

An astrocyte-neuron HSPB1 axis preserves lysosomal integrity and protects from tau pathology

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Supplementary Figures 1-4

Supplementary Tables 1-2

A

Target probe Pre-amplifier Amplifier Probe

UBS-C, DapB, HSPB1

FFPE human brain

B

DapB / DAPI UBQ-C / DAPI HSPB1 / DAPI

20 μm

C

ALDH1L1+

0-III V-VI HSPB1/DAPI

10 μm

D

Astrocytes with HSPB1 mRNA (Braak 0-VI)

% of HSPB1 mRNA+ cells that are ALDH1L1+

ALDH1L1+

E

NeuN+

0-III V-VI HSPB1/DAPI

10 μm

F

Neurons with HSPB1 mRNA (Braak 0-VI)

% of HSPB1 mRNA+ cells that are NeuN+

NeuN+

Supplementary Figure 2

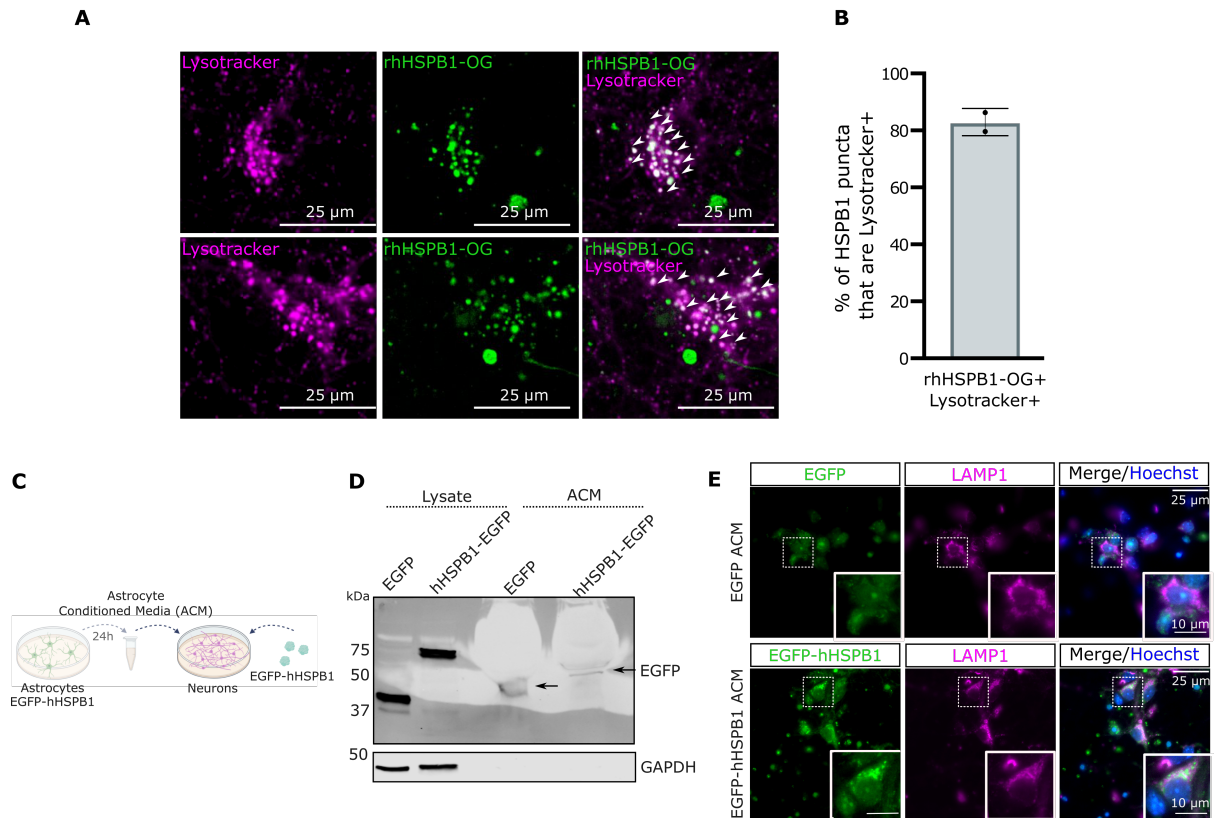


Figure S2. Primary neurons recruit exogenous rhHSPB1 to lysosomes. A. Representative images of primary neurons treated with rhHSPB1-OG for 6h. Functional lysosomes are stained with LysoTracker (magenta) and nuclei with Hoechst 33342. Arroheads indicate puncta positive for rhHSPB1-OG and LysoTracker. Scale bar: 25 μ m. **B.** Bar graph displays the percentage of number of rhHSPB1-OG that are LysoTracker positive. **C.** Schematic shows that primary mouse neurons were treated for 24 hours with conditioned medium from astrocytes transfected with EGFP-hHSPB1. **D.** Representative western blot shows the expression of EGFP or EGFP-hHSPB1 in astrocyte lysates or 24h conditioned medium from transfected astrocytes. GAPDH is used as a loading control for cell lysates. **E.** Representative images of primary neurons treated with conditioned media from astrocytes expressing HSPB1-EGFP or EGFP control and immunolabelled with LAMP1 (magenta). Nuclei are stained with Hoechst 33342. Scale bar: 25 μ m. Insets delimit areas displayed at higher magnification. Scale bar: 10 μ m.

