Supplementary Tables

Table S1. Control and ALS patient demographic and clinical information.

Case type	UoA case code	Age at onset (y)	Age at death (y)	Sex	PMD (h)	Clinical presentation or cause of death	Genetic mutation
Neurologically normal control	H211	N/A	41	M	8	Ischaemic heart disease	N/A
	H215	N/A	67	F	23.5	Ischaemic heart disease	N/A
	H231	N/A	65	M	8	Ischaemic heart disease	N/A
	H238	N/A	63	F	16	Dissecting aortic aneurysm	N/A
	H239	N/A	64	M	15.5	Ischemic Heart Disease	N/A
	H243	N/A	77	F	13	Ischaemic heart disease- coronary atherosclerosis	N/A
	H245	N/A	63	M	20	Asphyxia	N/A
	H248	N/A	98	M	10.5	Congestive heart failure	N/A
sALS	MN12	46	49	M	34	ALS	None found[a]
	MN13	55	55	M	10	ALS	None found[a]
	MN15	52	54	F	18	ALS + FTD	None found[a]
	MN30	84	84	F	17.5	ALS	N/D
UBQLN2-linked ALS	MN17	58	61	F	69	ALS + FTD	*UBQLN2* p.T487I
	IV:10	34	35	M	[b]	ALS	*UBQLN2* p.T487I
	V:7	29[c]	39	M	25.5	ALS + FTD	*UBQLN2* p.T487I

N/A, not applicable. N/D, not done.
[a] Negative for mutations in *C9orf72*, *TARDBP*, *SOD1* and *FUS*, as tested in Scotter et al. (2017).
[b] Information not available.
[c] Estimated; speech symptoms first reported at this time.

Table S2. Cases and regions for qualitative identification of subregions with ubiquilin 2-only aggregates.

Case type	Case	Region or sub-region	mtU2 aggs
Hippocampus			
UBQLN2-linked ALS/FTD	MN17, V:7	Dentate gyrus, Subiculum, Entorhinal cortex, Perirhinal cortex	**Yes**
Sporadic ALS	MN13, MN15	Dentate gyrus, Subiculum, Entorhinal cortex, Perirhinal cortex	
Non-neurodegenerative disease control	H238, H239, H245	Dentate gyrus, Subiculum, Entorhinal cortex, Perirhinal cortex	
Spinal Cord			
UBQLN2-linked ALS/FTD	MN17	Cervical segment 5, Thoracic segment 9, Lumbar segment 5	**Yes**
	V:7	Cervical segment, Thoracic segment, Lumbar segment	
	IV:10	Cervical segment	
Sporadic ALS	MN12	Cervical segment 8, Thoracic segment 9, Lumbar segment 5	
	MN13	Cervical segment 5, Thoracic segment 9, Lumbar segment 4	
	MN15	Cervical segment 5, Thoracic segment 9, Lumbar segment 2	
Non-neurodegenerative disease control	H211, H215, H248	Cervical segment 1	
Neocortex			
UBQLN2-linked ALS/FTD	MN17	Motor cortex 0, Motor cortex 3, Sensory cortex 0, Sensory cortex 3, Superior frontal gyrus 1, Middle frontal gyrus 0, Middle frontal gyrus 2, Inferior frontal gyrus 1, Superior temporal gyrus 1, Middle temporal gyrus 0, Middle temporal gyrus 3, Inferior temporal gyrus 1, Superior parietal 1, Inferior parietal 1, Visual cortex 1, Visual cortex 2, Auditory cortex 1, Cingulate gyrus 0, Cingulate gyrus 4, Orbito-frontal gyrus 1	**Yes**
	V:7	Motor cortex, Middle frontal gyrus	
	IV:10	Cortical regions A-C[a]	
Sporadic ALS	MN13	Motor cortex, Sensory cortex, Middle frontal gyrus 2, Middle temporal gyrus 2, Superior parietal 1, Visual cortex 2, Auditory cortex, Cingulate gyrus 0	
	MN15	Motor cortex 2, Sensory cortex 2, Middle frontal gyrus 2, Middle temporal gyrus 2, Superior parietal 1, Visual cortex 2, Auditory cortex, Cingulate gyrus 0, Cingulate gyrus 4	
Non-neurodegenerative disease control	H231, H238, H243	Sensory-motor cortex 0	
	H245	Sensory-motor cortex 3	
Striatum			
UBQLN2-linked ALS/FTD	MN17	Caudate nucleus, Putamen, Ventral striatum	**Yes**
	V:7	Caudate nucleus, Putamen	
Sporadic ALS	MN13, MN15	Caudate nucleus, Putamen, Ventral striatum	
Non-neurodegenerative disease control	H231, H238	Caudate nucleus, Putamen, Ventral striatum	
Medulla			

Case type	Case	Region or sub-region	mtU2 aggs
UBQLN2-linked ALS/FTD	MN17	Dorsal nucleus of vagus, Reticular formation (middle medulla), Raphe nuclei, Medial lemniscus, Inferior olivary nucleus, Medial accessory olivary nucleus, Medullary pyramids, Central grey, Gracile nucleus, Cuneate nucleus, Trigeminal nucleus, Reticular formation (lower medulla)	**Yes**
	V:7	Dorsal nucleus of vagus, Reticular formation, Raphe nuclei, Inferior olivary nucleus, Medial accessory olivary nucleus, Medullary pyramids	
Sporadic ALS	MN13, MN15	Dorsal nucleus of vagus, Reticular formation, Raphe nuclei, Medial lemniscus, Inferior olivary nucleus, Trigeminal nucleus, Medullary pyramids	
Substantia nigra			
UBQLN2-linked ALS/FTD	MN17 V:7 IV:10	Substantia nigra 1 Substantia nigra Midbrain-substantia nigra	**Yes**
Sporadic ALS	MN13 MN15	Substantia nigra Substantia nigra 1	
Globus Pallidus			
UBQLN2-linked ALS/FTD	MN17 V:7	Globus Pallidus Globus Pallidus externa	**No**
Sporadic ALS	MN13, MN15	Globus Pallidus	
Cerebellum			
UBQLN2-linked ALS/FTD	MN17	Cerebellum 3	**No**
	V:7, IV:10	Cerebellum	
Sporadic ALS	MN13, MN15,	Cerebellum	
Non-neurodegenerative disease control	H231, H243	Cerebellum	

[a]Tissue was provided in blocks from unspecified cortical regions. Cortical region A was confirmed to be motor cortex.

Abbreviations. mtU2 aggs, ubiquilin 2-only aggregates present in *UBQLN2*-linked ALS/FTD cases in this region.

Table S3. Cases and regions for quantitative analysis of ubiquilin 2 and TDP-43 aggregates.

Case type	Case	Region or sub-region
Hippocampus		
UBQLN2-linked ALS/FTD	MN17, V:7	Dentate gyrus, Subiculum, Entorhinal cortex, Perirhinal cortex
Sporadic ALS	MN13, MN15, MN30	Dentate gyrus, Subiculum, Entorhinal cortex, Perirhinal cortex
Spinal Cord		
UBQLN2-linked ALS/FTD	MN17	Cervical segment 5, Thoracic segment 9, Lumbar segment 5
	V:7	Cervical segment, Thoracic segment, Lumbar segment
	IV:10	Cervical segment
Sporadic ALS	MN13	Cervical segment 5, Thoracic segment 9, Lumbar segment 4
	MN15	Cervical segment 5, Thoracic segment 9, Lumbar segment 2
	MN30	Cervical segment 3, Thoracic segment 8, Lumbar segment 2
Motor cortex		
UBQLN2-linked ALS/FTD	MN17	Motor cortex 0, Motor cortex 3
	V:7	Motor cortex
	IV:10	Cortical region A[a]
Sporadic ALS	MN13	Motor cortex
	MN15	Motor cortex 2
	MN30	Motor cortex
Striatum		
UBQLN2-linked ALS/FTD	MN17	Caudate nucleus, Putamen, Ventral striatum
	V:7	Caudate nucleus, Putamen
Sporadic ALS	MN13, MN15, MN30	Caudate nucleus, Putamen, Ventral striatum

[a]Tissue was provided in blocks from unspecified cortical regions. Cortical region A was confirmed to be motor cortex.

Table S4. Primary antibodies used for fluorescent immunohistochemistry.

