Supporting information for:
The increase of wasteosomes in neurodegenerative diseases points to chronic glymphatic insufficiency as a key factor for these diseases

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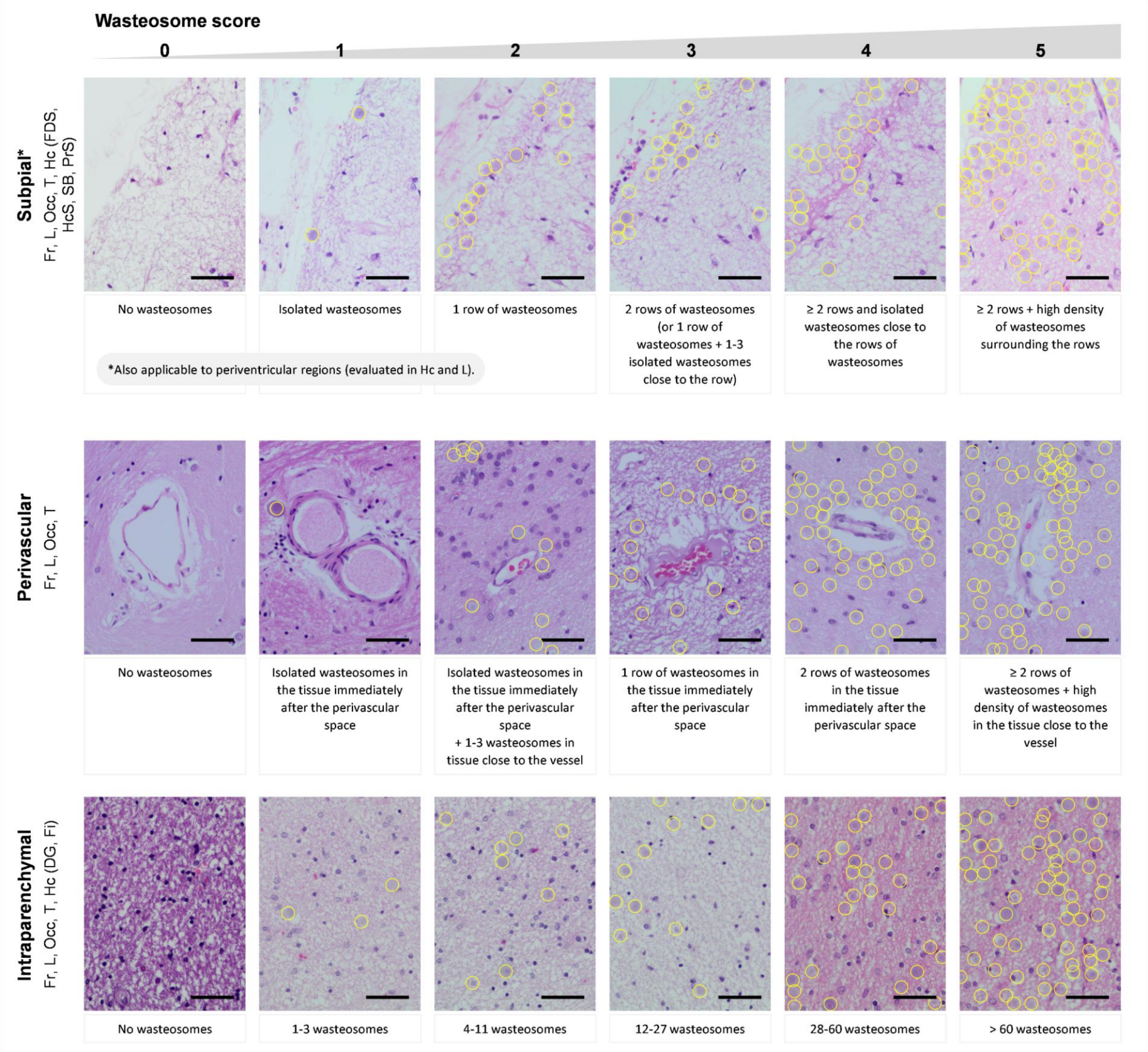
Figure S1. Wasteosome scoring across subpial, perivascular, and intraparenchymal regions is illustrated using representative haematoxylin and eosin-stained sections obtained from control brains and brains affected by neurodegenerative diseases. The top row shows five subpial fields from the temporal lobe, the middle row displays five perivascular fields from the lentiform nucleus, and the bottom row presents five intraparenchymal fields from the fimbria of the hippocampus. All fields are scored from 0 to 5, with the scoring criteria indicated beneath each representative image. Individual wasteosomes are highlighted with yellow circles. DG, dentate gyrus; FDS, fimbriodentate sulcus; Fi, fimbria; FOV, field of view; Fr, frontal (superior frontal gyrus); Hc, hippocampus; HcS, hippocampal sulcus; L, lentiform nucleus; Occ, occipital (calcarine sulcus of the occipital cortex); PrS, presubiculum; SB, subiculum; T, temporal (medial superior temporal cortex). Scale bar: 50 µm. Adapted from (1).