Supplementary Figure 3

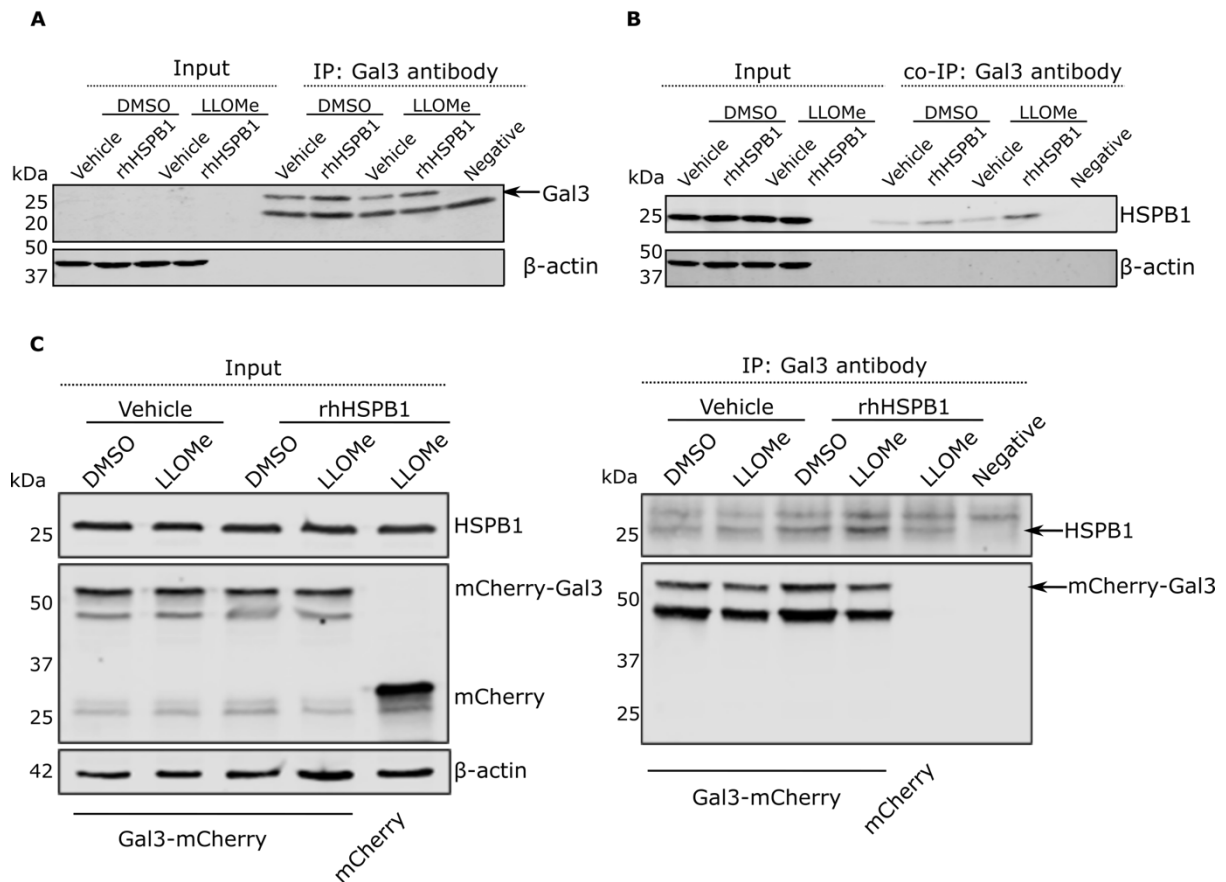


Figure S3. HSPB1-Gal3 interaction following lysosomal damage. HSPB1 and Gal 3 protein interaction was assessed in SH-SY5Y (A-B) or SH-SY5Y cells transfected mCherry-Gal3 (C-D) pre-treated with 50 ng/mL rhHSPB1 or vehicle (0.1% BSA) for 24 h and treated with 50 μ M LLOMe or vehicle (DMSO) for 1 h. **A-B.** Representative immunoblots of Gal3 (A) and HSPB1 (B) where total Gal3 and HSPB1 were detected in the protein lysates (input) with β -actin used as loading control, or in the eluted fraction after immunoprecipitation with a Gal 3 antibody. Negative control refers to IP with no lysates to distinguish IgG only bands. **C-D.** Representative immunoblots of mCherry and HSPB1 where total mCherry, mCherry-Gal 3 and HSPB1 were detected in lysates (input) with β -actin used as loading control (C) or in the eluted fraction after immunoprecipitation with a Gal 3 antibody (D). Cells transfected with mCherry were used as a control (note that HSPB1 interaction with endogenous Gal 3 is still detected but to a lesser extent). Negative control refers to IP with no lysates to distinguish IgG only bands.