Primary antibody	Species (isotype)	Company	Catalogue #	Clonality	Dilution
Qualitative analysis panels					
Spinal cord					
Ubiquilin 2	Mouse (Ig_{G2a})	Santa Cruz	SC-100612	Monoclonal	1:800
pTDP-43	Rat (IgG_{2a})	BioLegend	BL829901	Monoclonal	1:3,000
SMI-32 (NFH)	Mouse (IgG_1)	BioLegend	BL801701	Monoclonal	1:500
MAP2	Chicken (IgY)	Abcam	ab5392	Polyclonal	1:2,000
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500
All cortex					
Ubiquilin 2	Mouse (IgG_{2a})	Santa Cruz	SC-100612	Monoclonal	1:800
pTDP-43	Rabbit (IgG)	CosmoBio	TIP-PTD-PO2	Polyclonal	1:4,000
SMI-32 (NFH)	Mouse (IgG_1)	BioLegend	BL801701	Monoclonal	1:500
MAP2	Chicken (IgY)	Abcam	ab5392	Polyclonal	1:2,000
Subcortical grey matter					
Ubiquilin 2	Mouse (IgG_{2a})	Santa Cruz	SC-100612	Monoclonal	1:800
pTDP-43	Rat (IgG_{2a})	BioLegend	BL829901	Monoclonal	1:3,000
MAP2	Chicken (IgY)	Abcam	ab5392	Polyclonal	1:2,000
**Calbindin D-28k	Rabbit (IgG)	SWant	CB-38a	Polyclonal	1:2,000
**Leu-enkephalin	Rabbit (IgG)	Immunostar	20066	Polyclonal	1:500
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500
Quantitative analysis panel					
Round 1					
Ubiquilin 2	Mouse (IgG_{2a})	Santa Cruz	SC-100612	Monoclonal	1:800
Pan-TDP-43	Rat (IgG_{2a})	BioLegend	BL808301	Monoclonal	1:300
pTDP-43	Rat (IgG_{2a})	BioLegend	BL829901	Monoclonal	1:3,000
MAP2	Chicken (IgY)	Abcam	ab5392	Polyclonal	1:2,000
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500
Round 2					
***SMI-32 (NFH)	Mouse (IgG_1)	BioLegend	BL801701	Monoclonal	1:500
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500
Confocal imaging panel					
Ubiquilin 2	Mouse (IgG_{2a})	Santa Cruz	SC-100612	Monoclonal	1:800
Pan-TDP-43	Rat (IgG_{2a})	BioLegend	BL808301	Monoclonal	1:300
pTDP-43	Rat (IgG_{2a})	BioLegend	BL829901	Monoclonal	1:3,000
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500

Primary antibody	Species (isotype)	Company	Catalogue #	Clonality	Dilution
****Validation imaging panel					
Ubiquilin 2	Mouse (IgG_{2a})	Santa Cruz	SC-100612	Monoclonal	1:800
Ubiquilin 2	Mouse (IgG_1)	Novus Biologicals	NBP2-25164	Monoclonal	1:800
Pan-TDP-43	Rat (IgG_{2a})	BioLegend	BL808301	Monoclonal	1:300
pTDP-43	Rat (IgG_{2a})	BioLegend	BL829901	Monoclonal	1:3,000
*Histone H3	Rabbit (IgG)	Abcam	ab1791	Polyclonal	1:500

*Histone H3 used only for case V:7 and omitted when calbindin or enkephalin used.
**Calbindin used for cerebellum and enkephalin used for globus pallidus.
***SMI-32 only included for spinal cord and motor cortex sections.
****Co-labelling of either Santa Cruz ubiquilin 2 + Novus ubiquilin 2; Santa Cruz ubiquilin 2 + pooled pan- and pTDP-43; or Novus ubiquilin 2 + pooled pan- and pTDP-43 was carried out for validation imaging.
Abbreviations. MAP2, microtubule-associated protein 2; pTDP-43, phospho (S409/410) TDP-43; NFH, neurofilament heavy chain.

Table S5. Secondary antibodies used for fluorescent immunohistochemistry.

Secondary antibody or reagent	Conjugate	Company	Catalogue #	Dilution
Qualitative analysis panels				
Spinal cord				
Goat anti-mouse IgG_{2a}	AlexaFluor® 488	Invitrogen	A21131	1:500
Goat anti-mouse IgG_1	AlexaFluor® 546	Invitrogen	A21123	1:500
Goat anti-chicken IgY	AlexaFluor® 594	Invitrogen	A11042	1:500
Goat anti-rat IgG	AlexaFluor® 647	Jackson ImmunoResearch	112.605.167	1:500
*Goat anti-rabbit IgG	IRDye 800CW	LI-COR Biosciences	92632211	1:500
All cortex				
Goat anti-mouse IgG_{2a}	AlexaFluor® 488	Invitrogen	A21131	1:500
Goat anti-mouse IgG_1	AlexaFluor® 546	Invitrogen	A21123	1:500
Goat anti-chicken IgY	AlexaFluor® 594	Invitrogen	A11042	1:500
Goat anti-rabbit IgG	IRDye 800CW	LI-COR Biosciences	92632211	1:500
Subcortical grey matter				
Goat anti-mouse IgG	AlexaFluor® 488	Jackson ImmunoResearch	115-545-166	1:500
Goat anti-chicken IgY	AlexaFluor® 594	Invitrogen	A11042	1:500
Goat anti-rat IgG	AlexaFluor® 647	Jackson ImmunoResearch	112.605.167	1:500
Goat anti-rabbit IgG	IRDye 800CW	LI-COR Biosciences	92632211	1:500
Quantitative analysis panel				
Round 1				
*Goat anti-rabbit IgG	Biotin	Sigma-Aldrich	B7389	1:500
*Streptavidin	AlexaFluor® 405	ThermoFisher Scientific	S32351	1:200
Goat anti-rat IgG	AlexaFluor® 488	Jackson ImmunoResearch	112-545-167	1:500
Goat anti-mouse IgG	AlexaFluor® 594	Jackson ImmunoResearch	115-585-166	1:500
Goat anti-chicken IgY	AlexaFluor® 647	Invitrogen	A21449	1:500
Round 2				
*Goat anti-rabbit IgG	Biotin	Sigma-Aldrich	B7389	1:500
*Streptavidin	AlexaFluor® 405	ThermoFisher Scientific	S32351	1:200

Secondary antibody or reagent	Conjugate	Company	Catalogue #	Dilution
Goat anti-mouse IgG	AlexaFluor® 594	Jackson ImmunoResearch	115-585-166	1:500
Confocal imaging panel				
*Goat anti-rabbit IgG	Biotin	Sigma-Aldrich	B7389	1:500
*Streptavidin	AlexaFluor® 405	ThermoFisher Scientific	S32351	1:200
Goat anti-rat IgG	AlexaFluor® 488	Jackson ImmunoResearch	112-545-167	1:500
Goat anti-mouse IgG	AlexaFluor® 594	Jackson ImmunoResearch	115-585-166	1:500
Validation imaging panel				
Co-label 1 (Santa Cruz ubiquilin 2 + Novus ubiquilin 2)				
Goat anti-mouse IgG_{2a}	AlexaFluor® 647	Invitrogen	A21241	1:500
Goat anti-mouse IgG_1	AlexaFluor® 488	Invitrogen	A21121	1:500
Co-labels 2 and 3 (Santa Cruz ubiquilin 2 + pooled pan- and pTDP-43; Novus ubiquilin 2 + pooled pan- and pTDP-43)				
Goat anti-mouse IgG	AlexaFluor® 488	Jackson ImmunoResearch	115-545-166	1:500
Goat anti-rat IgG	AlexaFluor® 647	Jackson ImmunoResearch	112-605-167	1:500
All sections				
*Goat anti-rabbit IgG	Biotin	Sigma-Aldrich	B7389	1:500
*Streptavidin	AlexaFluor® 405	ThermoFisher Scientific	S32351	1:200
All panels				
Hoechst 33342	-	Cayman Chemicals	15547	5 µg/mL

* Goat anti-rabbit 800CW or goat anti-rabbit IgG biotin and streptavidin AlexaFluor® 405 used only for case V:7, with Hoechst 33342 being omitted.

Supplementary Figures

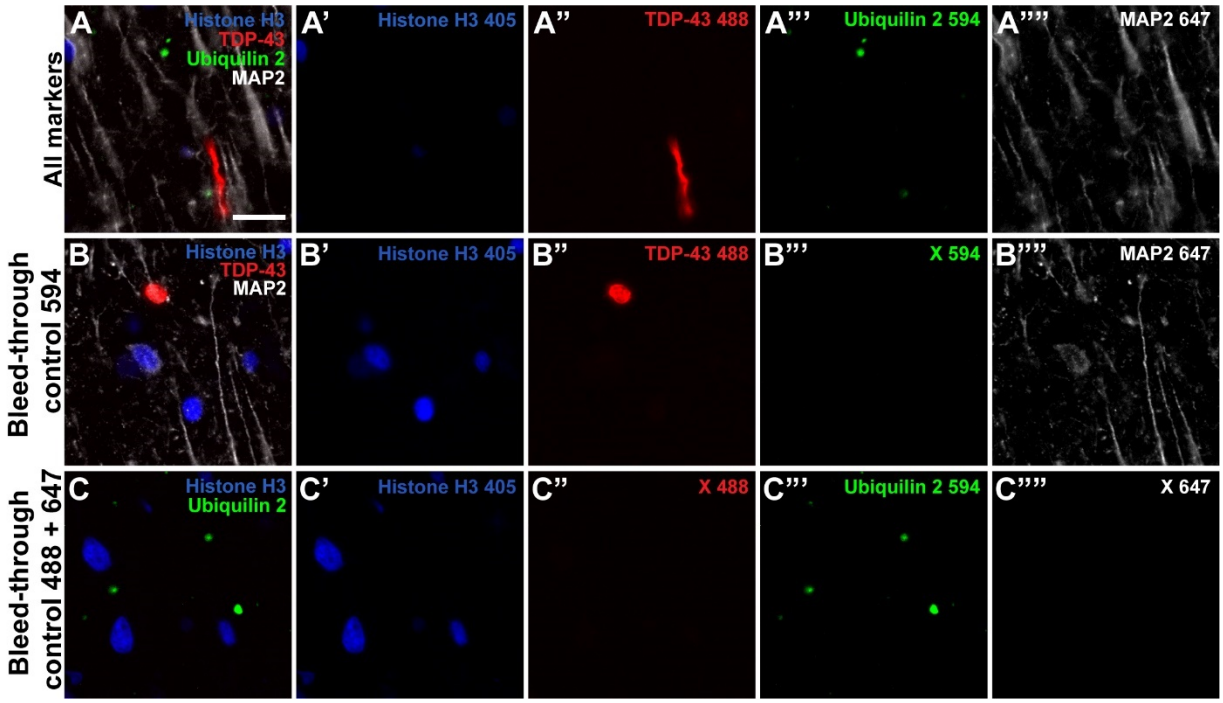

Figure S1. Representative bleed-through control.