Reference

1. R. Alsina, et al., Increase in wasteosomes (corpora amylacea) in frontotemporal lobar degeneration with specific detection of tau, TDP-43 and FUS pathology. Acta Neuropathol. Commun. 12, 97 (2024).

Figure S2. Box plot of Wasteosome scores by Regions. The box plot shows wasteosome scores across the different regions, including all available data for each region. Regions with very low semiquantitative values across all groups, often close to zero, and regions with a high proportion of missing values were excluded from inferential analysis due to limited signal resolution and lack of reliable dynamic range (red boxes and boxes where color cannot be seen). Statistical comparisons were therefore restricted to regions exhibiting sufficient signal intensity to allow robust quantification (green boxes). These selected regions include the intraparenchymatous region of the fimbria (IP_Fi), the subpial region of the fimbriodentate sulcus (SP_FDS), the subpial region of the hippocampal sulcus (SP_HpS), the subpial region of the subiculum (SP_Sb), the subpial region of the presubiculum (SP_PrS), a subpial region in the area of the brain containing the lentiform nucleus (SP_L), and the perivascular region around the thalamostriate vein (or its perforant branches) in the same brain area (PVa_TS). Importantly, semiquantitative data are available for all regions in the control, FTLD-tau, FTLD-FUS, and FTLD-TDP groups (146 out of 185 donors), whereas data from AD and ALS-TDP groups (39 out of 185) are semiquantified in the green regions, reflecting incomplete sample coverage rather than biological absence of signal. See the main text for further details.