Supplementary Figure 4

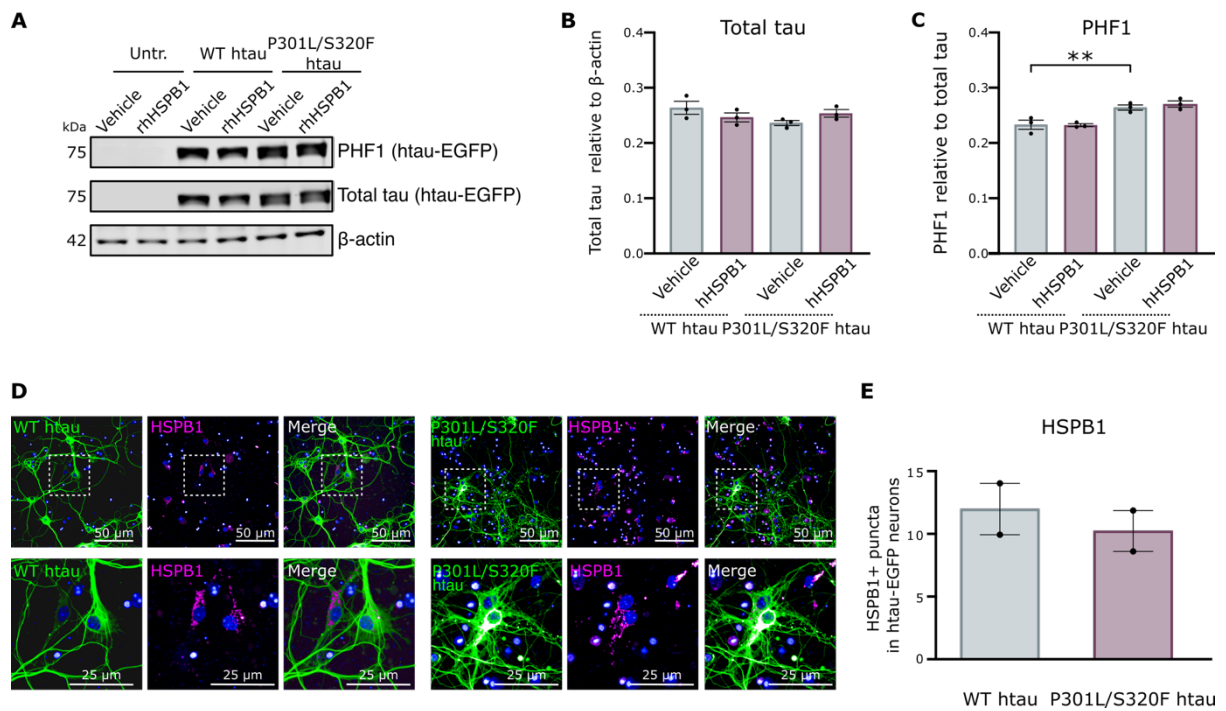


Figure S4. Internalized HSPB1 does not alter phosphorylation of mutant htau. A. Representative immunoblots of tau phosphorylated at PHF1 (Ser396/404) and total tau in primary neurons expressing WT or P301L/S320F-hTau-EGFP or non-transduced and treated with 50 ng/mL rhHSPB1 or vehicle (0.1% BSA) at DIV6, 8 and 10 and collected at DIV12. β -actin was used as the loading control. **B-C.** Bar graphs display the quantification of **(B)** total tau relative to β -actin or **(C)** phosphorylated tau (PHF1) relative to total tau. Data is mean $\pm$ SD. N=3. Two-way ANOVA with Tukey's multiple comparison test. **D.** Representative images of primary neurons expressing WT or P301L/S320F mutant htau-EGFP treated with rhHSPB1-Alexa568 for 1 h prior to imaging live. Nuclei are stained with Hoechst 33342. Scale bar: 50 μ m. Insets delimit areas displayed at higher magnification below. Scale bar: 25 μ m. **E.** Bar graph displays the average number of HSPB1-Alexa568 puncta in neurons expressing WT or mutant htau-EGFP. Data is mean $\pm$ SD. N=2.