Middle frontal gyrus sections from *UBQLN2* p.T487I-linked ALS/FTD case V:7 were labelled with all markers (**A**) or alternate markers (**B and C**) from Quantitative analysis panel Round 1 (See Tables S4 and S5). No bleed-through of fluorescence was observed from a given channel to the next channel that might be misidentified as co-localisation between ubiquilin 2, TDP-43, and/or MAP2. Scale bar 20 μm.

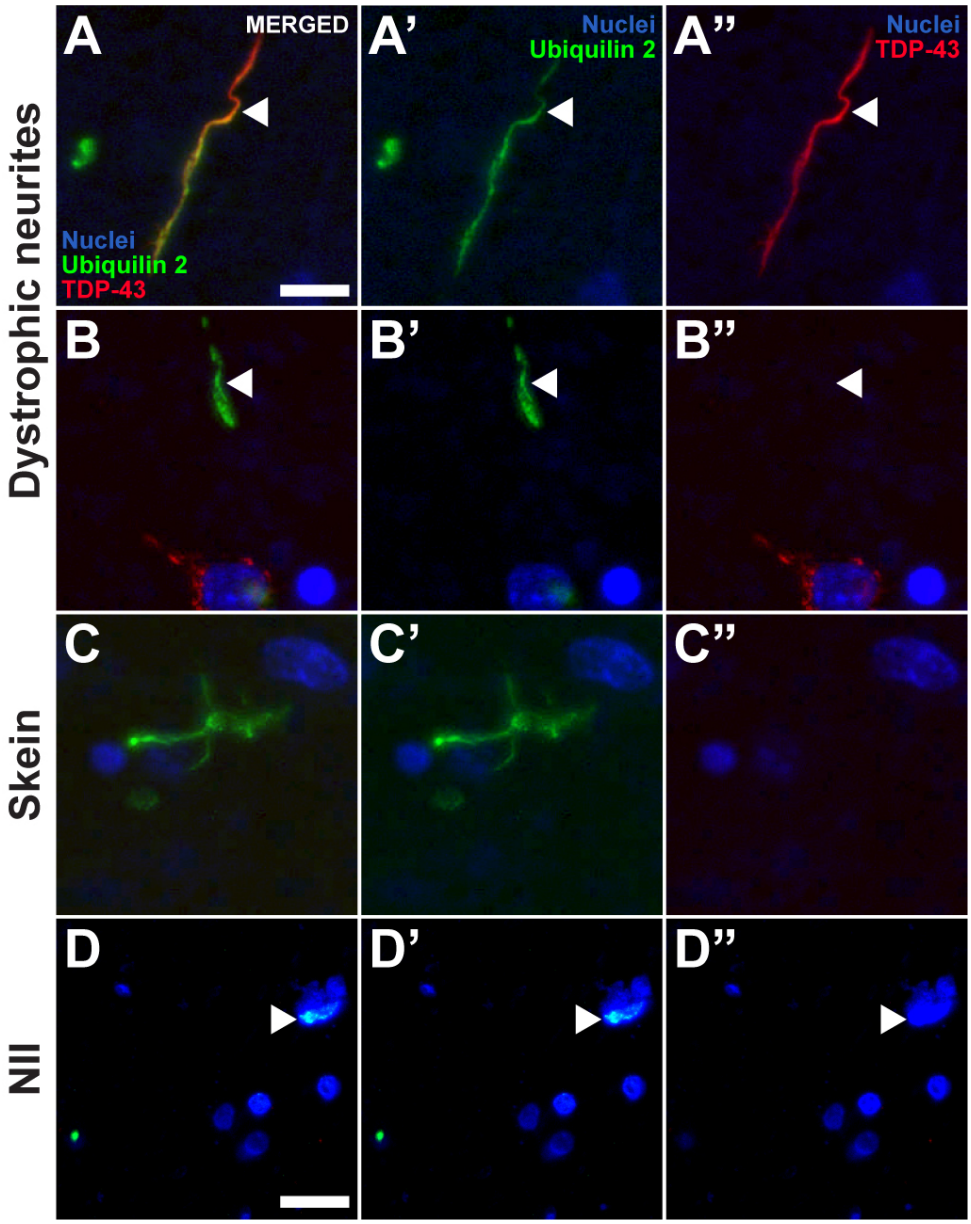

Figure S2. Rare non-punctate ubiquilin 2 aggregates.

Sections from *UBQLN2* p.T487I-linked ALS/FTD cases MN17 (**A-C**) and V:7 (**D**) demonstrated dystrophic neurites that were positive for TDP-43 (**A,** ventral striatum), dystrophic neurites that were negative for TDP-43 (**B,** ventral striatum), skeins that were negative for TDP-43 (**C,** ventral striatum but also seen in subiculum), or neuronal intranuclear inclusions that were negative for TDP-43 (**D,** cervical spinal cord ventral horn). Scale bar A-C, 10 µm; D, 20 µm. Abbreviation: NII, neuronal intranuclear inclusion.

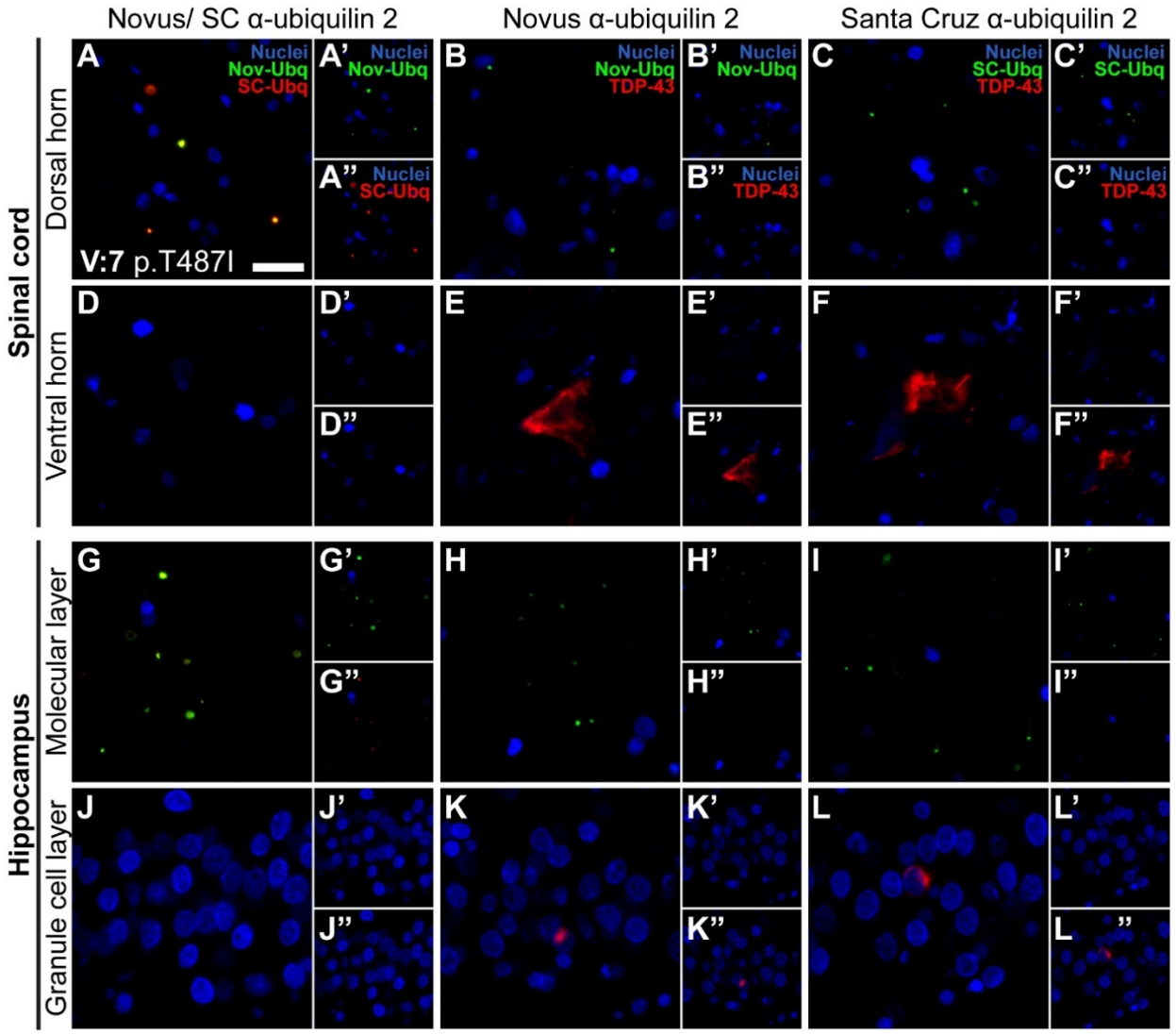

Figure S3. Alternative ubiquilin 2 antibody labelling of *UBQLN2* p.T487I-linked ALS/FTD spinal cord and hippocampus.

