

INCREASE IN WASTEOSOMES (CORPORA AMYLACEA) IN FRONTOTEMPORAL LOBAR DEGENERATION WITH SPECIFIC DETECTION OF TAU, TDP-43 AND FUS PATHOLOGY

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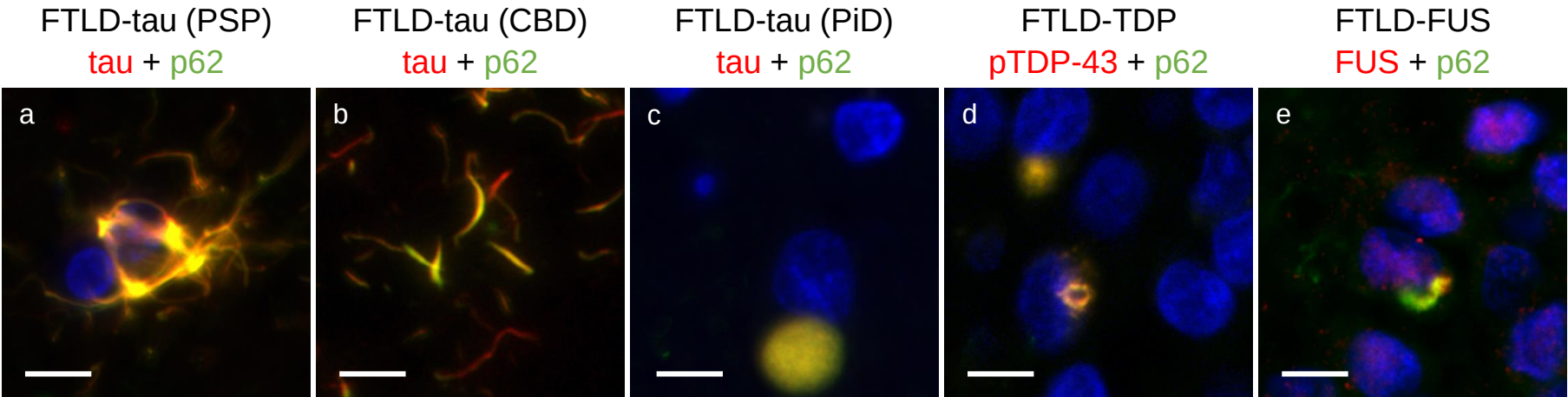
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Supplementary Figure 1 Confirmation of antibody specificity through detection of characteristic lesions of the different proteinopathies using immunofluorescence. **a** Tufted astrocyte stained with anti-p62 (green) and anti-tau (red) in the hippocampus of a PSP patient. **b** Astrocytic plaque stained with anti-p62 (green) and anti-tau (red) in the hippocampus of a CBD patient. **c** Pick body stained with (green) and anti-tau (red) in the hippocampus of a PiD patient. **d** pTDP-43 neuronal cytoplasmatic inclusion stained with anti-p62 (green) and anti-pTDP-43 (red) in the hippocampus of an FTLD-TDP patient. **e** FUS neuronal cytoplasmatic inclusion stained with anti-p62 (green) and anti-FUS (red) in the hippocampus of an FTLD-FUS patient. Nuclei are stained with Hoechst (blue). FTLD: frontotemporal lobar degeneration; PiD: Pick’s disease; PSP: progressive supranuclear palsy; CBD: corticobasal degeneration. Scale bar: 10 μ m.



Supplementary Table 1 Primary antibodies used for immunohistochemistry and immunofluorescence.

