Figure 6. Sensitivity of FUS antibodies for detection of the FUS inclusions in aFTLD-U. Confocal microscopy of the hippocampal sections from two aFTLD-U cases stained with antibodies against ubiquitin and FUS.

Fig. 7. Effect of the antigen retrieval protocols on detection of the inclusions. The representative ubiquitin- (A-F) and p62- (G-L) positive inclusions in spinal neurons of SALS cases were not apparently FUS-positive when antigen was retrieved using either boiling (A-C and G-I) or microwave (D-F and J-L) protocol. (M-O) Representative TDP43- and ubiquitin-positive inclusions are shown with microwave antigen retrieval protocol.

Supplementary figure 1. FUS-immunoreactive aggregates/inclusions in SALS, ALS/dementia and non-SOD1 FALS. Human spinal cord sections were analyzed with immunohistochemistry using an affinity purified anti-FUS antibody (11570-1-AP). Representative FUS-immunoreactive aggregates/inclusions are shown here in anterior horn neurons in patients with FALS (A-D), SALS (E-R) and ALS/dementia (S and T). Apparent skein-like, cytoplasmic and neuritic FUS-positive inclusions were observed in neurons with abundant lipofuscin (U and V). No FUS-positive inclusions were observed in six spinal cord samples from control cases without ALS (W-B'). Autopsy numbers are labeled on the top of each panel. (F), FALS; (S), SALS; (S+D), SALS/dementia; (C), controls. All images were taken with original magnification of 400X.

Supplementary figure 2. Lack of FUS pathology in spinal anterior neurons of SOD1-linked ALS cases and mutant SOD1 transgenic mice. FUS antibody 11570-1-AP and monoclonal anti-ubiquitin antibody were used for confocal microscopy. Ubiquitin-

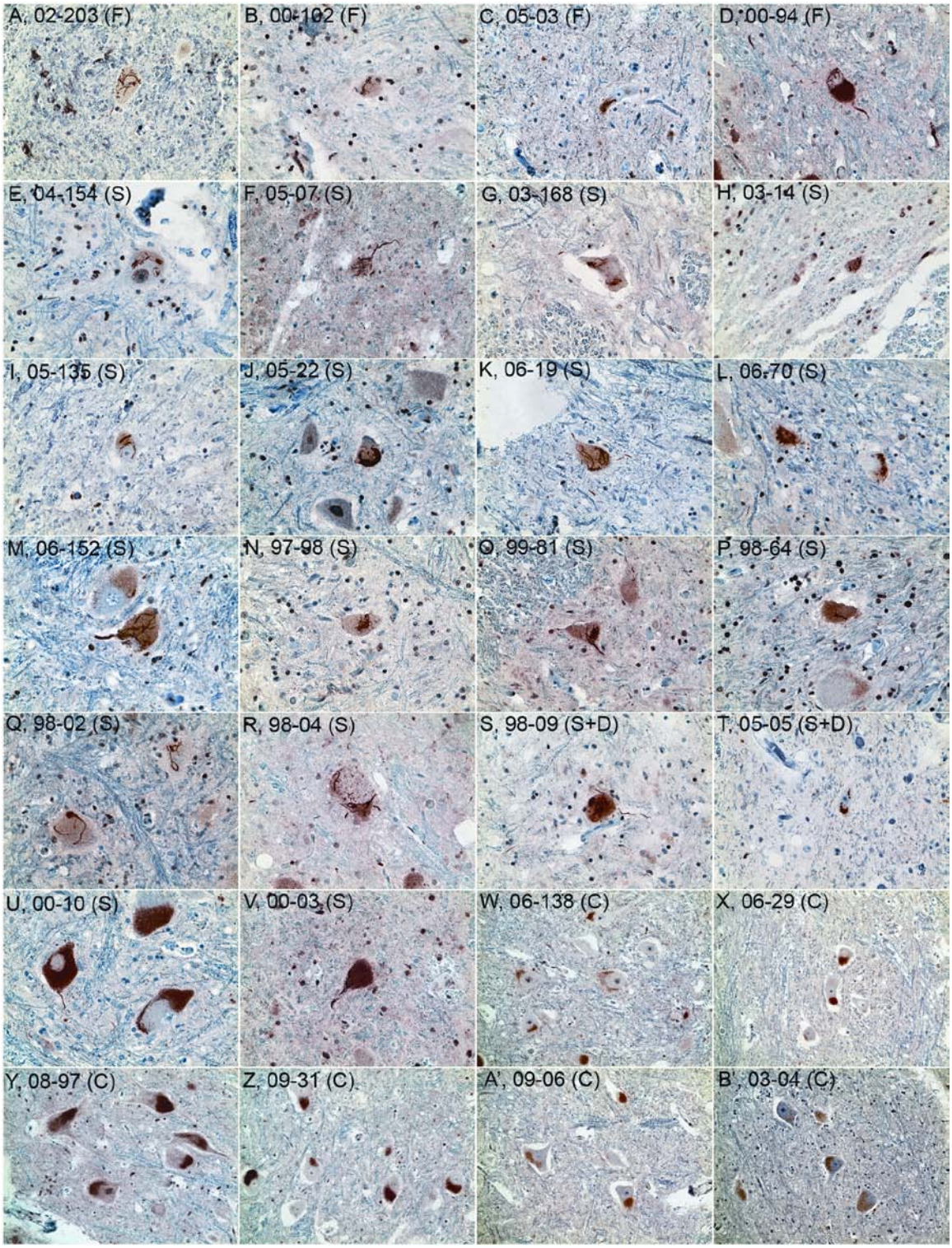
positive inclusions in spinal neurons were FUS-negative in patients with $SOD1^{G85R}$ (A-C) or $SOD1^{A4V}$ (D-F) mutation and in the transgenic mice overexpressing $SOD1^{G93A}$ (G-I) and $SOD1^{L126Z}$ (J-L). Ubiqutin-positive inclusions are indicated by arrows and nuclear staining of FUS is indicated by arrowheads.

