Supplemental Table 1

Antibody	Species	Company, catalog number	Dilution
Western blotting			
human TDP-43	mouse monoclonal	Abnova, H00023435-M01	1:1000
TDP-43	rabbit polyclonal	Proteintech, 10782-2-AP	1:2000
TDP-43 C-term	rabbit polyclonal	Proteintech, 12892-1-AP	1:2000
beta-actin	mouse monoclonal	Millipore, MAB1051	1:5000
Alix/AIP-1	mouse monoclonal	BD Transduction Lab, 611621	1:1000
Flotillin-1	mouse monoclonal	BD Transduction Lab, 610821	1:1000
CD63	mouse monoclonal	Thermo Scientific, MA5-11501	1:1000
Calnexin	rabbit polyclonal	Enzo, SPA-865	1:1000
GM130	rabbit polyclonal	Novus Bio, NB101-57021	1:1000
TGN46	rabbit polyclonal	Abcam, AB50595	1:1000
TGN38	rabbit polyclonal	Santa Cruz, sc-33784	1:1000
Rab27a	rabbit polyclonal	Synaptic System, 168 013	1:1000
LC3	rabbit polyclonal	MBL, PM036	1:1000
p62	guinea pig polyclonal	MBL, PM066	1:1000
V5	mouse monoclonal	Invitrogen, R960-25	1:2000
Immunofluorescent and immunohistochemistry			
SMI-31	mouse monoclonal	Covance, SMI31R	1:200
SV2	mouse monoclonal	DSHB, AB2315387	1:50
TDP-43	rabbit polyclonal	Proteintech, 10782-2-AP	1:1000
pTDP-43 (ser409/410)	rabbit polyclonal	Cosmo Bio, CAC-TIP-PTD-P01	1:1000
pTDP-43 (ser409/410)	mouse monoclonal	Cosmo Bio, CAC-TIP-PTD-M01	1:1000
NeuN	mouse monoclonal	Cell Signaling, D3531	1:1000
Laminin	rabbit polyclonal	Sigma Aldrich, L9393	1:200
ChAT	goat polyclonal	Millipore, AB144P	1:200
Immunoelectron microscopy			
V5	mouse monoclonal	Invitrogen, R960-25	1:1000
CD63	mouse monoclonal	Thermo Scientific, MA5-11501	1:100
Alix/AIP-1	mouse monoclonal	BD Transduction Lab, 611621	1:500
human TDP-43	mouse monoclonal	Abnova, H00023435-M01	1:500

Supplemental Table 2

Sporadic ALS

	Clinical diagnosis	Age/Gender	Duration
1	ALS-FTD	71/M	3 years
3	ALS	66/M	3 years
5	ALS	58/F	12 years

Disease control

	Clinical diagnosis	Age/Gender
2	MSA-C	63/F
3	FAP	71/M
4	CIDP	74/M

MSA-C: multiple system atrophy cerebellar dysfunction subtype
FAP: familial amyloid polyneuropathy
CIDP: Chronic inflammatory demyelinating polyneuropathy

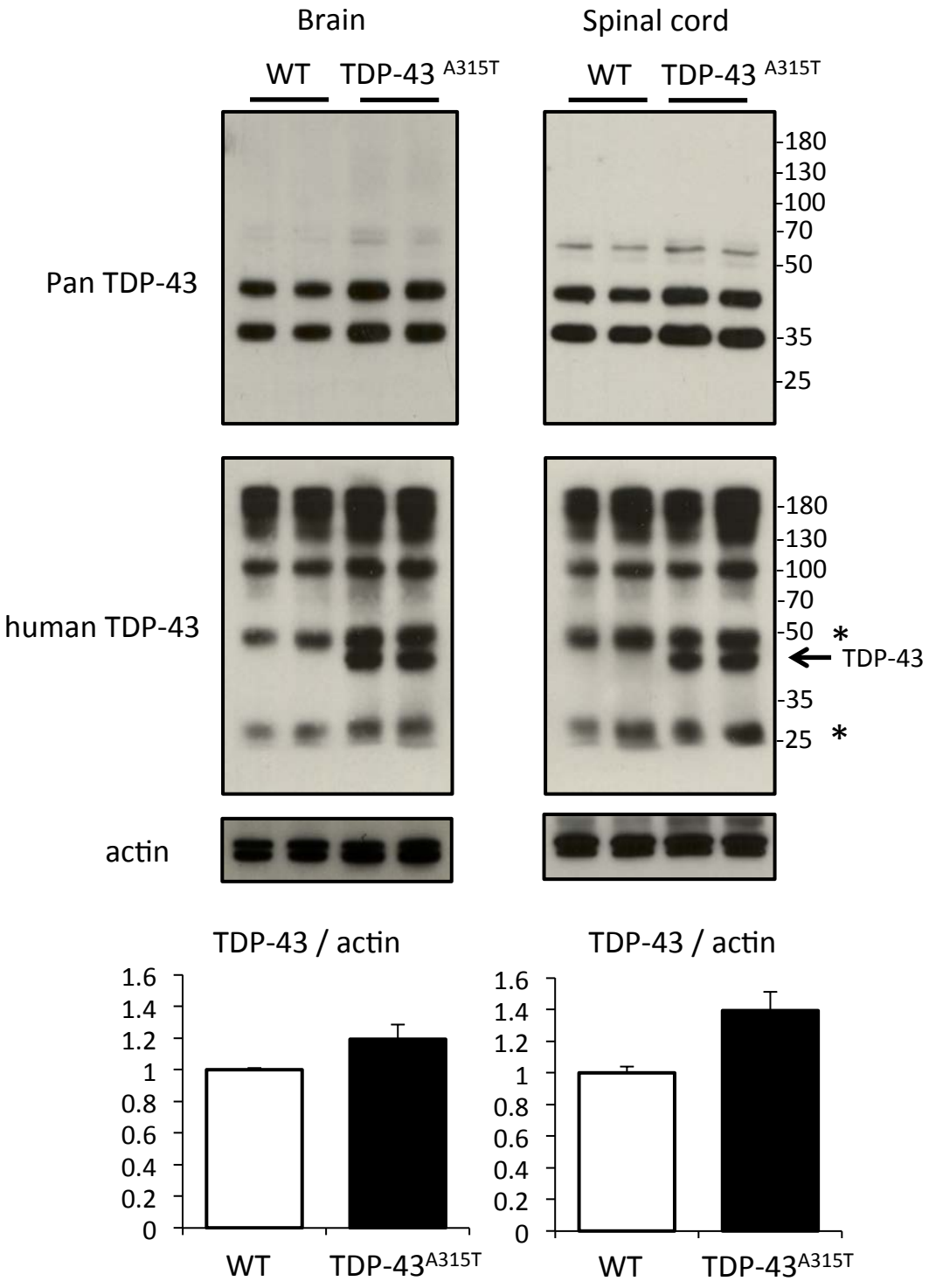


Figure S1. Immunoblots of brain and spinal cord of WT or TDP-43^{A315T} mice. Asterisks indicate non-specific immunoglobulin bands.