Validation of the monoclonal anti-ubiquilin 2 antibody used in this study (Santa Cruz #SC-100612, clone 5F5, IgG2a: SC-Ubq) was carried out by immunohistochemically co-labelling spinal cord (**A-F**) and hippocampus (**G-L**) sections from *UBQLN2* p.T487I-linked ALS/FTD case V:7 with another monoclonal anti-ubiquilin 2 antibody (Novus Biologicals #NBP2-25164, clone 6H9, IgG1: Nov-Ubq). To confirm the identified patterns of ubiquilin 2 and TDP-43 aggregation, we carried out three double-labels: Novus anti-ubiquilin 2 with Santa Cruz anti-ubiquilin 2; Novus anti-ubiquilin 2 with pooled anti-pan- and pTDP-43, and Santa Cruz anti-ubiquilin 2 with pooled anti-pan- and pTDP-43. Antibodies were visualised using AlexaFluor® 488 and 647 antibodies, respectively, to ensure spectral separation of markers. As seen with the Santa Cruz monoclonal anti-ubiquilin 2 antibody #SC-100612 in Figure 4, spinal cord sections from *UBQLN2*-linked cases demonstrated punctate aggregates in the dorsal horn that were positive for ubiquilin 2 only (**A-C**), with aggregates being immunoreactive for both the Santa Cruz and Novus anti-ubiquilin 2 antibodies. In the same sections, cytoplasmic aggregates in the ventral horn were positive for TDP-43 (**E", F",** red)

and negative for ubiquilin 2 (**E', F',** green), irrespective of whether ubiquilin 2 was being visualised using the Santa Cruz or Novus antibody. The same pattern of ubiquilin 2 and TDP-43 aggregation was observed in the hippocampus, with ubiquilin 2-only aggregates identified in the molecular layer (**G-I**) and TDP-43-only aggregates identified in the granule cell layer (**J-L**), irrespective of whether ubiquilin 2 was visualised using the Santa Cruz or Novus antibody. Scale bars 20 μm.

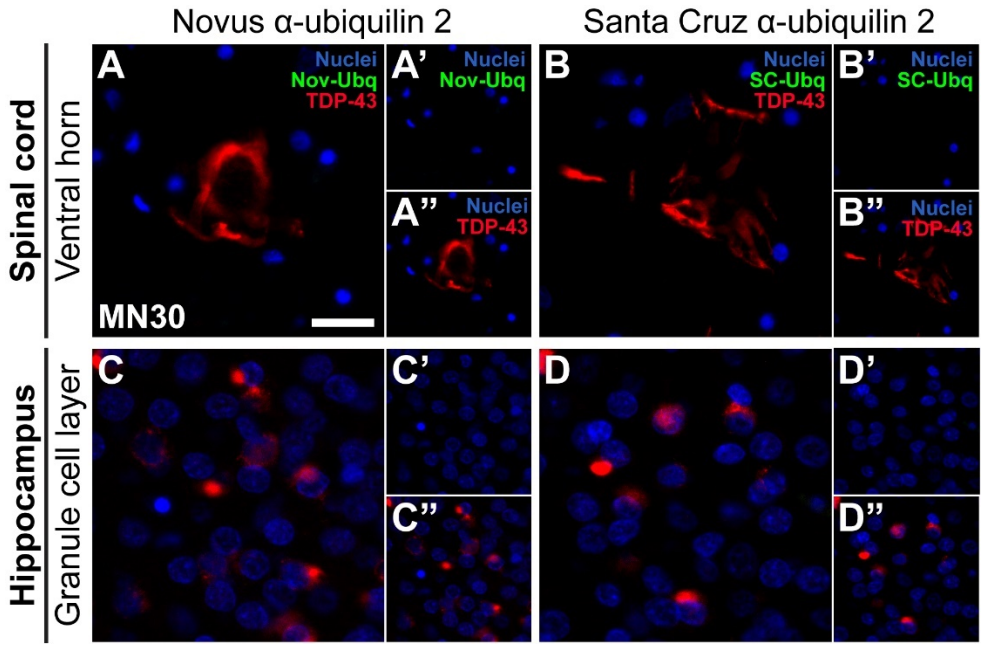

Figure S4. Alternative ubiquilin 2 antibody staining of stage 4 sALS spinal cord and hippocampus.

Validation of the monoclonal anti-ubiquilin 2 antibody used in this study (Santa Cruz #SC-100612, clone 5F5, IgG2a: SC-Ubq) was carried out by immunohistochemically co-labelling spinal cord (**A-B**) and hippocampus (**C-D**) sections from stage 4 sALS case MN30 with another monoclonal anti-ubiquilin 2 antibody (Novus Biologicals #NBP2-25164, clone 6H9, IgG1: Nov-Ubq). To confirm the identified patterns of ubiquilin 2 and TDP-43 aggregation, we carried out three double-labels: Novus anti-ubiquilin 2 with Santa Cruz anti-ubiquilin 2; Novus anti-ubiquilin 2 with pooled anti-pan- and pTDP-43, and Santa Cruz anti-ubiquilin 2 with pooled anti-pan- and pTDP-43. Antibodies were visualised using AlexaFluor® 488 and 647 antibodies, respectively, to ensure spectral separation of markers. Cytoplasmic aggregates in the ventral horn were positive for TDP-43 (**A", B",** red) and negative for ubiquilin 2 (**A', B',** green), irrespective of whether ubiquilin 2 was being visualised using the Santa Cruz or Novus antibody. The same pattern of ubiquilin 2 and TDP-43 aggregation was observed in the hippocampus, with TDP-43-only aggregates identified in the granule cell layer (**C-D**), irrespective of whether ubiquilin 2 was visualised using the Santa Cruz or Novus antibody. Scale bars 20 μm.

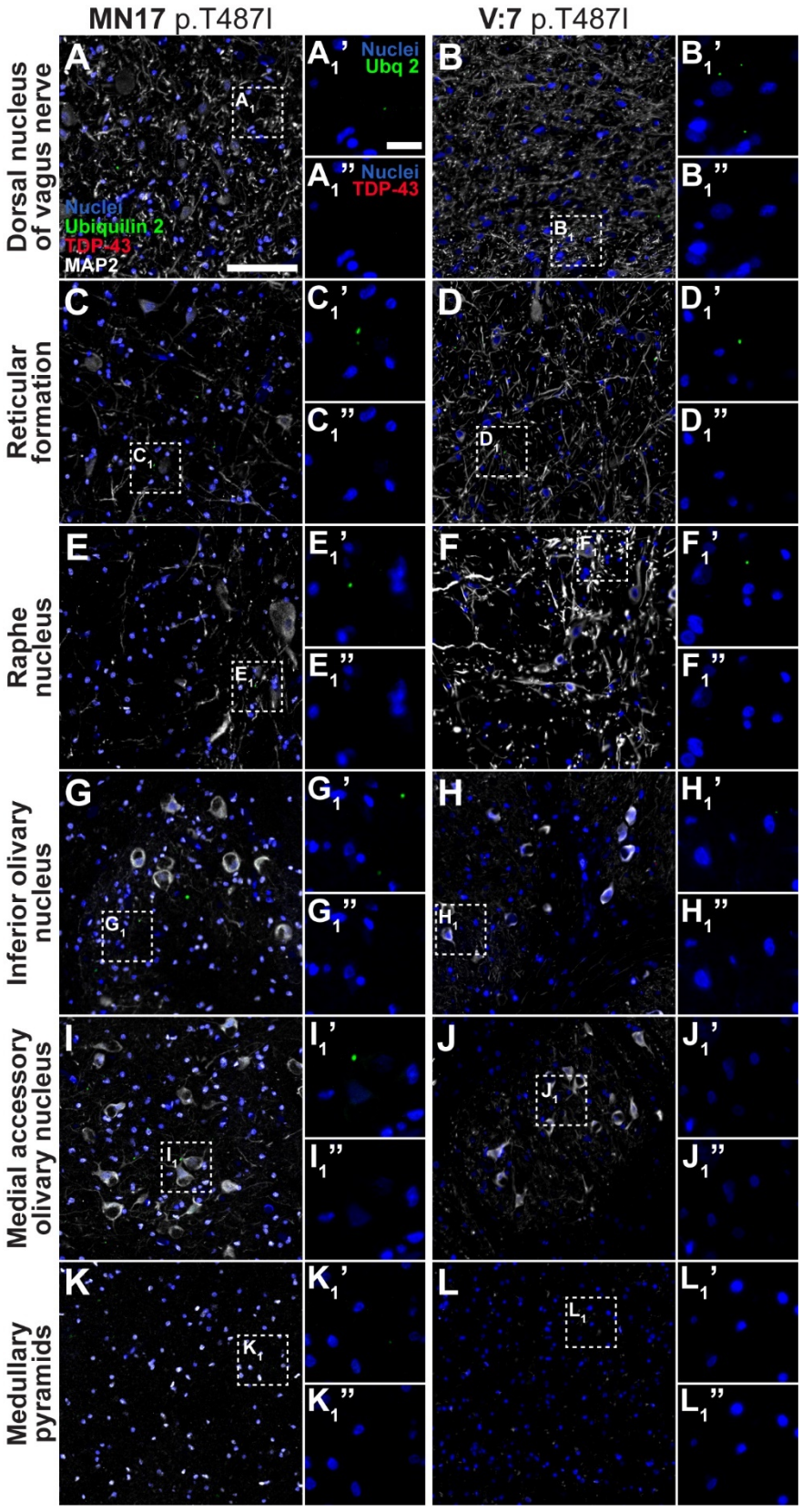
MN17 p.T487I
V:7 p.T487I
Dorsal nucleus of vagus nerve
Reticular formation
Raphe nucleus
Inferior olivary nucleus
Medial accessory olivary nucleus
Medullary pyramids
Nuclei
Ubiquilin 2
TDP-43
MAP2
Nuclei
Ubq 2
Nuclei
TDP-43