Supplementary Figure 5

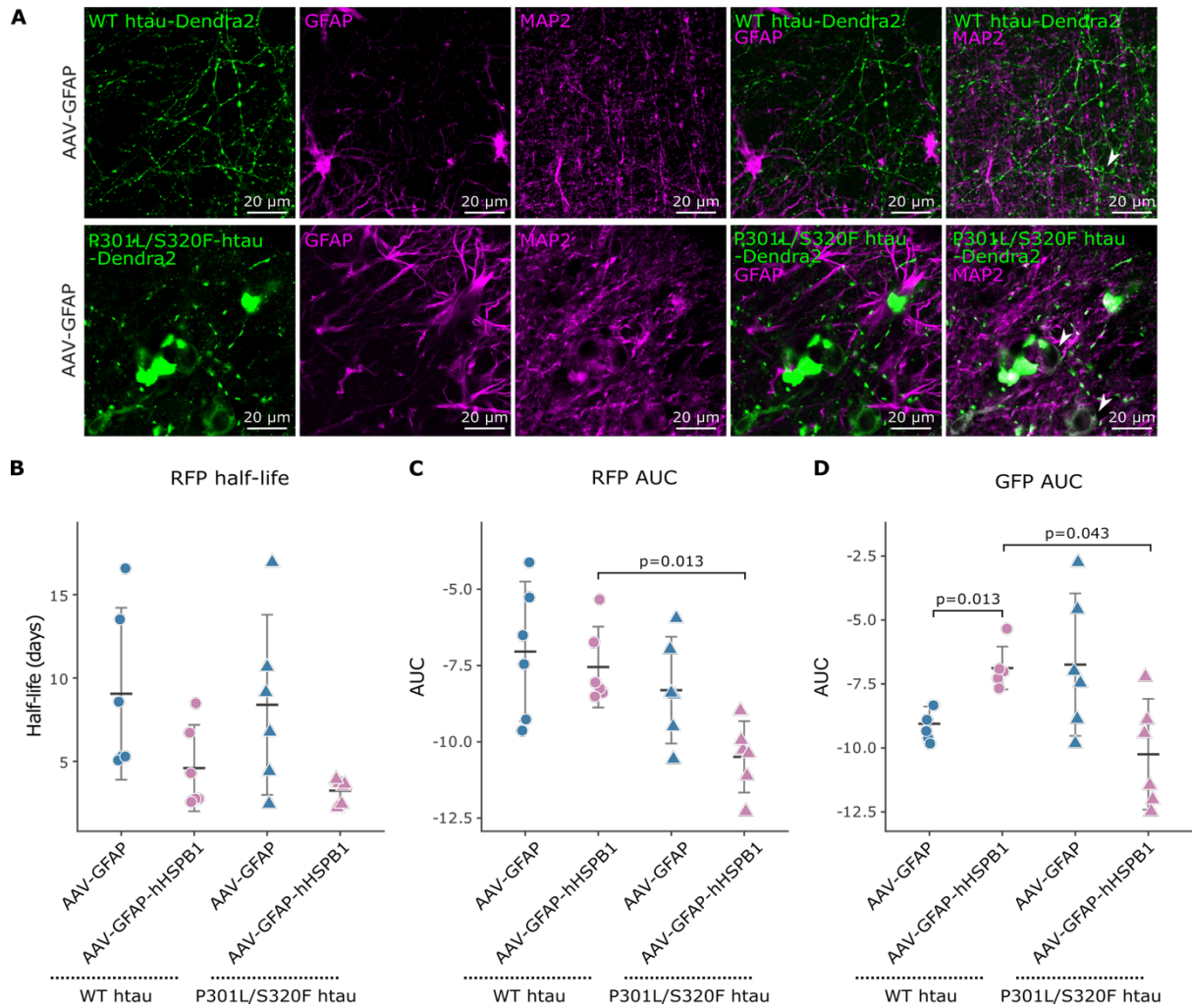


Figure S5. Photoconverted and unconverted htau-Dendra2 signal dynamics. A. Representative immunofluorescence images of fixed mouse organotypic brain slice cultures expressing Dendra2-tagged WT or P301L/S320F mutant htau (green) and AAV-GFAP control, immunolabelled with antibodies against GFAP (magenta) and MAP2 (magenta). Arrowheads indicate overlap of Dendra2-tagged htau and MAP2-positive neurons. N = 2 organotypic slice cultures per group. Scale bar is 20 μ m. **B.** Half-life in days of the photoconverted Dendra2-htau. **C.** RFP area under the curve (AUC), referenced to the immediate post-photoconversion measurement at 0 to 21 days post-conversion. **D.** GFP AUC, referenced to the pre-photoconversion measurement, 0 to 21 days post-conversion. Blue, AAV-GFAP; pink, AAV-GFAP-hHSPB1. Circles, WT-htau; triangles, P301L/S320F-htau. Each point is an individual organotypic slice culture and bars show the mean with 95% confidence interval. N=6 organotypic slice cultures per group. Kruskal-Wallis omnibus test followed by Holm adjusted pairwise comparisons. Omnibus p-values are shown on each panel. p-values are the four comparisons of interest: the effect of construct within each tau background, and the effect of tau background within each construct.