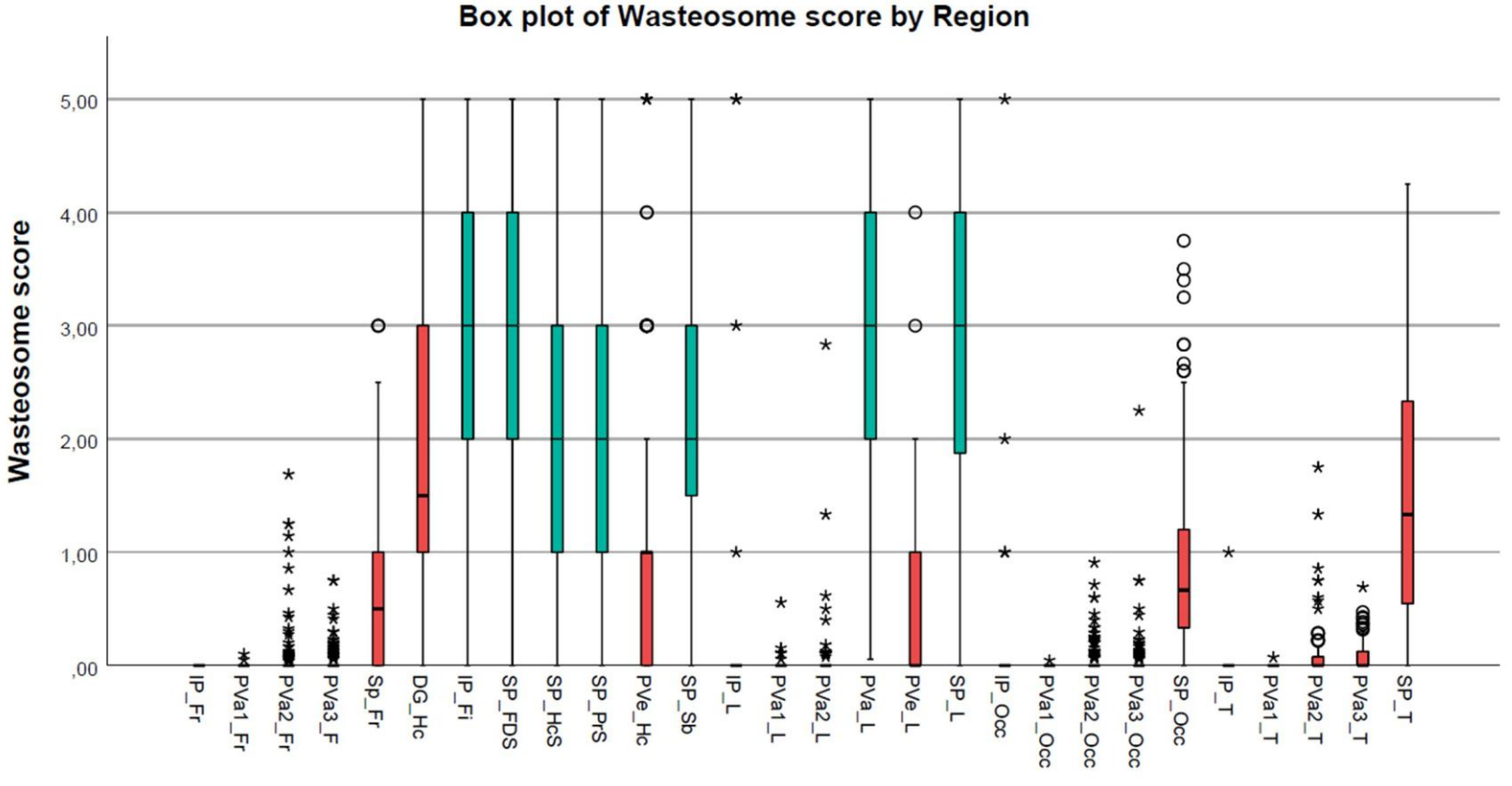


Table S1. Inclusion and exclusion criteria. ALS = amyotrophic lateral sclerosis; CBD = corticobasal degeneration; FTLD = frontotemporal lobar degeneration; NIA-AA = National Institute on Aging-Alzheimer's Association; PSP = progressive supranuclear palsy.

		Inclusion criteria	Exclusion criteria
AD		NIA-AA ABC score: A3B3C3	Genetic cases McKeith type: limbic or neocortical
FTLD-Tau	FTLD-CBD	All cases available	Genetic cases
	FTLD-Pick	All cases available	Genetic cases
	FTLD-PSP	Minimal copathology	Genetic cases
FTLD-TDP		All cases available	Genetic cases
ALS-TDP		Brettschneider stage: 2 or 3	Brettschneider stage: 1 or 4

Table S2. Characteristics of the donors. AD = Alzheimer's disease; ALS = amyotrophic lateral sclerosis; FTLD = frontotemporal lobar degeneration.

	Controls	AD	FTLD-Tau	FTLD-TDP	ALS-TDP
Participants (N)	29	20	75	42	19
Age at death (years, mean ± SD)	73.2 ± 14.6	85.1 ± 9.0	72.7 ± 8.5	72.5 ± 11.1	61.4 ± 15.7
Disease duration (years, mean ± SD)	-	9.3 ± 4.5	8.1 ± 4.1	7.8 ± 4.7	3.8 ± 2.4
Post-mortem delay (hours, mean ± SD)	12.3 ± 6.9	11.5 ± 5.9	8.5 ± 4.1	9.0 ± 4.5	12.8 ± 3.6
Sex (% female)	58.6	75	57.3	38.1	47.4

Table S3. Primary antibodies used for neuropathological examination.

Antibody	Clone	Company	Host
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-p-α-synuclein	pSyn#64	Wako Chemicals, Richmond, VA, USA	Mouse
anti-FUS	3A10B5	Proteintech, Rosemont, IL, USA	Mouse
anti-p-TDP-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	Thermo Scientific, Rockford, IL, USA	Mouse