Figure S5. Ubiquilin 2-only aggregates in the *UBQLN2* p.T487I-linked ALS/FTD middle medulla.

Middle medulla sections from 2 *UBQLN2* p.T487I-linked ALS/FTD cases demonstrated punctate aggregates in the dorsal nucleus of the vagus nerve, reticular formation, raphe nucleus, and inferior olivary nucleus, that were positive for ubiquilin 2 only (**A_1'-H_1',** green). In case MN17 but not case V:7, the medial accessory olivary nucleus and medullary pyramids also harboured punctate aggregates that were positive for ubiquilin 2 only (**I_1'-L_1',** green). Scale bar in main images, 100 μm; in insets 20 μm.

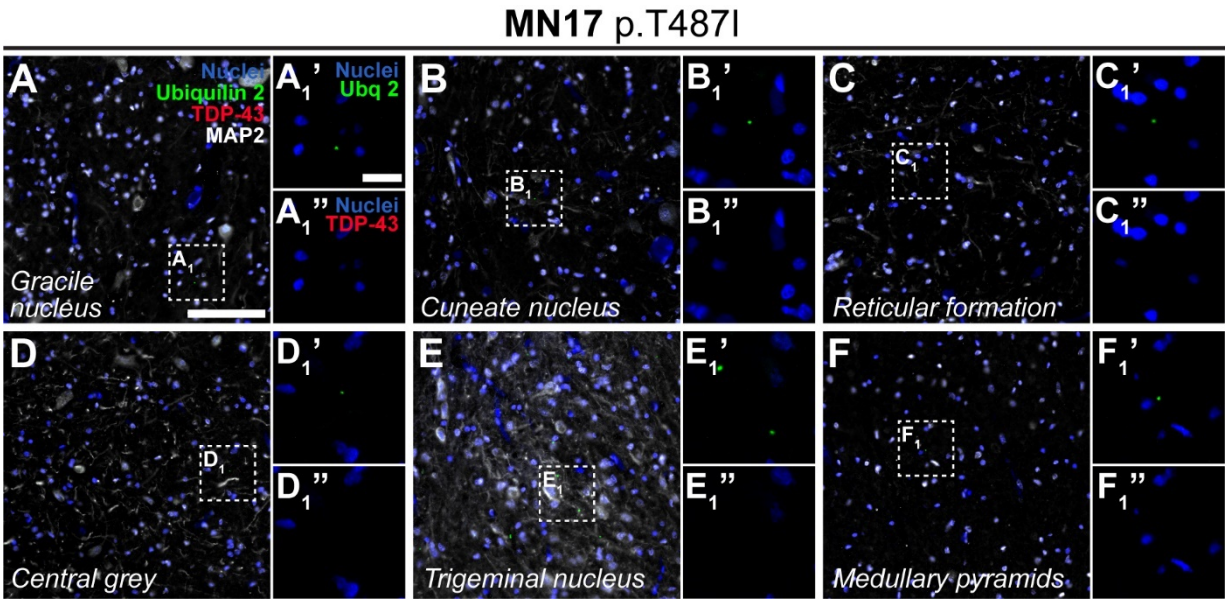

Figure S6. Ubiquilin 2-only aggregates in the *UBQLN2* p.T487I-linked ALS/FTD lower medulla.

Lower medulla sections from 1 *UBQLN2* p.T487I-linked ALS/FTD case demonstrated punctate aggregates in the gracile nucleus, cuneate nucleus, reticular formation, central grey, trigeminal nucelus, and medullary pyramids, that were positive for ubiquilin 2 only (**A'1-F'1,** green). Scale bar in main images, 100 µm; in insets 20 µm.

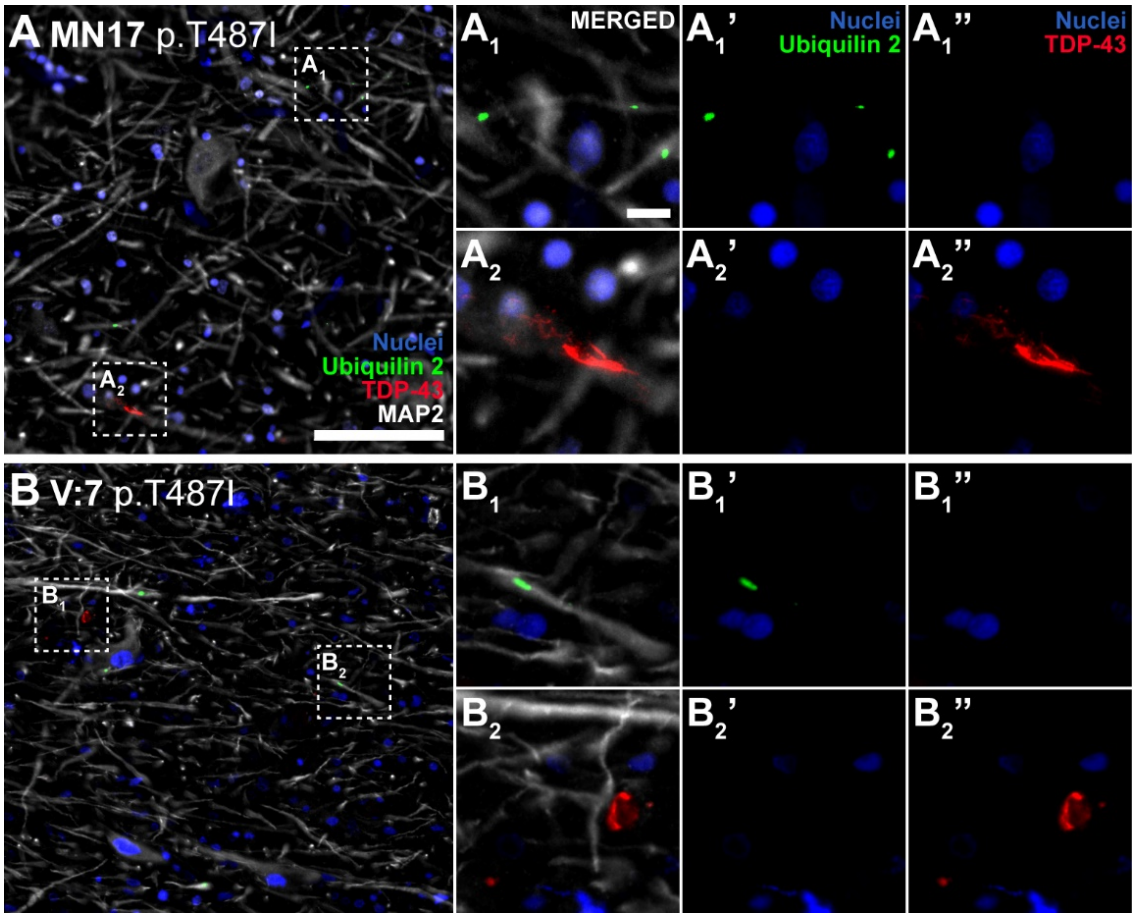

Figure S7. Ubiquilin 2-only and TDP-43-only aggregates in the *UBQLN2* p.T487I-linked ALS/FTD substantia nigra.

Substantia nigra sections from 2 *UBQLN2* p.T487I-linked ALS/FTD cases demonstrated punctate neuritic aggregates that were positive for ubiquilin 2 (**A_1'-B_1',** green) or TDP-43 only (**A_2"-B_2",** red), but not both. Scale bar in main images, 100 µm; in insets 10 µm.