Antibody	Clone	Company	Host
Neuropathology			
anti-beta-amyloid	6F/3D	Dako, Glostrup, Denmark	Mouse
anti-phospho-tau	AT8	Thermo Scientific, Rockford, USA	Mouse
anti-tau (3-repeat isoform, tau RD3)	8E6/C11	Millipore, Temecula, CA, USA	Mouse
anti-tau (4-repeat isoform, tau RD4)	1E1/A6	Millipore, Temecula, CA, USA	Mouse
anti- α -synuclein	KM51	Leica, Barcelona, Spain	Mouse
anti- α -synuclein	5G4	Roboscreen, Leipzig, Germany	Mouse
anti-phosphorylated- α -synuclein	pSyn#64	Wako Chemicals, Richmond, VA, USA	Mouse
anti-FUS	3A10B5	Proteintech, Rosemont, IL, USA	Mouse
anti-pTDP-43	11-9	Cosmo Bio, Tokyo, Japan	Mouse
anti-ubiquitin	Polyclonal	Dako, Glostrup, Denmark	Rabbit
anti-p62	3/P62 LCK ligand	BD Biosciences, San Jose, USA	Mouse
anti- α -internexin	2E3	Novex, Invitrogen, Thermo Scientific, Rockford, IL, USA	Mouse
Immunofluorescence for protein detection in wasteosomes			
anti-tau	Tau5	Thermo Fisher Scientific; Rockford, IL, USA	Mouse
anti-FUS	3A10B5	Proteintech, Rosemont, IL, USA	Mouse
anti-pTDP-43	11-9	Cosmo Bio, Tokyo, Japan	Mouse
anti-p62	2C11	Abcam, Cambridge, UK	Mouse

Supplementary Table 2 Description of the wasteosome score according to each area. CS: calcarine sulcus; DG: dentate gyrus; FDS: fimbriodentate sulcus; Fi: fimbria; FOV: field of view; Hc: hippocampus; HS: hippocampal sulcus; Lent: lentiform nucleus; MST: medial superior temporal; PSB: pre-subiculum; SB: subiculum; SFG: superior frontal gyrus.

Regions		Area																			
		Subpial								Periventricular		Perivascular				Intraparenchymal					
		SFG	Lent	CS	MST	Hc				Hc	Lent	SFG	Lent	CS	MST	SFG	Lent	CS	MST	Hc	
						FDS	HS	SB	PSB											DG	Fi
Score per area	0	No wasteosomes								No wasteosomes				No wasteosomes							
	1	Isolated wasteosomes								Isolated wasteosomes in the tissue immediately after the perivascular space				1-3 wasteosomes							
	2	1 row of wasteosomes								Isolate wasteosomes in the tissue immediately after the perivascular space + 1-3 wasteosomes in tissue close to the vessel				4-11 wasteosomes							
	3	2 rows of wasteosomes (or 1 row of wasteosomes + 1-3 isolated wasteosomes close to the row)								1 row of wasteosomes in the tissue immediately after the perivascular space				12-27 wasteosomes							
	4	≥ 2 rows and isolated wasteosomes close to the rows of wasteosomes								2 rows of wasteosomes in the tissue immediately after the perivascular space				28-60 wasteosomes							
	5	≥ 2 rows + high density of wasteosomes surrounding the rows								≥ 2 rows of wasteosomes + high density of wasteosomes in the tissue close to the vessel				> 60 wasteosomes							
Wasteosome score		Mean value of the score given to each depth of sulci						Score given to the FOV at 400x with highest wasteosomes accumulation		Mean value of the score given to vessels of the 3 FOV at 100x				Score given to the FOV at 400x with highest accumulation of wasteosomes							

Supplementary Table 3 Demographic and neuropathological data about the brain donors used for immunofluorescence. ARTAG: aging-related tau astrogliopathy; PMD: post-mortem delay; CBD: corticobasal degeneration; FTLD: frontotemporal lobar degeneration; FUS: Fused in Sarcoma; LATE: limbic-predominant age-related TDP-43 encephalopathy; PSP: progressive supranuclear palsy.

Subject	Sex	Age at death	PMD (hh:mm)	Neuropathological diagnosis	Copathology			
					ABC score	ARTAG in the hippocampus	LATE	FUS pathology
1	Female	70	11:00	FTLD-TDP (Type C)	A0B0C0	No	No	No
2	Male	77	07:15	FTLD-TDP (Type C)	A2B0C1	No	No	No
3	Male	64	10:30	FTLD-TDP (Type AB)	A1B0C0	No	No	No
4	Male	72	7:00	FTLD-CBD	A0B0C0	No	No	No
5	Female	71	04:30	FTLD-CBD	A0B0C0	No	No	No
6	Male	77	7:00	FTLD-PSP	A1B0C0	No	No	No
7	Male	78	11:00	FTLD-Pick	A1B0C1	No	No	No
8	Male	56	16:25	FTLD-FUS	A0B0C0	No	No	Yes
9	Female	54	5:47	FTLD-FUS	A1B0C0	No	No	Yes
10	Male	43	4:00	FTLD-FUS	A0B0C0	No	Yes	Yes