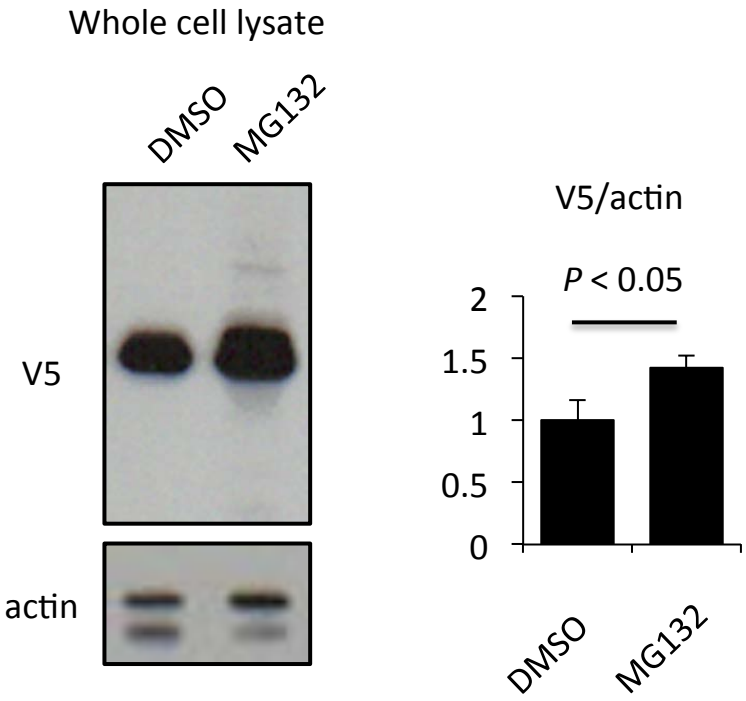


Figure S2. Immunoblots of whole cell lysate of Neuro2a cells treated with DMSO or MG132, and its densitometric analysis.

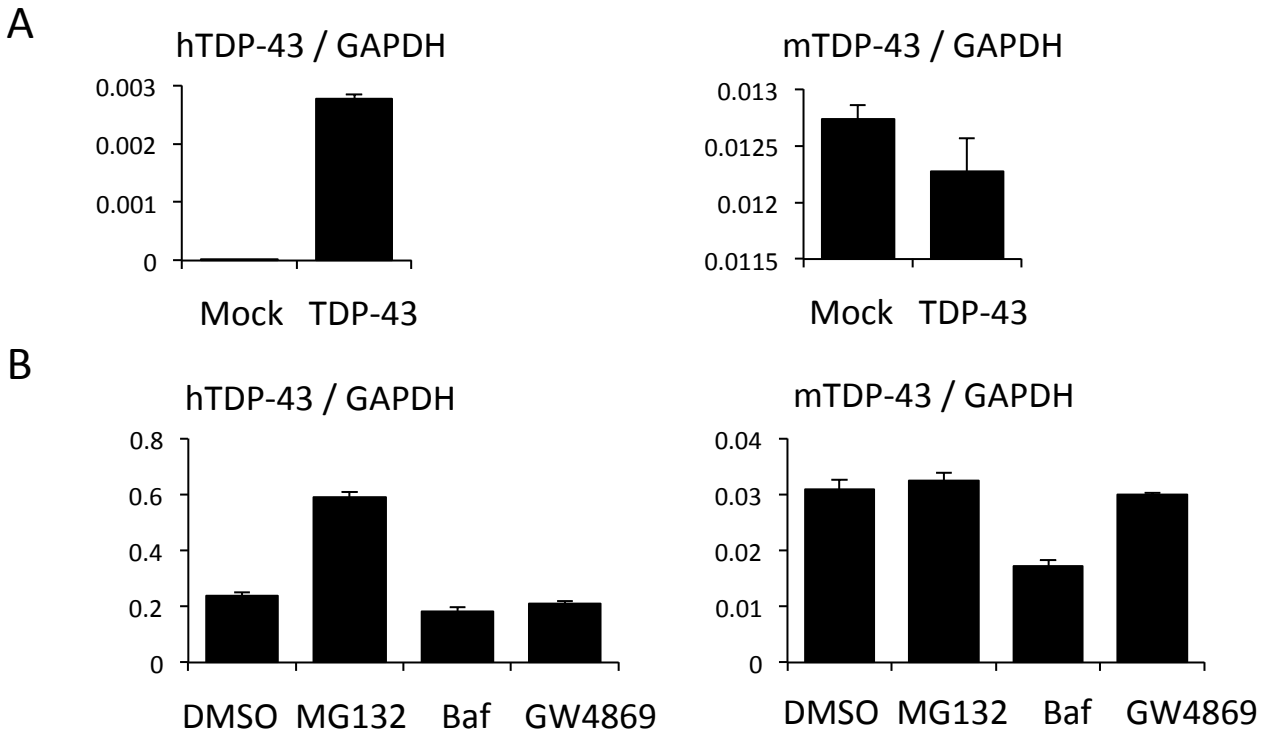


Figure S3. RT qPCR analysis of human and mouse TDP-43 in Neuro2a cells. (A) Neuro2a cells transfected with mock or human TDP-43 plasmid were analyzed. (B) Neuro2a cells treated with DMSO, MG132, Bafilomycin, or GW4869 were analyzed. All cells were transfected with human TDP-43.