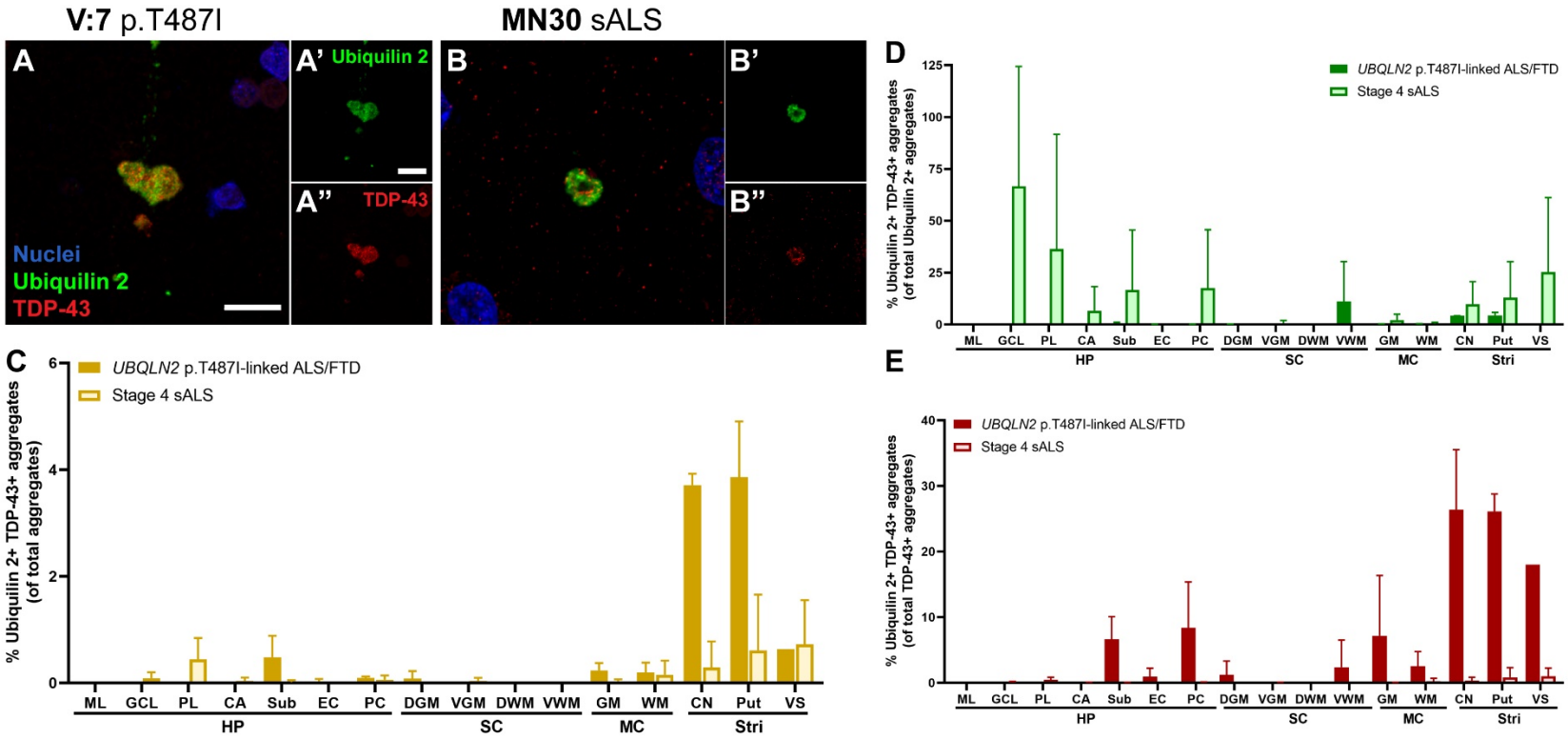

Figure S8. Ubiquilin 2- and TDP-43-double immunopositive aggregates in the *UBQLN2* p.T487I-linked ALS/FTD and stage 4 sALS CNS.

Confocal analysis of ubiquilin 2- and TDP-43-double immunopositive aggregates demonstrated that they were larger and more diffuse than punctate ubiquilin 2-only aggregates, similar in size to nuclei, and were present in both *UBQLN2* p.T487I-linked ALS/FTD and stage 4 sALS (**A, B**). Quantification of double immunopositive aggregates in sections from 2-3 *UBQLN2* p.T487I-linked ALS/FTD cases and 3 stage 4 sALS cases demonstrated these aggregates to be very rare as a proportion of all aggregates (<4%), and mostly found in striatum in *UBQLN2* p.T487I-linked ALS/FTD (**C**). Of all ubiquilin 2 aggregates, double immunopositivity with TDP-43 was common in stage 4 sALS hippocampus, but not in *UBQLN2* p.T487I-linked ALS/FTD (**D**). Of all TDP-43 aggregates, double immunopositivity with ubiquilin 2 was most common in *UBQLN2* p.T487I-linked ALS/FTD striatum (**E**). Scale bars 10 µm. Abbreviations: HP, hippocampus; ML, molecular layer; GCL, granule cell layer; PL, polymorphic layer; CA, cornu ammonis; Sub, subiculum; EC, entorhinal cortex; PC, piriform cortex; SC, spinal cord; DGM, dorsal grey matter; VGM, ventral grey matter; DWM, dorsal white matter; VWM, ventral white matter; MC, motor cortex; GM, grey matter; WM, white matter; Stri, striatum; CN, caudate nucleus; Put, putamen; VS, ventral striatum.

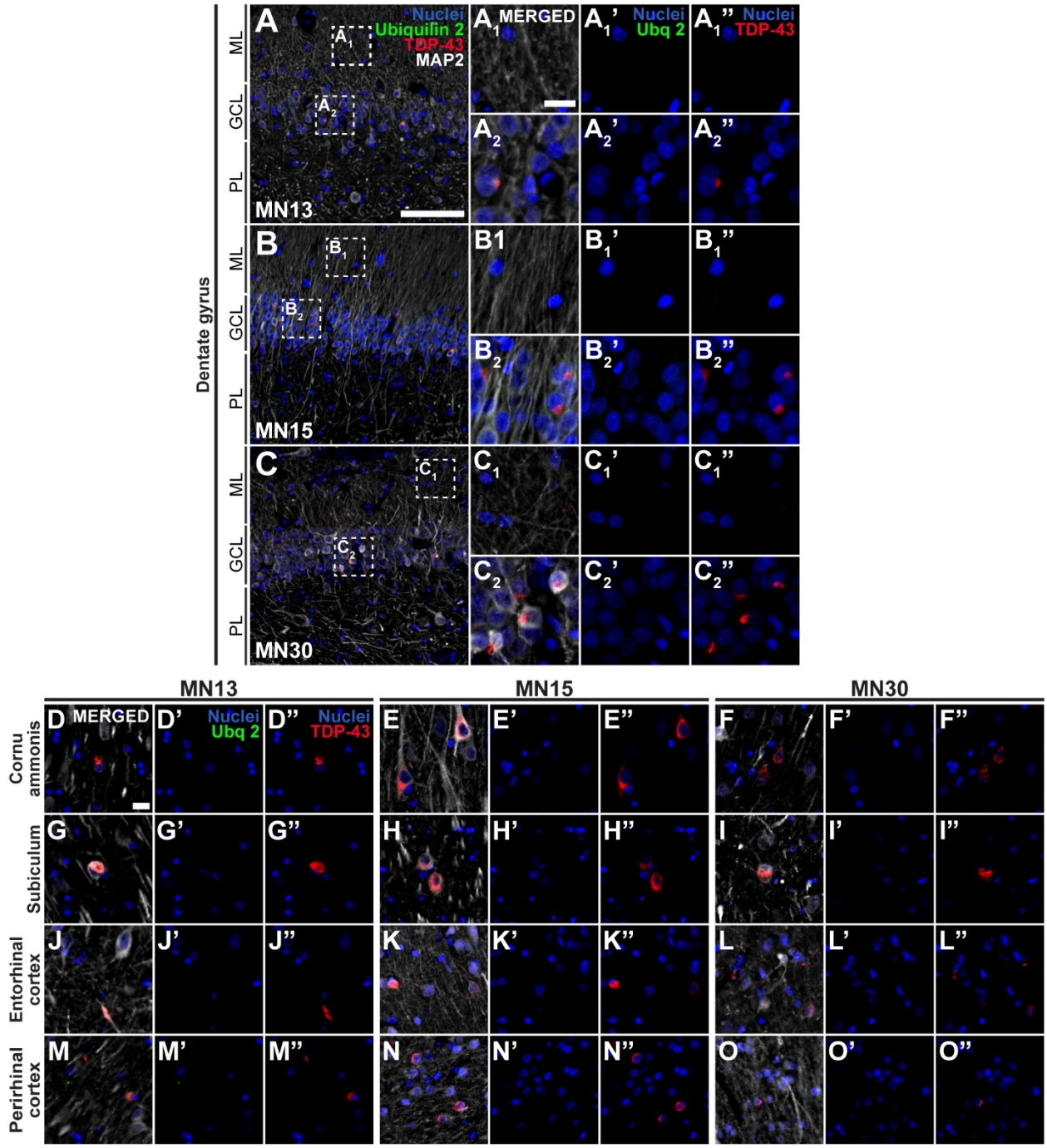

Figure S9. Ubiquilin 2-only and TDP-43-only aggregates in the stage 4 sALS hippocampus.