Supplementary figure 3. Differential sensitivities of FUS antibodies for detection of FUS pathology in spinal neurons of SALS. Representative images showing that the 11570-1-AP antibody (A-C) yielded apparently stronger FUS signals than the HPA008784 (D-F) and sc-47711 (G-I). In some cells with TDP43-positive inclusions, the FUS signals yielded by HPA008784 and sc-47711 were co-localized with strong TDP43 signals (arrows), but they were much less robust when TDP43 signals were weak (arrowheads) (D-I). Nuclear staining of FUS in a representative cell with FUS- and TDP43-positive inclusions is indicated by a large arrow (F), but no nuclear staining of the TDP43 in the same cell. The FUS signals are not apparent for the TDP43-positive inclusions when other FUS antibodies were used (J-R).

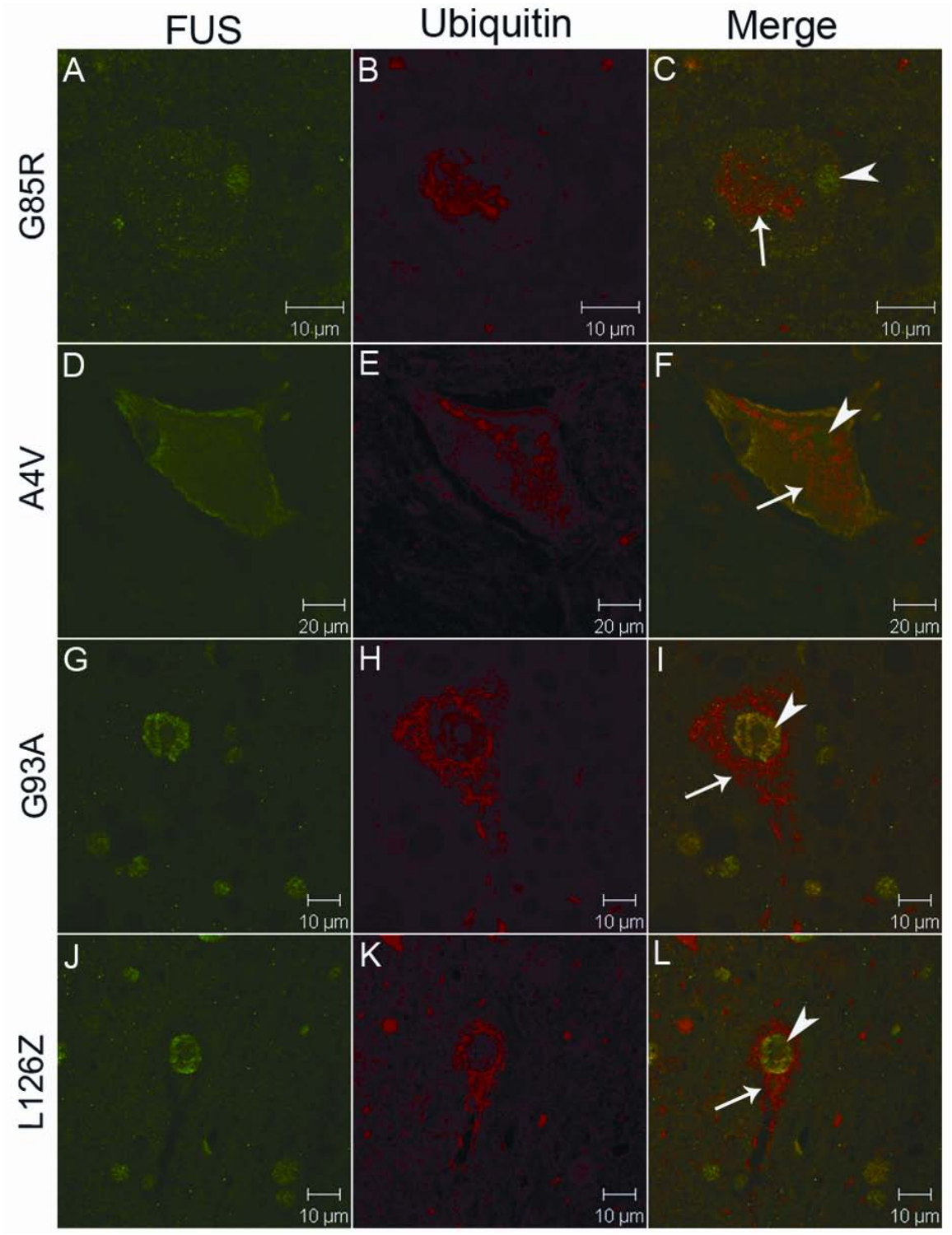
Supplementary figure 4. Location of polypeptides for antibody production. A full sized FUS (526 amino acids) is labeled on the top. The location of polypeptides for antibody production is individually indicated. The antibodies HPA008784 and sc-47711, which recognize the inclusions, are generated using two polypeptides that are 61 amino acids apart.

Supplementary figure 5. FUS pathology in brain of patients with FTLD-U and ALS/dementia. (A-F) Representative FUS pathology is shown in frontal lobe sections from patients with (A-C) or without (D-F) mutations in progranulin. (G-L) FUS