Figure S4

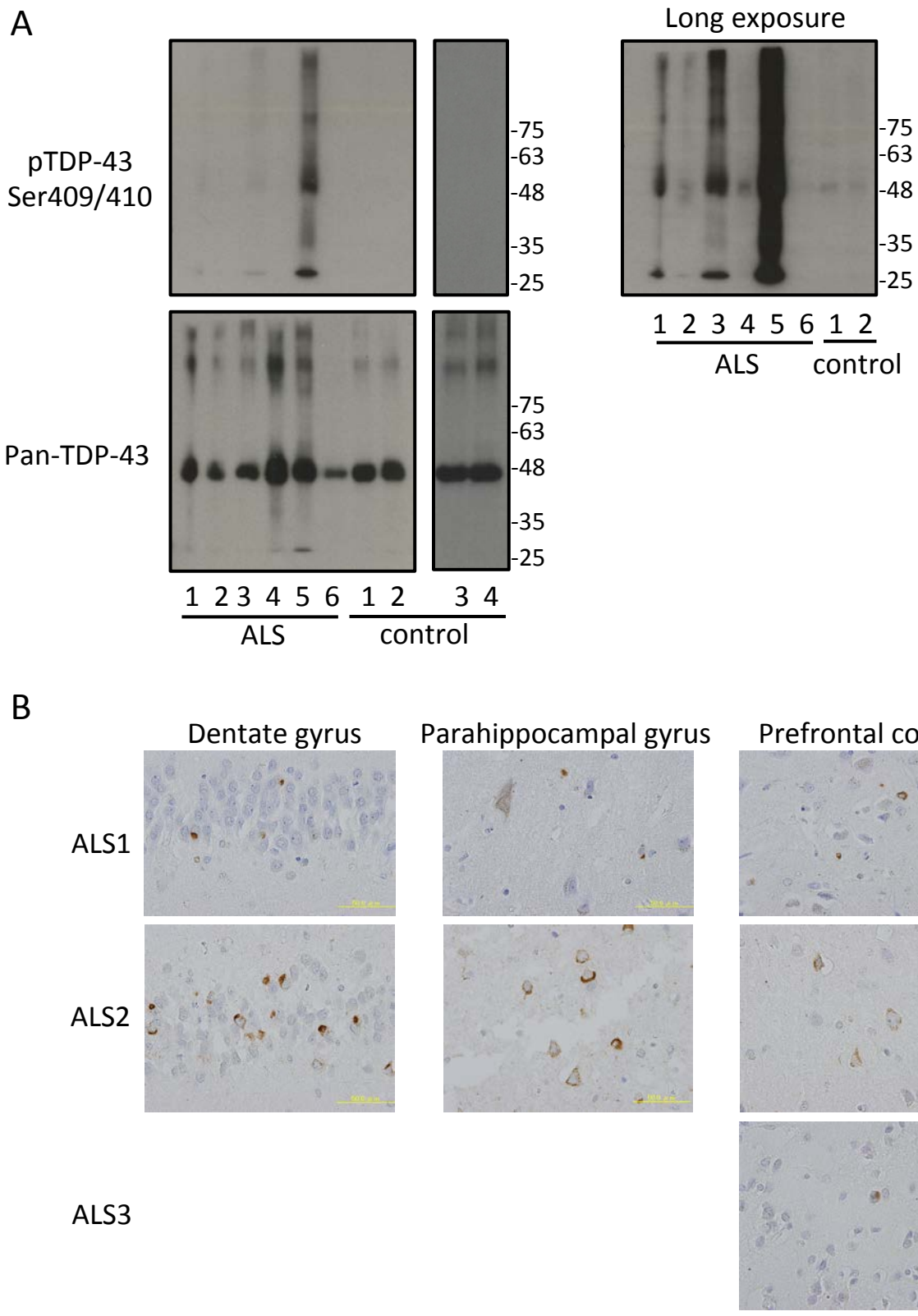


Figure S4. (A) Immunoblots of salkosyl-insoluble fraction of control or ALS brains. ALS-1, 3, and 5 were positive for phosphorylated TDP-43. (B) Immunohistochemistry of dentate gyrus, parahippocampal gyrus, and prefrontal cortex of ALS patients using phospho-specific TDP-43 antibody.

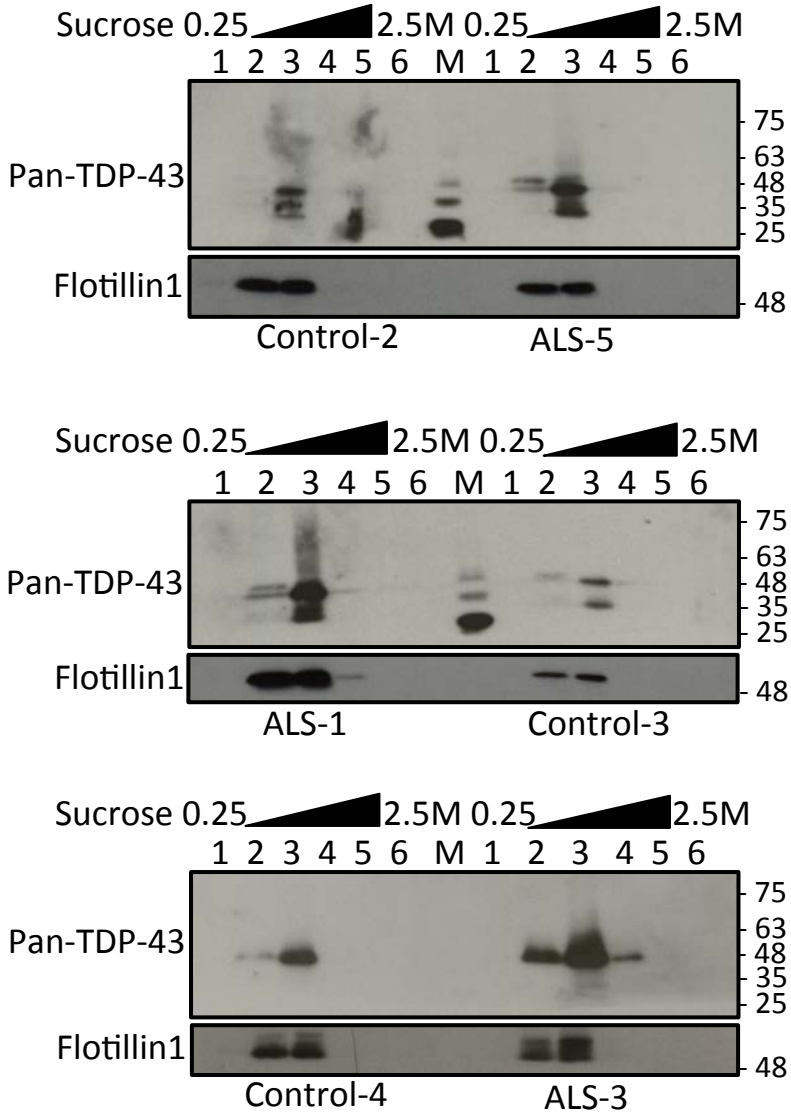


Figure S5. Sucrose density gradient fraction of extracellular matrix of control or ALS brains. TDP-43 and Flotillin 1 were predominantly detected in the second and third fractions.

