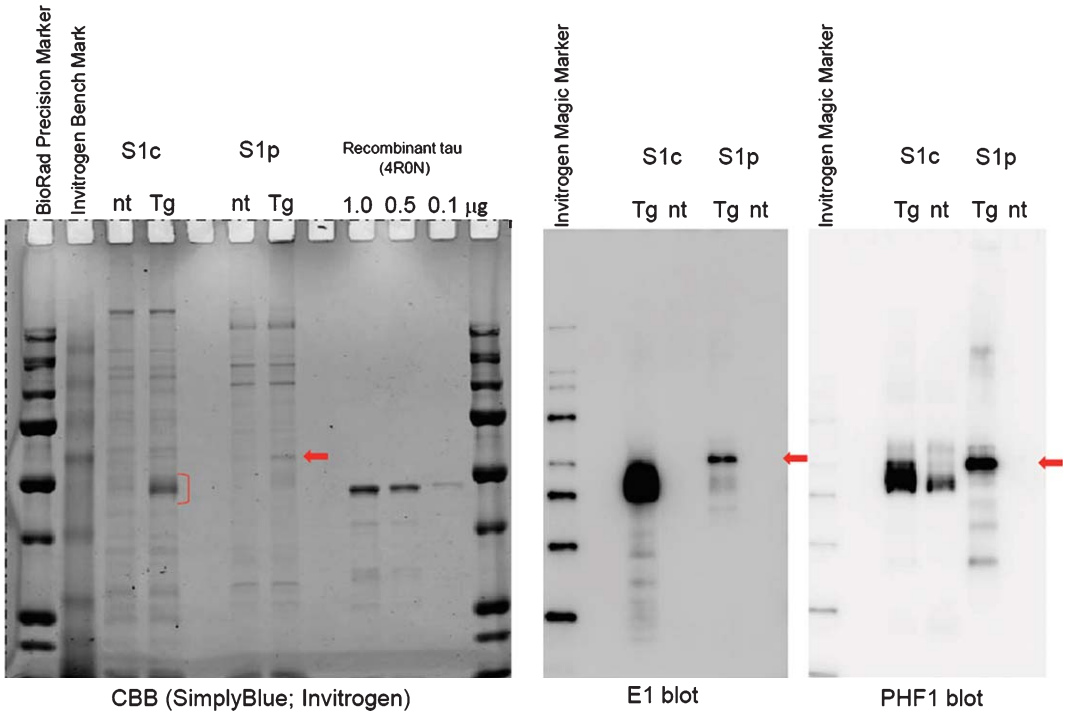
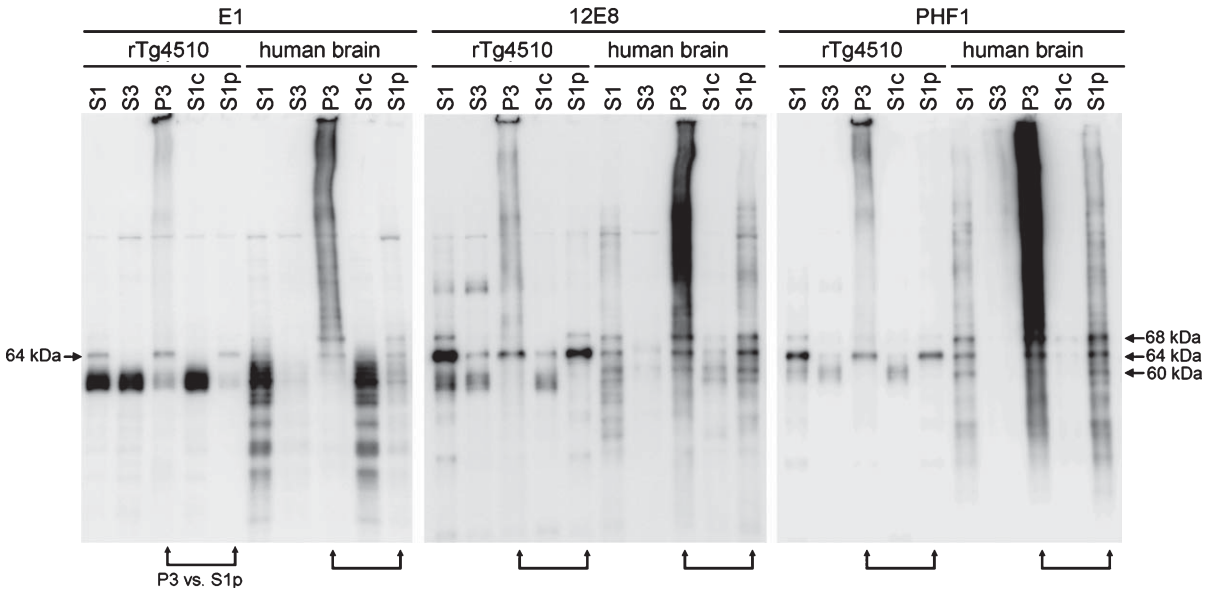


Supplementary Figure 1. Thermo-stability of tau in S1c and S1p fractions. Both S1c and S1p fractions from 6 month-old rTg4510 brain and 6 month-old non-Tg brain were incubated at 95°C for 10 min. After centrifugation, supernatants were prepared for SDS-PAGE samples and loaded into 10% Tris-Glycine SDS-PAGE gel. A) Gel was stained with Simply Blue staining kit (Invitrogen). B) Western blot with hTau specific antibody E1. C) Western blot with phosphorylated tau specific antibody PHF1. Arrows indicated 64 kDa tau.