Hippocampal sections from 3 stage 4 sALS cases (**A-O"**) demonstrated cytoplasmic aggregates in the dentate gyrus and surrounding hippocampus that were positive for TDP-43 only (**A1"-O",** red) and negative for ubiquilin 2 (**A1'-O'**). As shown in Figure 3L, quantification of the density of aggregates showed that ubiquilin 2-only aggregates were rare or absent but TDP-43-only aggregates were found throughout the hippocampal formation, particularly in the granule cell layer. Scale bars in A, B, C, 100 µm; others 20 µm. Abbreviations: ML, molecular layer; GCL, granule cell layer; PL, polymorphic layer.

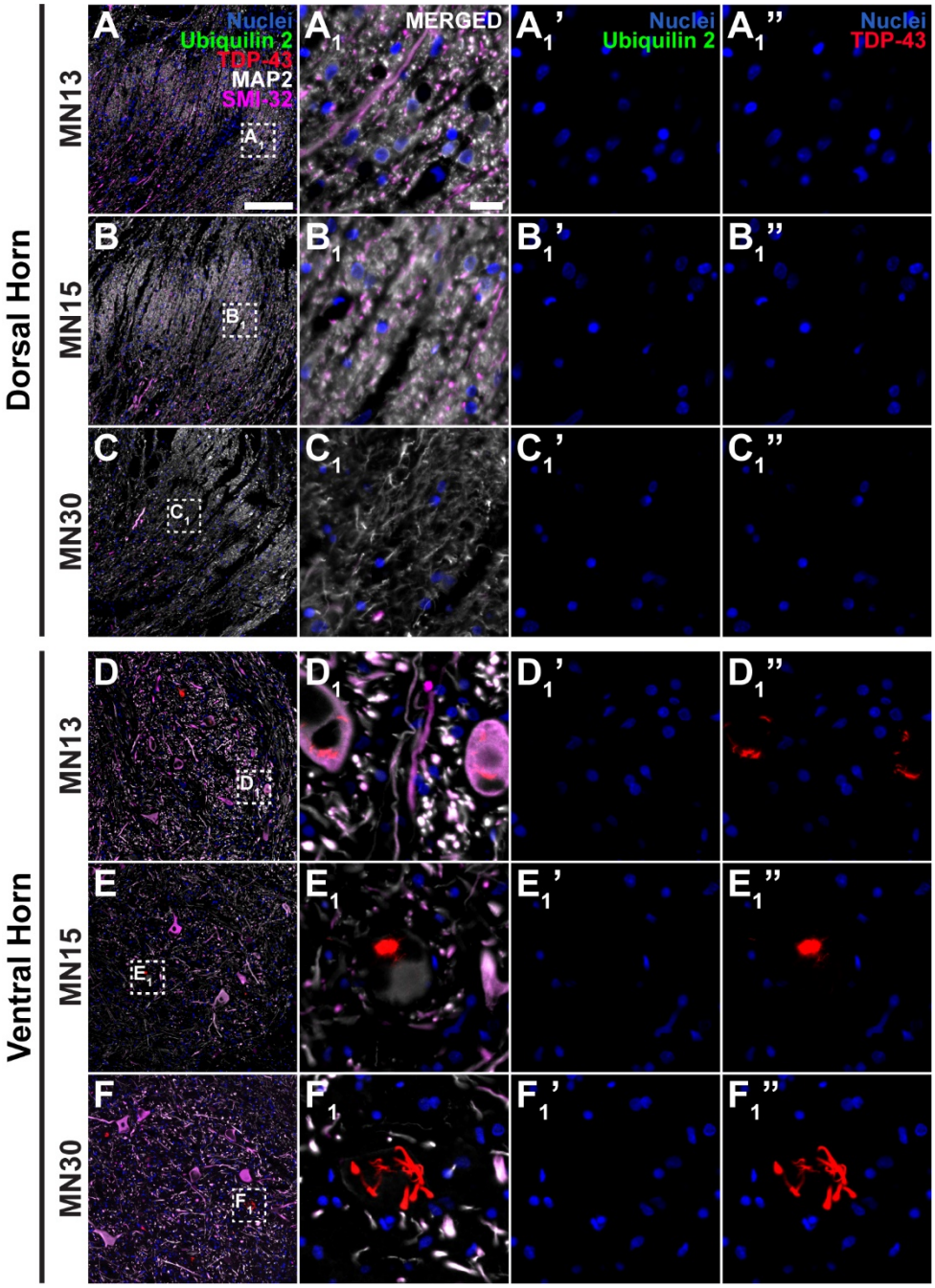

Figure S10. Ubiquilin 2-only and TDP-43-only aggregates in the *UBQLN2* p.T487I-linked ALS/FTD spinal cord.

Spinal cord sections from 3 stage 4 sALS cases (**A-F'1**) demonstrated cytoplasmic aggregates in the motor neurons of the ventral horn (magenta) that were positive for TDP-43 only (**D1"-F1",** red) and negative for ubiquilin 2 (**D1'-F1'**, green). As shown in Figure 4H, quantification of the density of aggregates showed that ubiquilin 2-only aggregates were rare or absent, being most common in the dorsal grey matter, while TDP-43-only aggregates were abundant in the ventral grey matter. Scale bar in main images, 200 µm; in insets 20 µm.

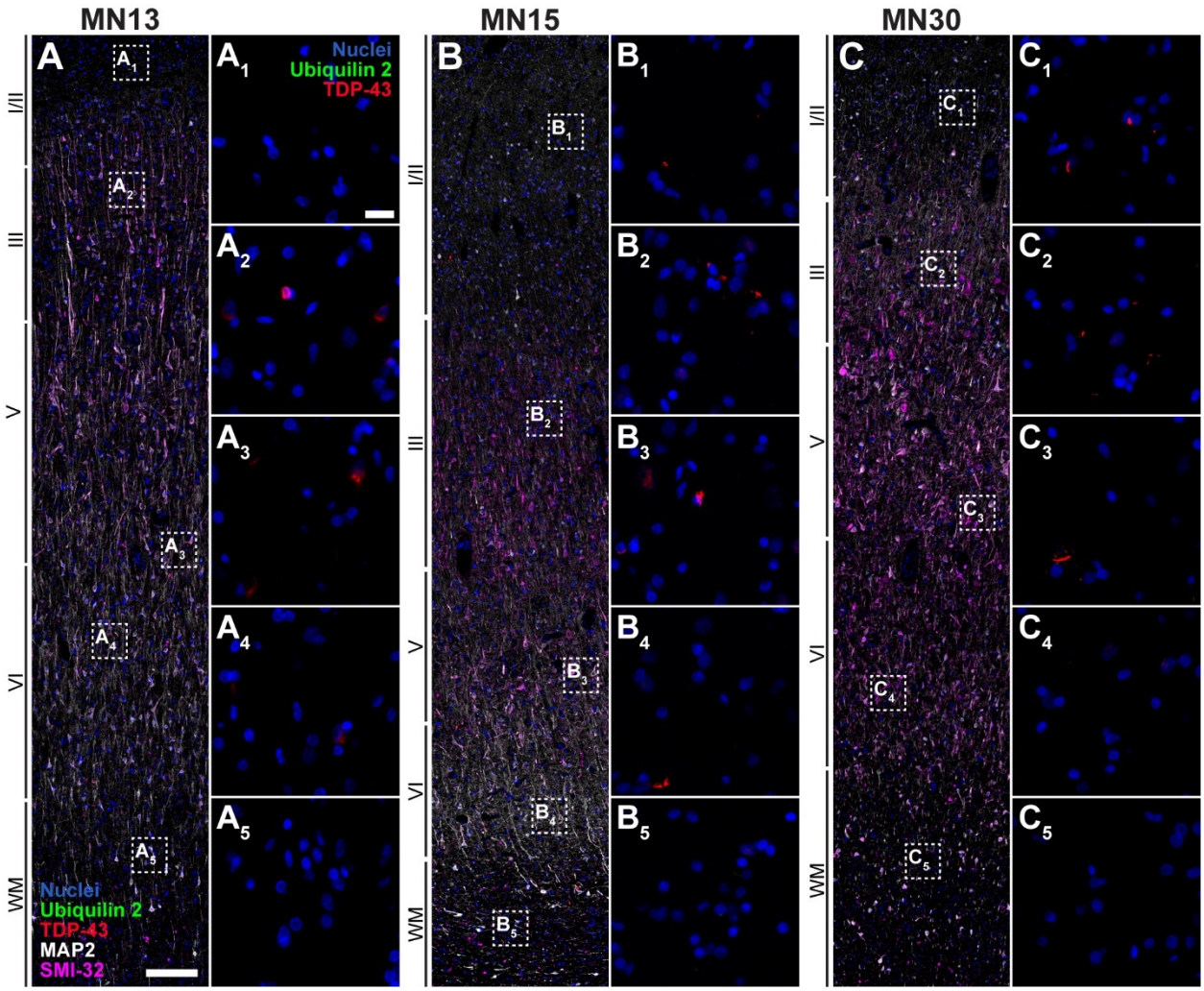

Figure S11. Ubiquilin 2-only and TDP-43-only aggregates in the stage 4 sALS motor cortex.