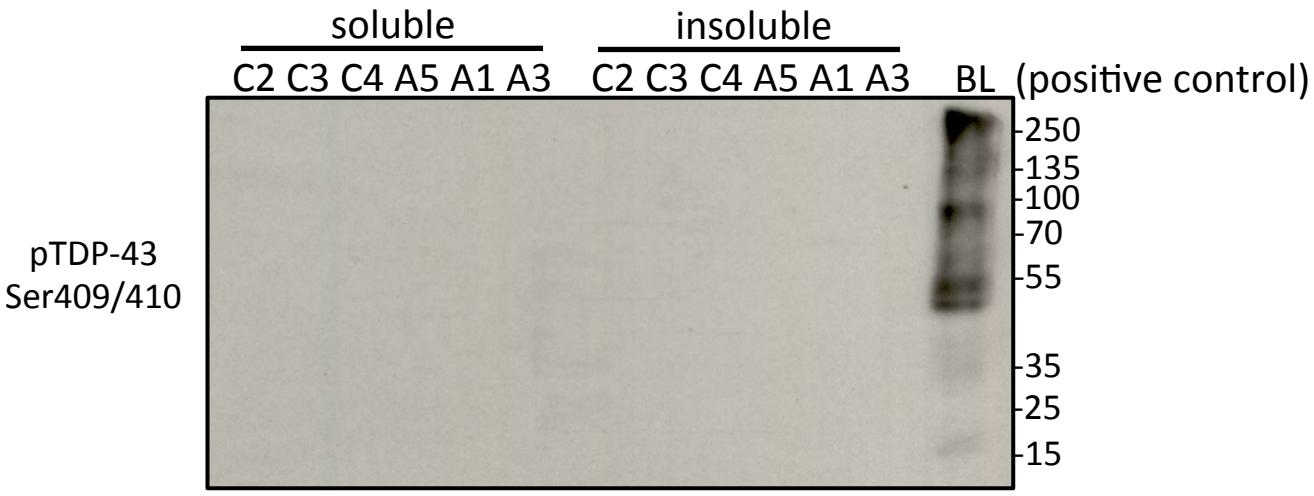


Figure S6. Immunoblot of exosome fractions of control or ALS brains with salkosyl-insoluble brain lysate (BL) of ALS-5 (positive control).

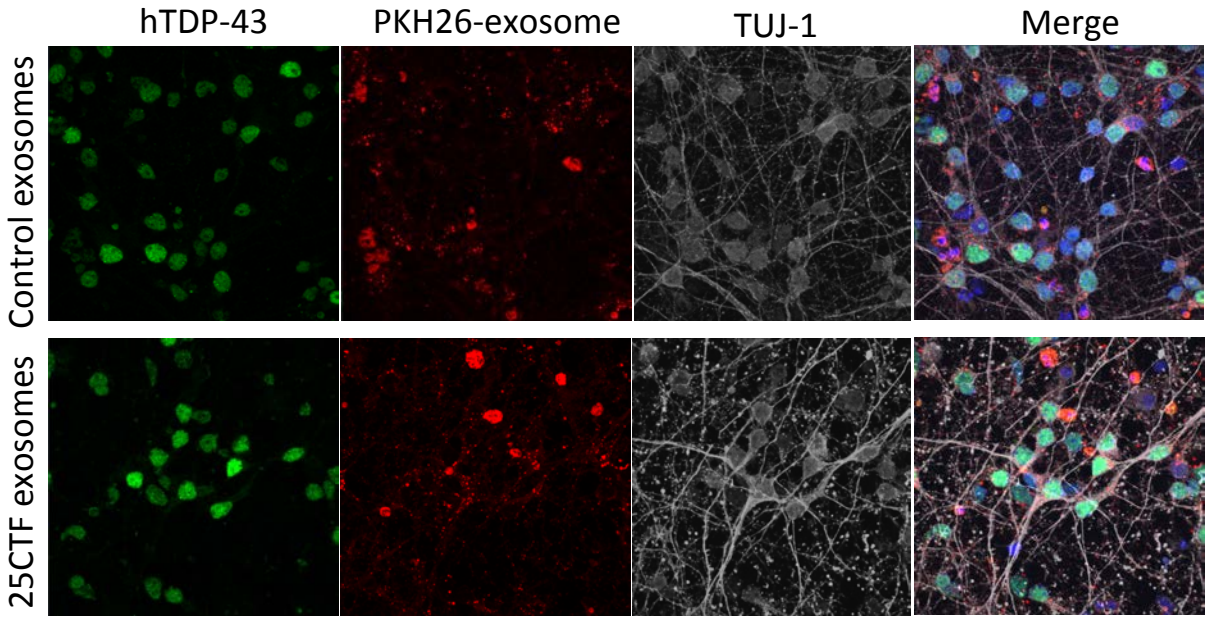


Figure S7. Immunofluorescent images of primary cortical neurons of TDP-43^{G348C} mouse treated with control or TDP-43 25CTF-containing exosomes.

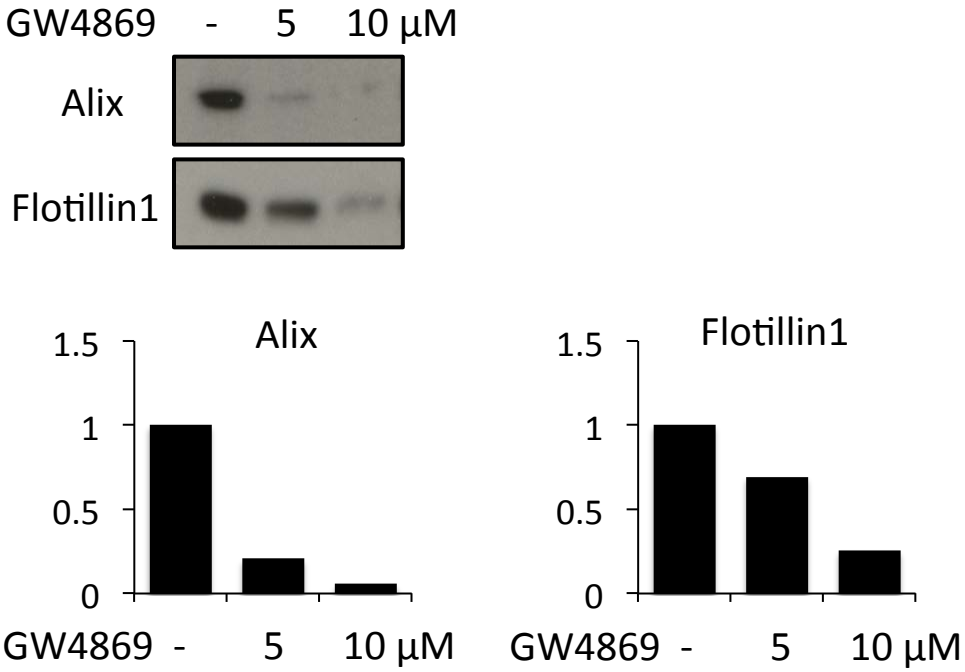


Figure S8. Immunoblots of exosome fraction purified from Neuro2a cells treated with GW4869 for 24 h.