Supplementary Table 1

Sex	Age (y)	PMD (h)	Braak Stage	Pathology/Diagnosis	Brain Area	Use
M	78	10	0/I	Control. Ageing changes (mild).	BA21	WB
M	90	45	0	Control brain. Mild age-related changes.	BA9	WB
F	77	21	I	Control adult brain.	BA9	WB
M	82	47	I	Control case. Very early Alzheimer disease pathology – BNE stage I) with focal amyloid angiopathy.	BA9	WB
F	73	27	I	Control. Early ageing changes BNE stage I.	BA9	WB
F	81	17	I	Control. Mild ageing changes Braak I.	BA21	WB
M	84	53	I/II	Control. Control case but with hypoxic-type changes and amyloid angiopathy.	BA9	WB
M	80	48.2 5	I/II	Control. Normal adult brain consistent with ageing.	BA9	WB
F	89	41	I/II	Control with mild focal amyloid angiopathy.	BA9	WB
M	82	24	II	Control. Old infarcts in the right cerebral hemisphere.	BA9	WB
F	92		II	Control adult case.	BA9	WB
M	79	47	II	Early tau pathology, Braak II, no neuritic plaques.	BA9	WB
M	93	33	II	Mild Alzheimer's-type changes Braak II.	BA21	WB
F	90	50	II	Control brain, mild Alzheimer-type changes and mild amyloid angiopathy.	BA21	WB