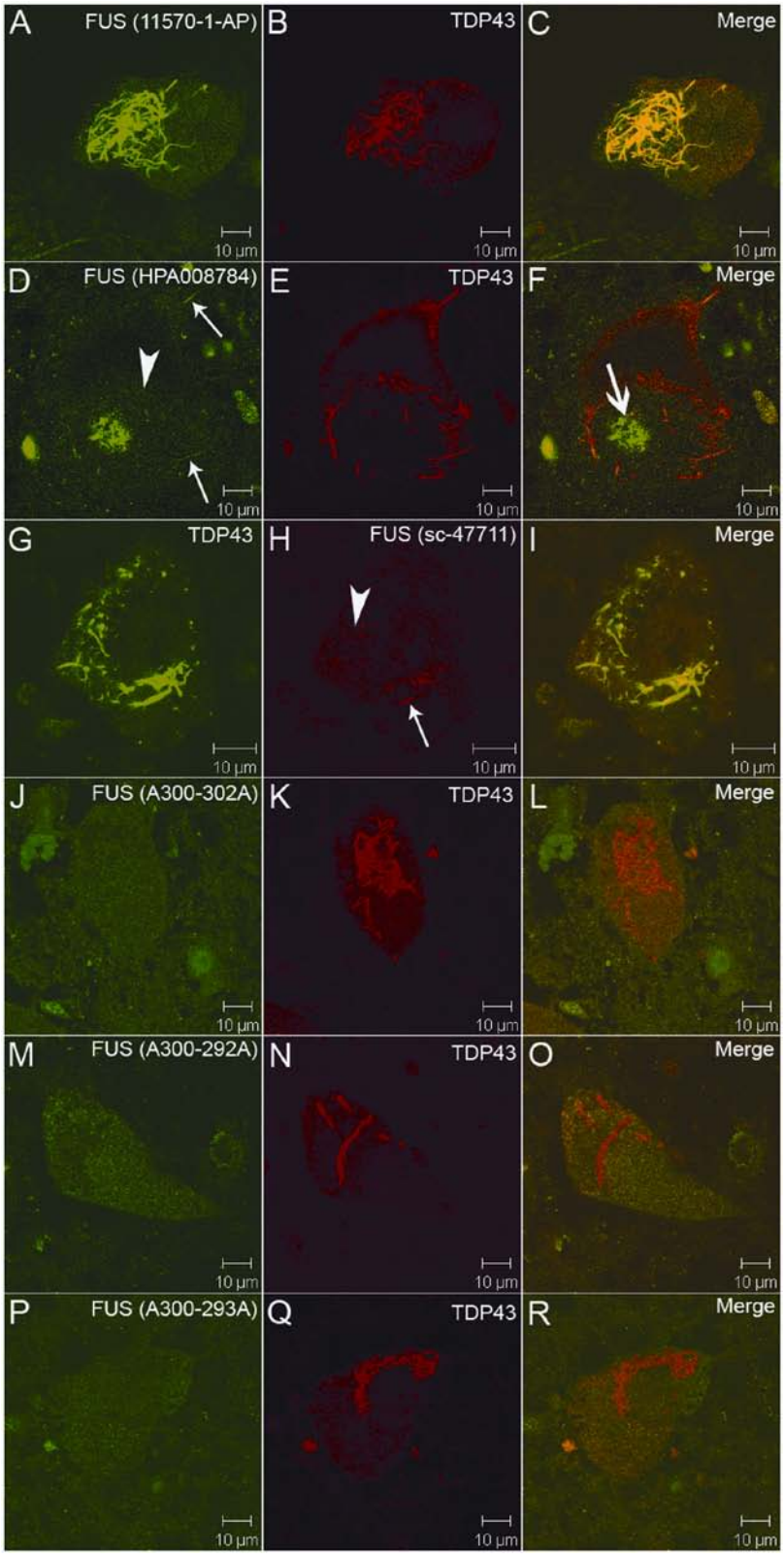
pathology is shown in sections of the neocortex (G-I) and hippocampus (J-L) from patients with ALS/dementia.