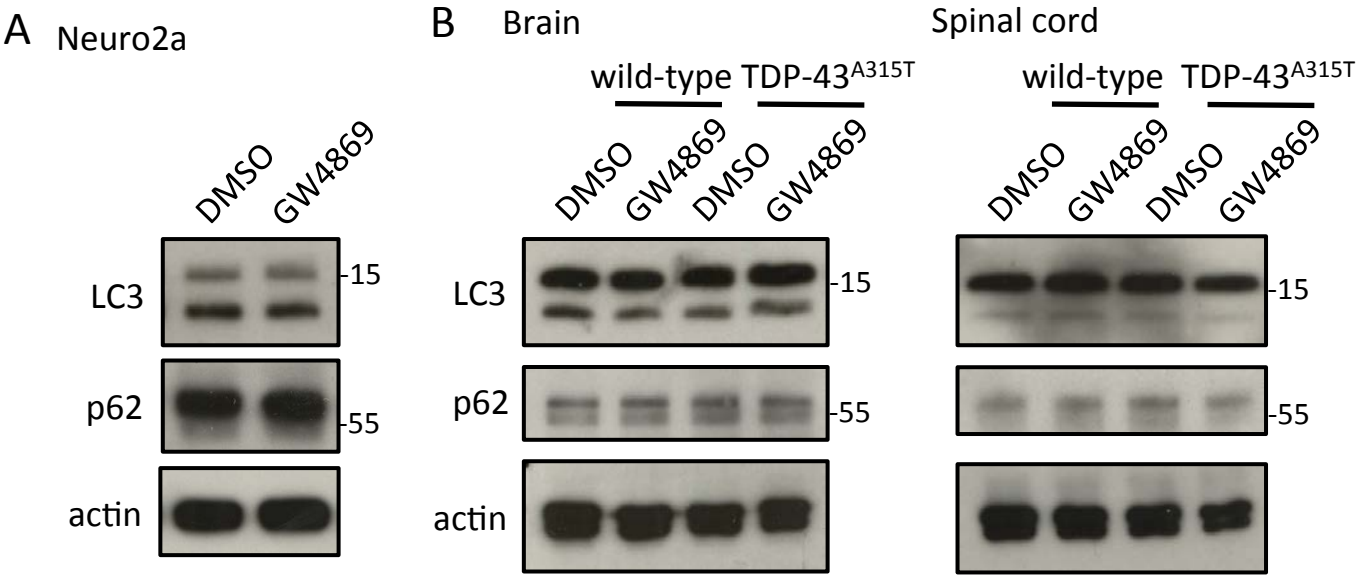


Figure S9. (A) Immunoblots of cell lysate (RIPA-soluble fraction) of Neuro2a cells treated with DMSO or GW4869. (B and C) Immunoblots of mouse brain and spinal cord (RIPA-soluble fraction) of wild-type or TDP-43^{A315T} mice treated with DMSO or GW4869.

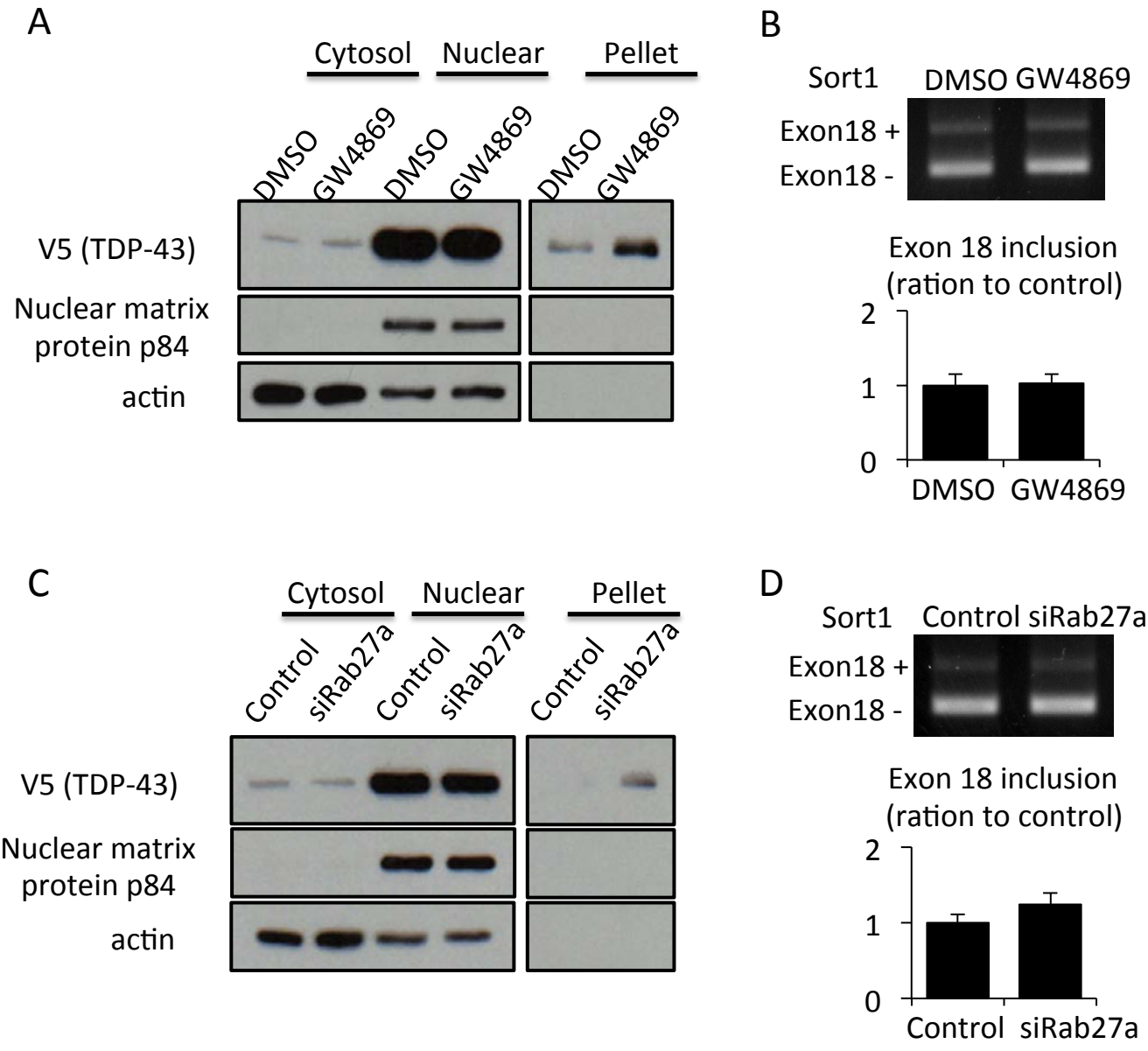


Figure S10. (A) Immunoblots of cytosol, nuclear fractions and insoluble pellet of Neuro2a cells treated with DMSO or GW4869. (B) Semi-quantitative RT-PCR of Sort1 showing two splice variants, and quantification of exon18 inclusion from three biological replicas per group. (C) Immunoblots of cytosol, nuclear fractions and insoluble pellet of Neuro2a cells silencing Rab27a or its control. (D) Semi-quantitative RT-PCR of Sort1 showing splice changes, and quantification of exon18 inclusion from three biological replicas per group.