M	81	12.5	III	Alzheimer changes consistent with Braak III and amyloid angiopathy.	BA9	WB
						CRR
F	92	19.5	III	Alzheimer's disease (moderate – limbic stage) Braak III.Cerebral infarcts.	BA9	WB
F	98	3.5	III	Alzheimer's disease BNE IV, CERAD probable: TDP-43 pathology (hippocampus and amygdala).	BA9	CRR
F	89	36.5	III	Alzheimer's disease pathology (BNE stage IV) with amyloid angiopathy and limbic TDP-43 pathology but cognitively not significantly impaired.	BA9	CRR
M	86	52	III	Ageing changes (AD modified Braak III).	BA9	CRR
					BA21	WB
M	92	11	III	Mild Alzheimer-type changes Braak III.	BA21	WB
M	88	79	III/IV	Alzheimer's Disease (modified Braak (BNE) stage III-IV. Mild amyloid angiopathy.	BA9	WB
M	84	86	IV	Alzheimer's disease (modified Braak (BNE) stage IV) with moderate amyloid angiopathy.	BA9	WB
						CRR
F	95	47	IV	Alzheimer's disease (modified Braak (BNE) stage IV) with moderate amyloid angiopathy and moderate to severe cerebrovascular changes and limbic TDP-43 pathology (Joseph stage II).	BA9	WB
						CRR
F	86	55.5	IV	Alzheimer's disease (modified Braak (BNE) stage IV) with mild amyloid angiopathy.	BA9	WB
						CRR

M	91	48	IV	Alzheimer's disease pathology (modified Braak (BNE) stage IV), Thal phase IV with moderate to severe amyloid angiopathy.	BA9	WB
M	98	53	IV	Alzheimer's Disease (modified Braak (BNE) stage IV, Mild amyloid angiopathy. Mild limbic TDP-43 proteinopathy, watershed infarcts.	BA9	WB
						CRR
M	92	70	IV	Alzheimer's disease (BNE stage 4).	BA9	CRR
M	82	28	IV	Alzheimer's disease (modified Braak stage IV) with limbic TDP-43 pathology. Widespread severe amyloid angiopathy; diffuse microvascular cerebrovascular pathology.	BA9	WB
					BA21	WB
F	92	29.5	IV	Alzheimer's disease Braak IV. Tau positive grain disease.	BA9	WB
F	83	22	IV	AD (limbic stage-modified Braak IV) with moderate-severe amyloid angiopathy.	BA9	CRR
					BA21	WB
M	86	53	IV	AD (modified Braak IV) with extensive severe amyloid angiopathy.	BA9	CRR
					BA21	WB
M	83	77	V	Alzheimer's disease BNE modified Braak stage V with extensive amyloid angiopathy. mild to moderate small vessel disease in frontal and occipital white matter.	BA9	WB
						CRR
M	86	26	V	AD Braak V with moderate amyloid angiopathy.	BA9	CRR
F	84	24	V	Alzheimer's disease Braak V.	BA9	CRR
F	82	24	V	Alzheimer's disease Braak V.	BA9	CRR
F	80	13	V		BA9	CRR

				AD Braak V with mild amyloid angiopathy.	BA21	WB
F	82	69	V	AD Braak V with mild amyloid angiopathy.	BA9	CRR
					BA21	WB
M	86	70	V	Alzheimer's disease stage V with moderate to severe amyloid angiopathy	BA9	WB
M	73	35	V/VI	Alzheimer's disease Braak VI BNE 5.	BA9	WB
F	68	43	VI	Alzheimer's disease Braak VI	BA9	WB
F	83	26.5	VI	Alzheimer's disease Braak VI	BA9	WB
						CRR
M	72	5.5	VI	Alzheimer's disease Braak VI with marked amyloid angiopathy.	BA9	WB
F	95	48	VI	Alzheimer's disease (modified Braak stage VI, Thal phase V with mild amyloid angiopathy and limbic stage. Dementia with Lewy bodies and limbic TDP pathology.	BA9	WB
						CRR
M	85	27	VI	Alzheimer's disease Braak VI, Thal phase V and moderate degree of cerebrovascular pathology and limbic and early cortical TDP-43 pathology.	BA9	WB
F	89	38.5	VI	Alzheimer's disease (modified Braak (BNE) stage VI) with moderate amyloid angiopathy and dementia with Lewy bodies (limbic stage) and limbic TDP-43 pathology.	BA9	WB
						CRR
M	81	74	VI	AD Braak VI.	BA9	CRR
M	72	5	VI	AD Braak VI with marked amyloid angiopathy.	BA9	CRR