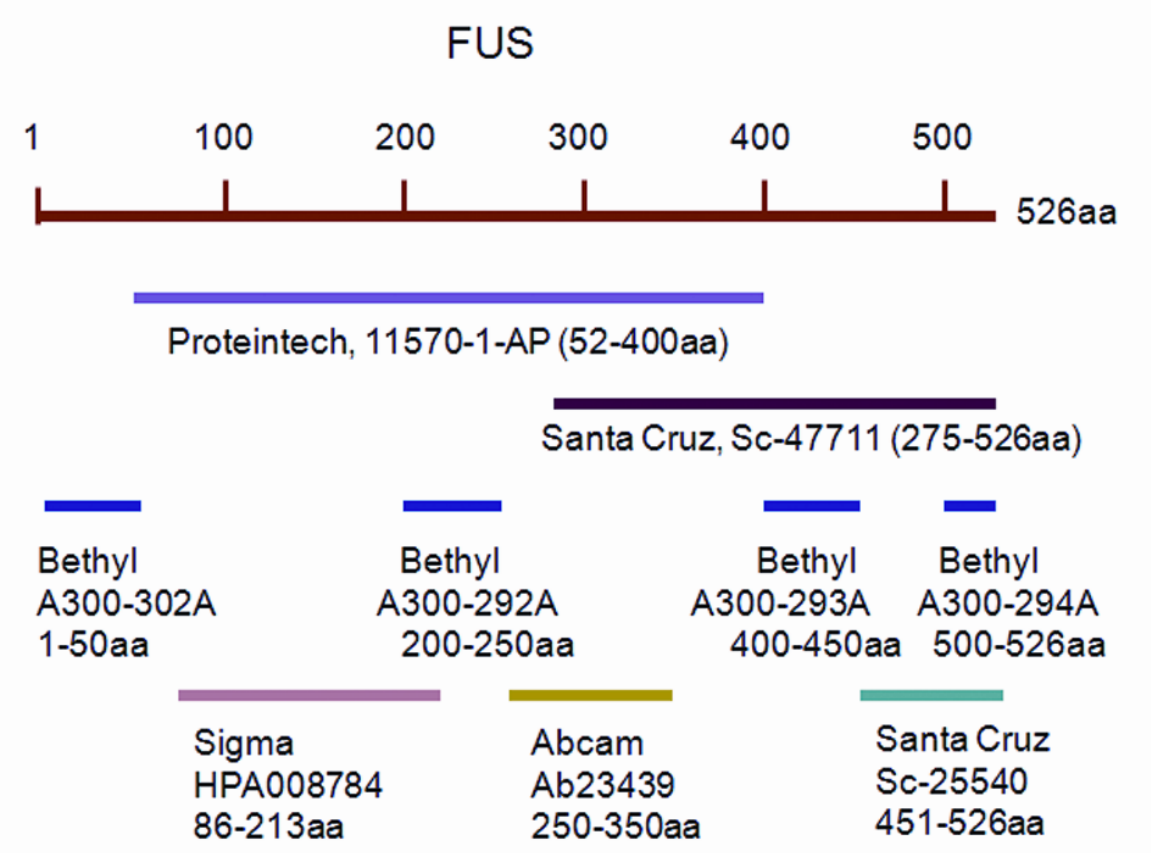
Supplementary Fig. 1.