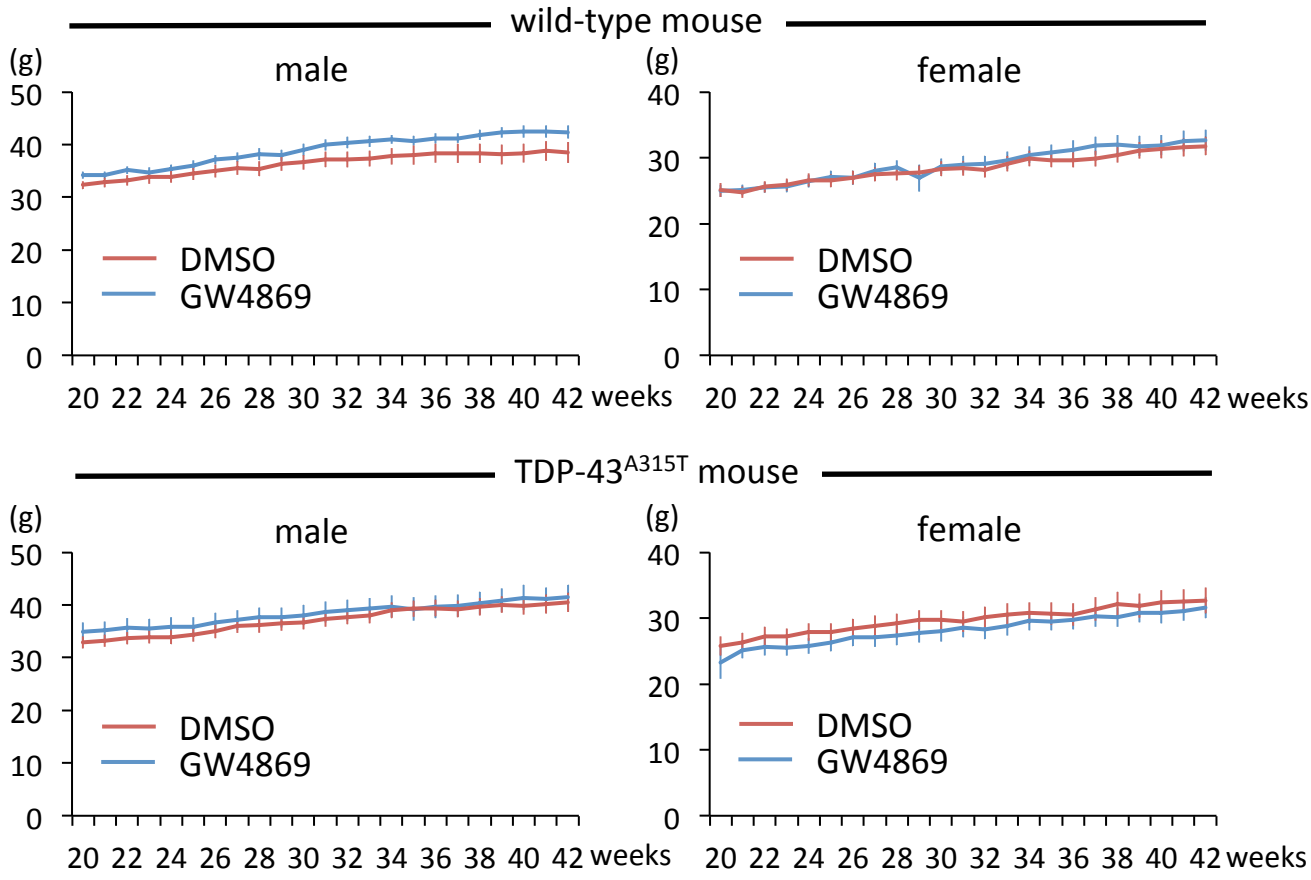


Figure S11. Body weight of wild-type and TDP-43^{A315T} mice treated with DMSO or GW4869.