Motor cortex sections from 3 stage 4 sALS cases (**A-C$_5$**) demonstrated cytoplasmic TDP-43-only aggregates (red) that were negative for ubiquilin 2. As shown in Figure 5E, quantification of the density of aggregates showed that ubiquilin 2-only aggregates (green) were very rare in (or absent from) all cortical layers, while TDP-43-only aggregates (red) were abundant throughout. Case MN30 showed occasional deposition of ubiquilin 2 in layers I-II and case MN15 showed greater deposition of TDP-43 in layers I-II. Scale bar in main images, 200 µm; in insets 20 µm. Abbreviations: WM, white matter.

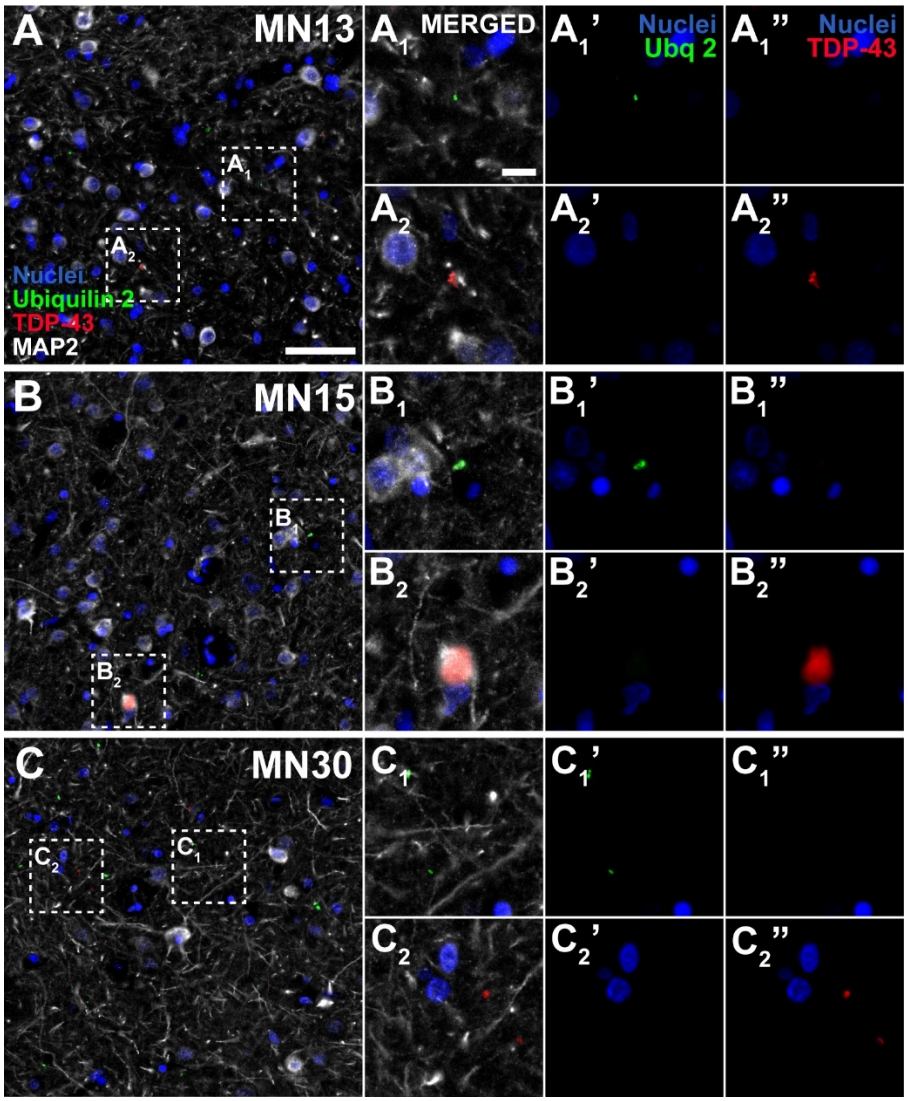

Figure S12. Ubiquilin 2-only and TDP-43-only aggregates in the stage 4 sALS striatum.

Striatal sections from 3 stage 4 sALS cases demonstrated punctate aggregates in the ventral striatum, that were positive for ubiquilin 2 only (**A', A_1', A_2'- C', C_1', C_2'**, green). Striatal sections also demonstrated cytoplasmic aggregates in that were positive for TDP-43 only (**A", A_1", A_2"- C", C_1", C_2"**, red). As shown in Figure 6G, quantification of the density of aggregates showed that ubiquilin 2-only aggregates (green) were most abundant in the putamen and ventral striatum of case MN30, while TDP-43-only aggregates (red) were abundant in these regions in case MN15. Scale bar in main images, 50 µm; in insets 5 µm.

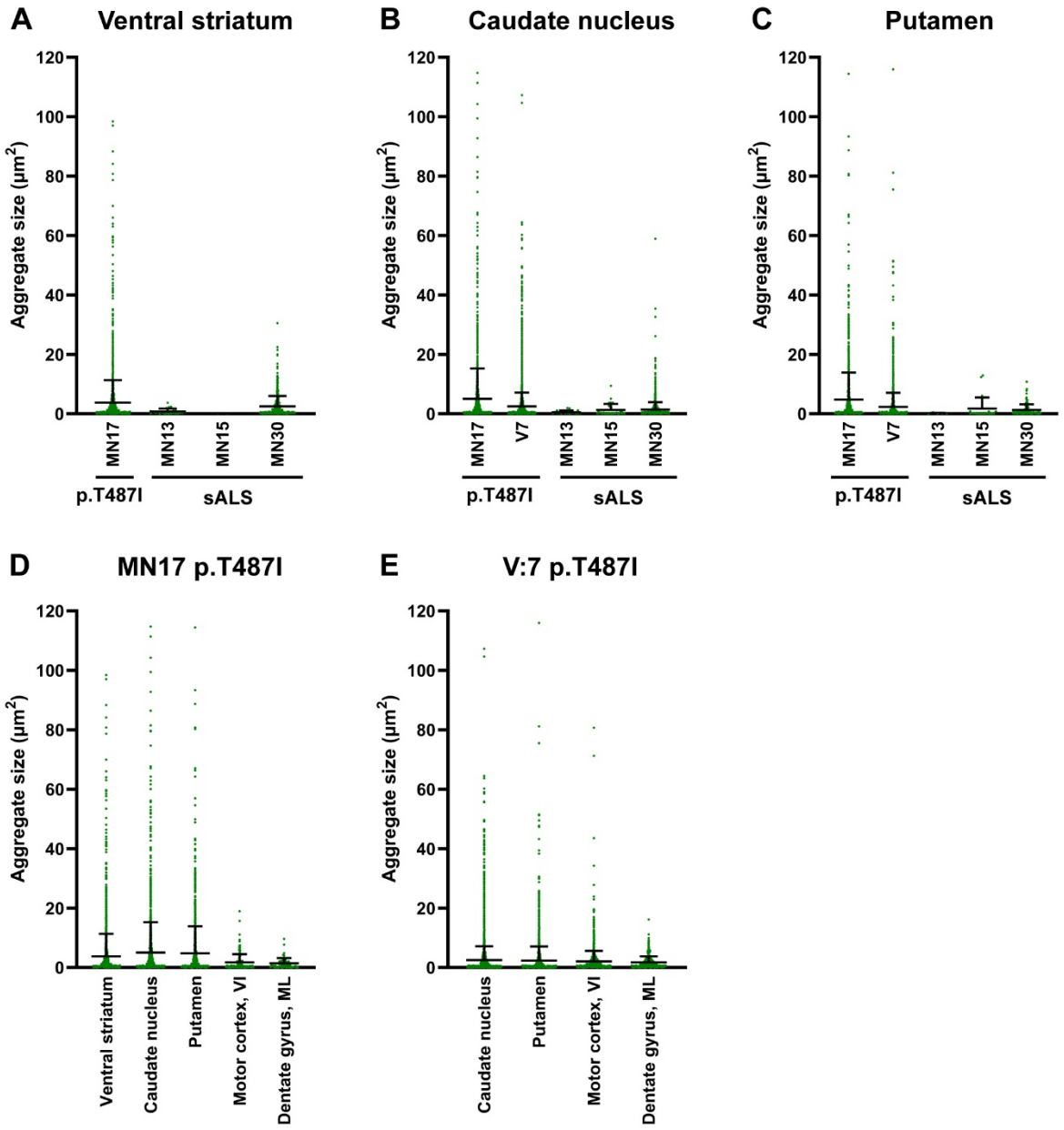

Figure S13. Ubiquilin 2-only aggregate size distributions.

Ubiquilin 2-only aggregate sizes were estimated in striatal sections from 2 *UBQLN2* p.T487I-linked ALS/FTD cases and 3 stage 4 sALS cases (**A-C**). Large ubiquilin 2-only aggregates (>40 µm^2) were a feature of the striatum in *UBQLN2* p.T487I-linked cases (**D, E**).