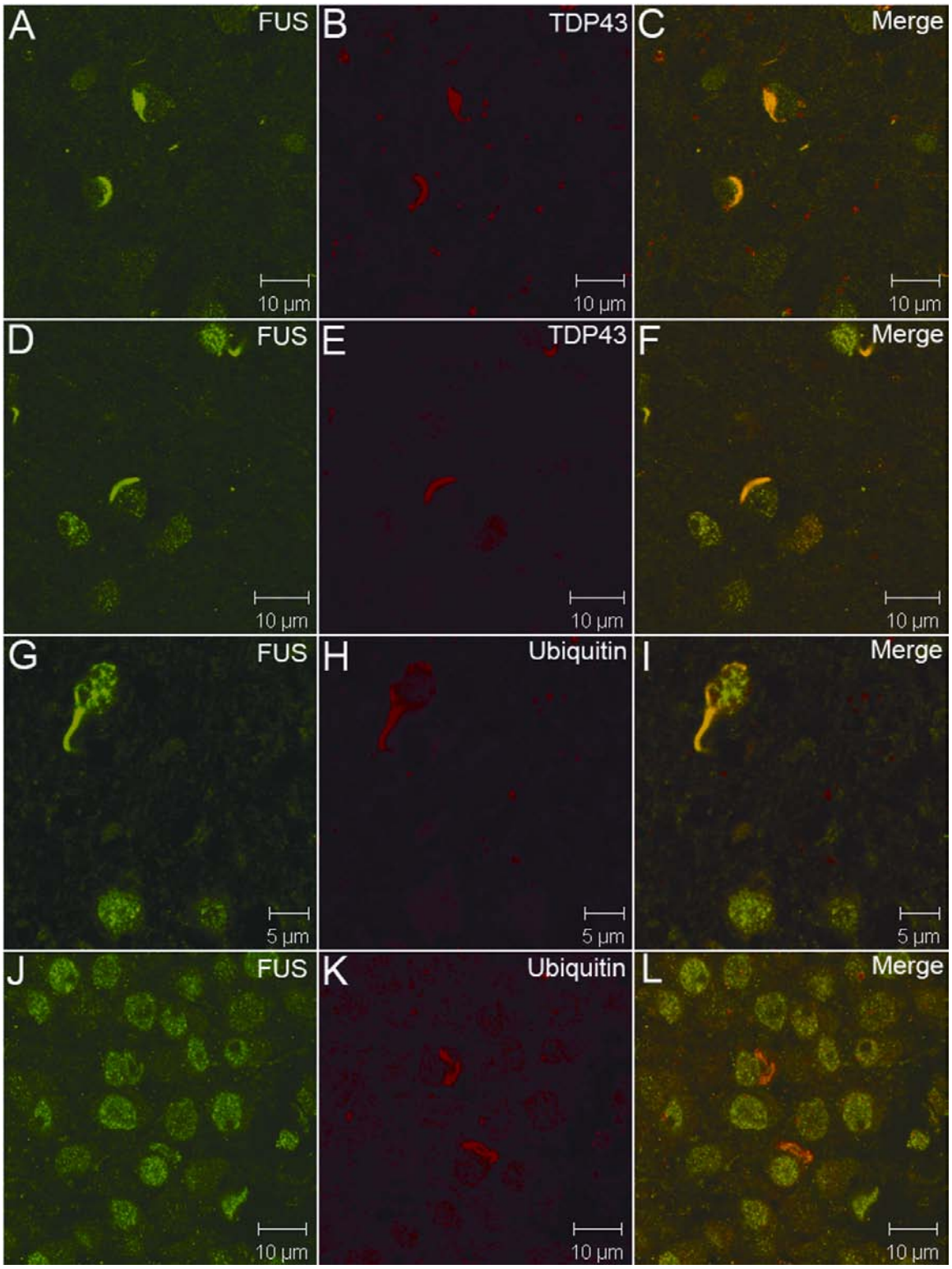
Supplementary Fig. 2.



Supplementary Fig. 3.



Supplementary Fig. 4.



Supplementary Fig. 5.

Supplementary table 1. Immunostaining of different antibodies to FUS in brain and spinal cord sections

Company	Product no.	Type	Epitope (aa 1-526)	IHC (concentration), ALS, spinal cord	IF (intensity), ALS, spinal cord	IF (intensity), aFTLD-U, Brain
Proteintech	11570-1-AP	RP (AP)	52-400	++++ (0.3-3µg/ml)	++++	++++
Sigma-Aldrich	HPA008784	RP (AP)	86-213	++ (0.4µg/ml)	+++	+++
Santa Cruz Biotech	sc-47711	MM	275-526*	- (2-4µg/ml)	++	++
Santa Cruz Biotech	sc-25540	RP (IgG)	451-526	- (2-8µg/ml)	-	-
Abcam	ab23439	RP (AP)	250-350	- (0.2-2µg/ml)	-	+
Bethyl Laboratories	A300-302A	RP (AP)	1-50	- (0.5-1µg/ml)	-	++
Bethyl Laboratories	A300-292A	RP (AP)	200-250	- (0.5-1µg/ml)	-	-
Bethyl Laboratories	A300-293A	RP (AP)	400-450	- (0.5-1µg/ml)	-	-
Bethyl Laboratories	A300-294A	RP (AP)	500-526	- (0.5-2µg/ml)	-	-

These antibodies detect nuclear staining of FUS, but with variable sensitivity for detection of inclusions.

RP, rabbit polyclonal; AP, affinity-purified; MM, mouse monoclonal; IHC, immunohistochemistry;

IF, immunofluorescence; *the exact epitope remains to be determined.

Semiquatitative rating of the degree of pathological FUS inclusions is scoreed as none (-), rare (+), occasional (++), common (+++) or numerous (++++) for immunohistochemistry, and none (-), visible (+), weak (++), strong (+++) or very strong (++++) for immunofluorescence.