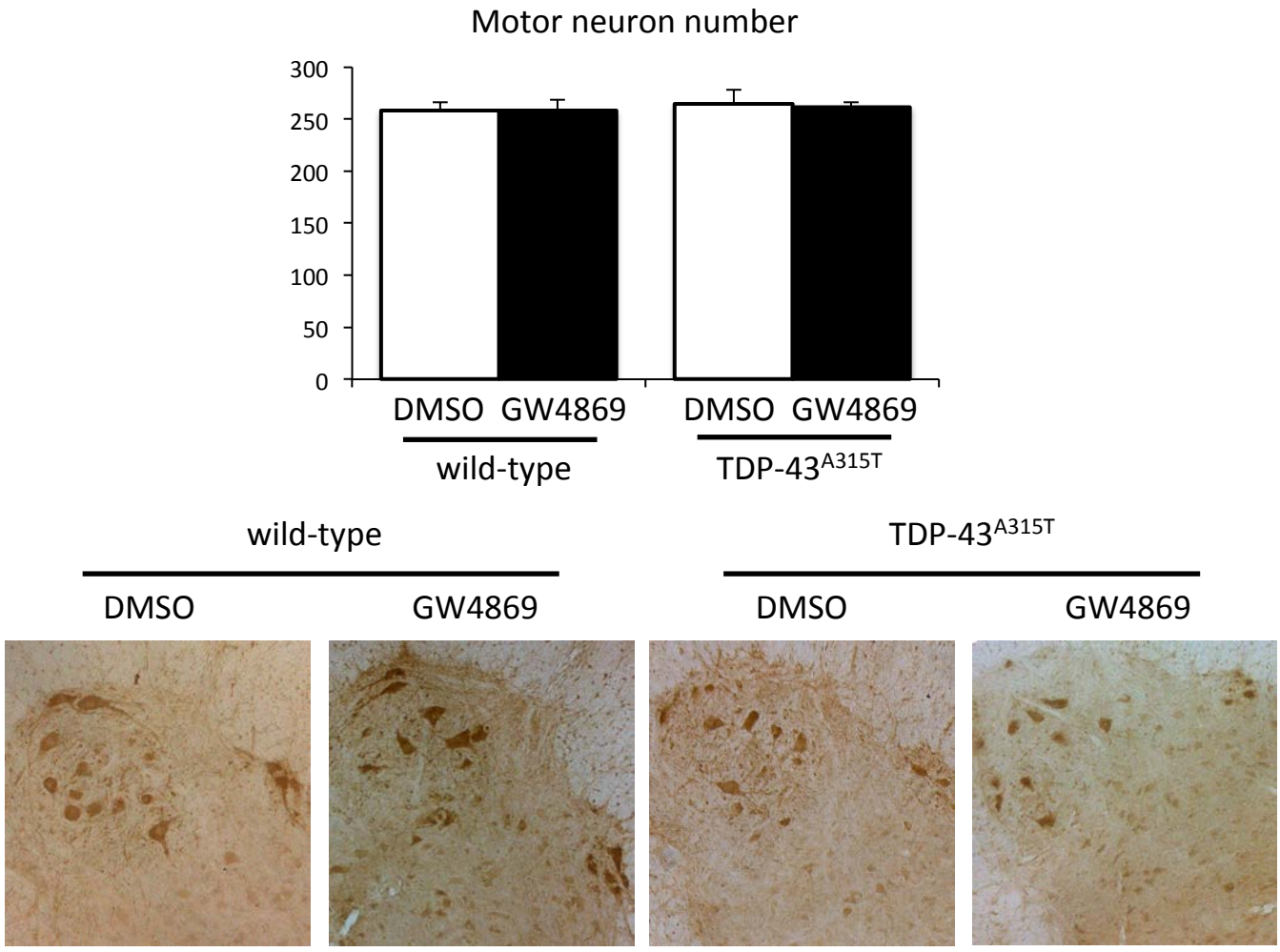


Figure S12. Motor neuron number in lumbar spinal cord. Immunohistochemistry of ChAT was done with every tenth 25-µm-thick section from 100 consecutive sections of lumber spinal cord, and total number of motor neuron was analyzed.

Fig.1D

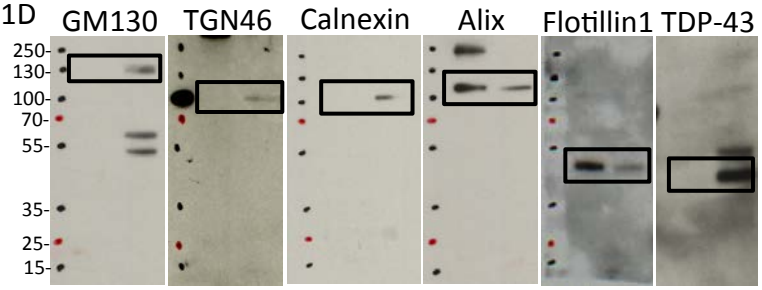


Fig.1E

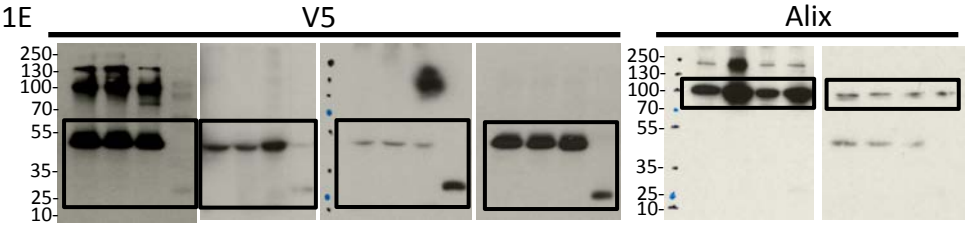


Fig.1F

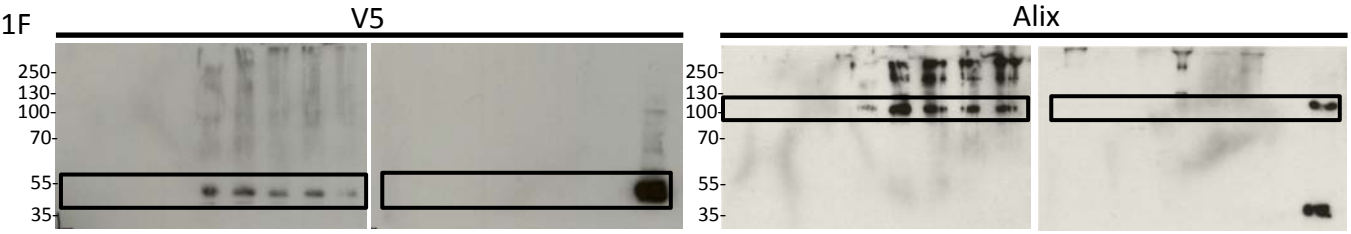


Fig.1I

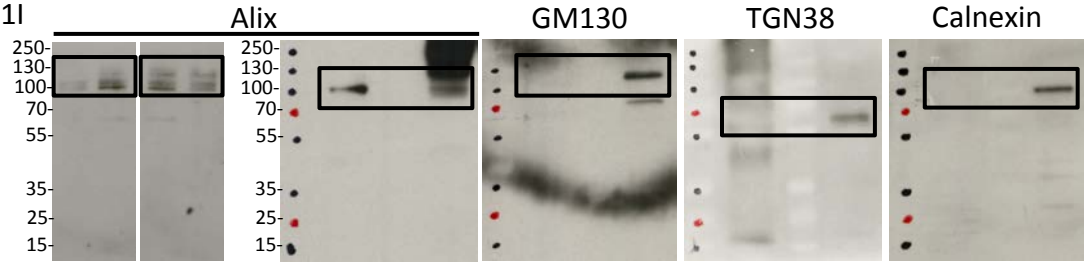


Fig.2B

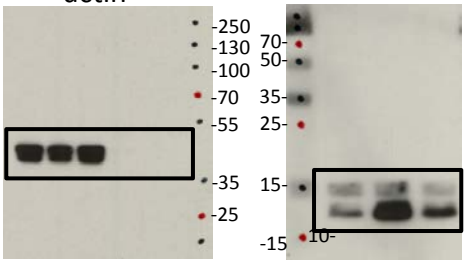


Fig.2D

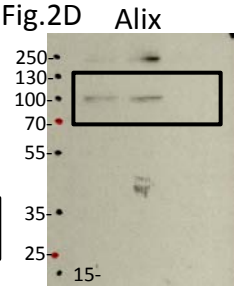


Fig.2F

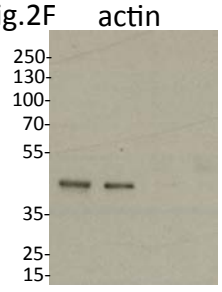


Fig.2I

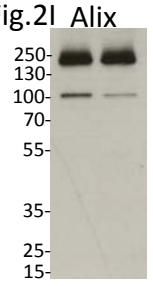


Fig.2L

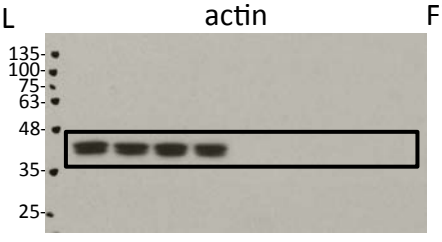
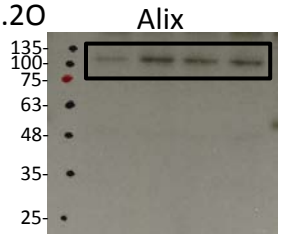