F	91	28.5	VI	Alzheimer's disease Braak VI.	BA9	CRR
F	69	16	VI	Alzheimer's disease Braak VI.	BA21	WB
F	85	79	VI	Alzheimer's disease stage VI with mild amyloid angiopathy and mild small vessel disease	BA9	WB
F	84	36	VI	Alzheimer's disease Braak VI.	BA21	WB
M	80	41	VI	Alzheimer's disease Braak VI with severe amyloid angiopathy.	BA9	CRR

Supplementary Table 1. Characteristics of human post-mortem samples employed for biochemical analysis. Age is shown in years (y), post-mortem delay (PMD) in hours (h), sex, pathology/diagnosis and Braak stage. BA9 and BA21 tissues used for protein quantification and correlation analysis are indicated as follows: western blotting (WB), correlation analysis of HSPB1 and tau species (CRR).

Supplementary Table 2

Sex	Age (y)	PMD (h)	Braak Stage	Pathology/Diagnosis	Brain Area	Use
F	86	45	II	Control brain. Alzheimer type changes. Mild small vessel disease, mild amyloid angiopathy.	BA21	IF
F	84	53	II	Control brain. Alzheimer changes consistent with patient's age.	BA21	IF
F	84	35	II	Control brain. Alzheimer changes consistent with normal ageing.	BA21	IF
M	73	49	I-II	Early Alzheimer-type changes.	BA21	IF
M	77	28.5	I	Mild to moderate amyloid angiopathy, capillary subtype.	BA21	IF
						RNA
F	78	6	I-II	Control brain. Primary Age related tauopathy (PART). Mild small vessel disease.	BA21	RNA
M	82	26	III	Alzheimer's type pathology, stage III. Moderate small vessel disease and moderate cerebral amyloid angiopathy.	BA21	RNA
M	97	20	0	Control case but with mild to moderate cerebrovascular disease.	BA21	RNA
F	82	13	0	Multiple system atrophy, argyrophilic grain disease and moderate small vessel disease.	BA21	RNA
M	75	59	V	Alzheimer's Disease Stage V. Thal phase 5 with moderate-severe amyloid angiopathy.	BA21	IF
M	72	41	VI	Alzheimer's Disease Braak Stage VI with extensive capillary amyloid angiopathy and significant cerebrovascular pathology.	BA21	IF
F	79	63	VI	Alzheimer's disease Braak Stage VI.	BA21.	IF

F	83	58	VI	Alzheimer's disease Braak Stage VI.	BA21	IF
F	85	12	VI	Alzheimer's disease Braak Stage VI.	BA21	IF
						RNA
M	72	6	VI	Alzheimer's disease Braak VI with marked amyloid angiopathy.	BA21	RNA
M	80	15	VI	Alzheimer's disease Braak VI.	BA21	RNA
F	96	20	VI	Alzheimer type changes corresponding with Stage VI. Very mild capillary type of cerebral amyloid angiopathy.	BA21	RNA
F	74	9	VI	Alzheimer's disease Stage VI with moderate amyloid angiopathy type 1 and moderate cerebrovascular disease.	BA21	RNA

Supplementary Table 2. Characteristics of human post-mortem samples employed for immunohistochemistry. Age is shown in years (y), post-mortem delay (PMD) in hours (h), sex, pathology/diagnosis and Braak stage. Tissues used for immunofluorescence or in-situ hybridisation (ISH) and immunofluorescence are indicated as follows: immunofluorescence (IF), ISH and immunofluorescence (RNA).