Supplementary Figure 2. Western blots showing tau protein extracted from rTg4510 mouse and human brains. The mouse samples came from 6 month-old mice. We compared S1, S3, P3, S1c, and S1p fractions by examining blots stained with E1, 12E8, and PHF1 antibodies. Sample loading volumes were adjusted for the detection of hTau based on original tissue weight. For rTg4510 mouse brain samples, S1, S3, P3, S1c, and S1p fractions were derived from 0.01, 0.16, 0.5, 0.01, and 0.05 mg of tissue, respectively. For human brain samples, S1, S3, P3, S1c, and S1p fractions were derived from 0.2, 0.4, 10, 0.2, and 1.0 mg of tissue, respectively. Because S3 fractions derived from human tissue almost reached the maximum loading level, the ratios between mouse brain and human brain samples were different. Arrows indicated bands of 68, 64, 60 kDa tau.

Supplementary Table 1
List of anti-tau antibodies

Antibody	Epitope	Immunoblotting	ImmunoEM	dot blotting	Source
E1	19–33	1 : 5,000	1 : 20		[1]
WKS44	162–178	1 : 5,000			[1, 2]
Tau5	218–225	1 : 2,000			Dr. Lester I. Binder (Northwestern University Medical School)
WKS46	359–370	1 : 5,000			[1]
Tau46	420–436	1 : 5,000	1 : 50		Zymed
AT270	pThr181	1 : 2,000			Innogenetics Inc.
Tau1	Ser199	1 : 5,000			[3–5], Dr. Lester I. Binder (Northwestern University Medical School)
CP13	pSer202/pThr205	1 : 200			[6], Dr. Peter Davies (Albert Einstein College of Medicine)
pS212	pSer212	1 : 2,000			Invitrogen
AT100	pThr212/pSer214	1 : 1,000			Innogenetics Inc.
PHF6	pThr231	1 : 5,000			AbCam
12E8	pSer262/pSer356	1 : 2,000			Dr. Peter Seubert (Elan Pharmaceuticals)
PHF1	pSer396/pSer404	1 : 2,000			[4, 5, 7], Dr. Peter Davies (Albert Einstein College of Medicine)
pS400	pSer400	1 : 1,000			Invitrogen
pS422	pSer422	1 : 2,000			Invitrogen
Tau13	2–18	1 : 5,000		1 : 5,000	Covance
MC1	conformation (7–9 & 326–330)			1 : 500	[8], Dr. Peter Davies (Albert Einstein College of Medicine)
Ab39	tau tangles			1 : 250	[9, 10]

REFERENCES

[1] Kenessey A, Nacharaju P, Ko LW, Yen SH (1997) Degradation of tau by lysosomal enzyme cathepsin D: Implication for Alzheimer neurofibrillary degeneration. *J Neurochem* **69**, 2026-2038.

[2] Ko LW, Ko EC, Nacharaju P, Liu WK, Chang E, Kenessey A, Yen SH (1999) An immunochemical study on tau glycation in paired helical filaments. *Brain Res* **830**, 3013-3013.

[3] Binder LI, Frankfurter A, Rebhun LI (1985) The distribution of tau in the mammalian central nervous system. *J Cell Biol* **101**, 1371-1378.

[4] Ko LW, Liu WK, Georgieff IS, Yen SH (1996) Modulated induction of tau proteins in cultured human neuroblastoma cells. *Brain Res* **707**, 256-265.

[5] Ko LW, Liu WK, Georgieff IS, Yen SH (1997) Modulated induction of tau proteins in cultured human neuroblastoma cells. *Brain Res* **707**, 256-265.

[6] Weaver CL, Espinoza M, Kress Y, Davies P (2000) Conformational change as one of the earliest alterations of tau in Alzheimer's disease. *Neurobiol Aging* **21**, 719-727.

[7] Greenberg SG, Davies P, Schein JD, Binder LI (1992) Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau. *J Biol Chem* **267**, 564-569.

[8] Jicha GA, Bowser R, Kazam IG, Davies P (1997) Alz-50 and MC-1, a new monoclonal antibody raised to paired helical filaments, recognize conformational epitopes on recombinant tau. *J Neurosci Res* **48**, 128-132.

[9] Yen SH, Crowe A, Dickson DW (1985) Monoclonal-antibodies to Alzheimer neurofibrillary tangles. 1. Identification of polypeptides. *Am J Pathol* **120**, 282-291.

[10] Yen SH, Dickson DW, Crowe A, Butler M, Shelanski ML (1987) Alzheimer's neurofibrillary tangles contain unique epitopes and epitopes in common with the heat-stable microtubule associated proteins tau and MAP2. *Am J Pathol* **126**, 81-91.