Fig.2O



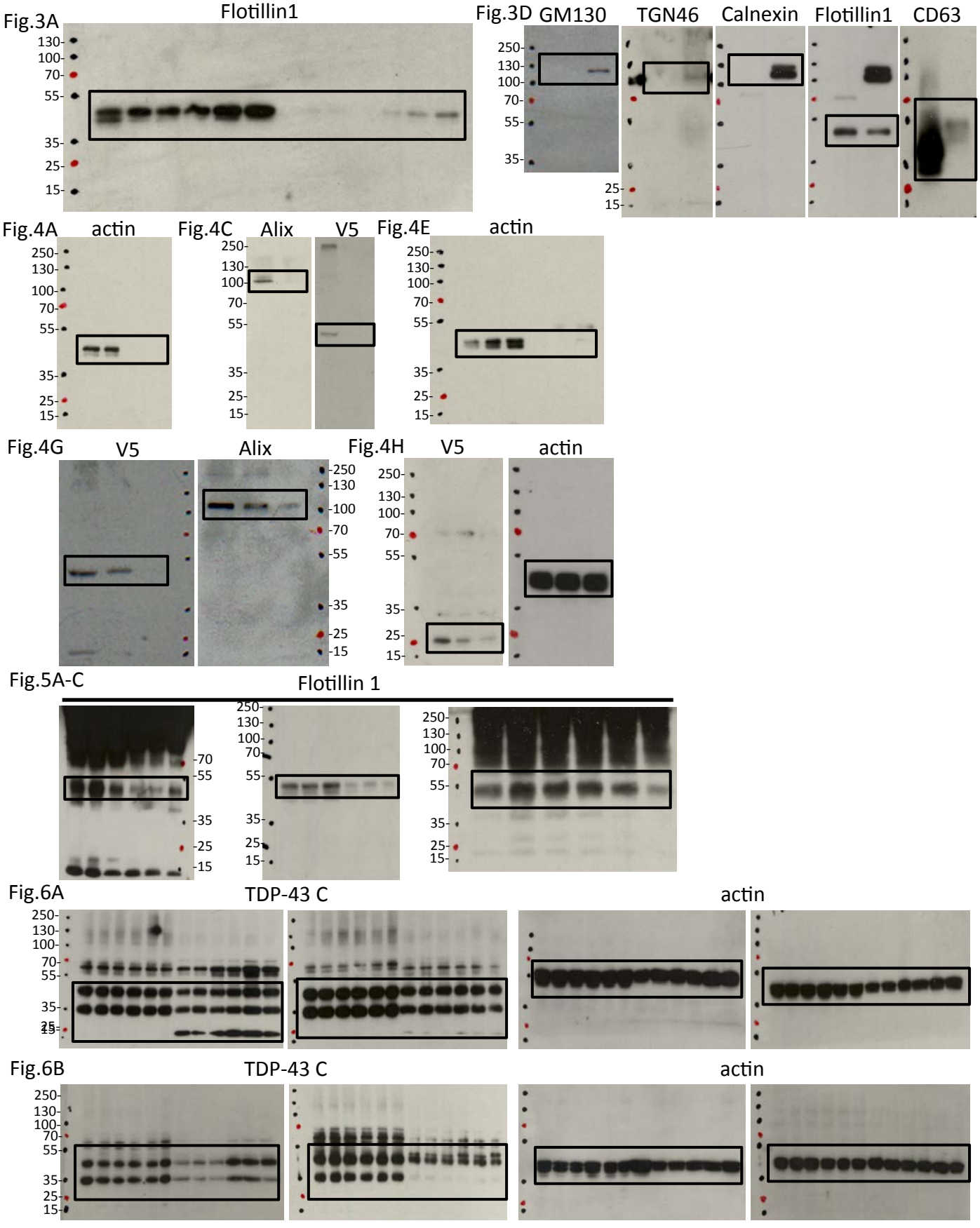


Figure S13. Uncropped full-length blots of main figures.

Supplementary materials and methods

Analysis of insoluble fraction of human brain

To confirm the existence of TDP-43 pathology we checked sarkosyl insoluble fractions of brain samples. First, 100 mg of brain tissues were homogenized with 1ml of buffer A (10mM Tris-HCl, pH 7.5 with 1mM EGTA, 10% sucrose, 0.8M NaCl). Next, we added 1ml of 2% TritonX-100 buffer (in buffer A), incubated for 30min at 37°C, and centrifuged at 100,000g for 30min at 25°C. The supernatant was discarded, and the pellet was resuspended with 1% Sarkosyl buffer (in buffer A). The homogenate was incubated for 30min at 37°C, and centrifuged at 100,000g for 30min at 37°C. After the supernatant was discarded, the pellet was resuspended with 150 µl of PBS. Ten-µl of that solution was used for immunoblots with anti-phospho TDP-43 (Ser409/Ser410) monoclonal antibody (Cosmo Bio) and pan-TDP-43 polyclonal antibody (Proteintech).

Nuclear and Cytoplasmic Extractions

Cells cultured in 6-well plate were harvested with 500 µl of buffer A (10mM HEPES pH 7.9, 1.5mM $MgCl_2$, 10mM KCl, 0.1mM EDTA, 0.1% NP-40, 1mM DTT), incubateed at 4°C for 30 min, and centrifuged at 1000 g for 5min. Supernatant was kept for cytoplasmic fraction. The pellet was resuspended with 200 µl of buffer B (20mM HEPES pH 7.9, 1.5mM MgCl2, 400mM NaCl, 0.1mM EDTA, 0.1% NP-40, 10% Glycerol, 1mM DTT), incubated at 4°C for 30 min, and sonicated. The suspension was centrifuged at 15,000g for 5min. Supernatant was nuclear fraction. The pellet was resuspended with 200 µl of 3% SDS sample buffer. Twenty-µg protein was loaded and separated by SDS-PAGE.

Immunohistochemistry

The human brain tissues were fixed in 20% neurtral-buffered formalin. The paraffin-embedded tissue blocks were cut at a thickness of 4.5µm. Mouse lumbar spinal cords were taken from 4% paraformaldehyde-perfused mice. For the analysis of spinal motor neuron immunohistochemistry of ChAT was done with every tenth 25-µm-thick section of lumbar spinal cord from 100 consecutive sections of lumber spinal cord, and total number of motor neuron was analyzed.

RT-PCR for alternative splicing analysis

Total RNA was isolated from cells using Absolutely RNA Miniprep Kit (Agilent). cDNA was synthesized from 1 µg of total RNA with the Oligo-dT primer (Invitrogen). The sequences of primers against Sort1 are ATGAATCCCGCCAGAGAAG and GACAAGCATCAGTCCCACGA. Semi-quantitative RT-PCR was performed using Taq DNA polymerase (Bio Basic).