

Figure S1. Intra-section heterogeneity of pTDP-43 staining in ARSACS CNS tissue sections, and negative glioblastoma control staining. Representative immunohistochemistry images show intra-section heterogeneity of pTDP-43 staining in different ARSACS brain tissue sections. Negatively stained neurons (red circles) can be observed in all panels. Neuron with clear cytoplasmic pTDP-43 pathology (white arrow) is observed alongside neighboring neurons lacking such accumulation, as indicated in the third panel. This striking juxtaposition highlights the regionally variable nature of TDP-43 aggregation within the same tissue section. A separate panel (far left) shows staining performed on a glioblastoma brain sample used as a negative control, which was processed in parallel using the same pTDP-43 antibody and protocol. Acquisitions were digitally zoomed to enlarge cells and enhance visibility. Scale bars: 50 µm. Magnification: 400X.

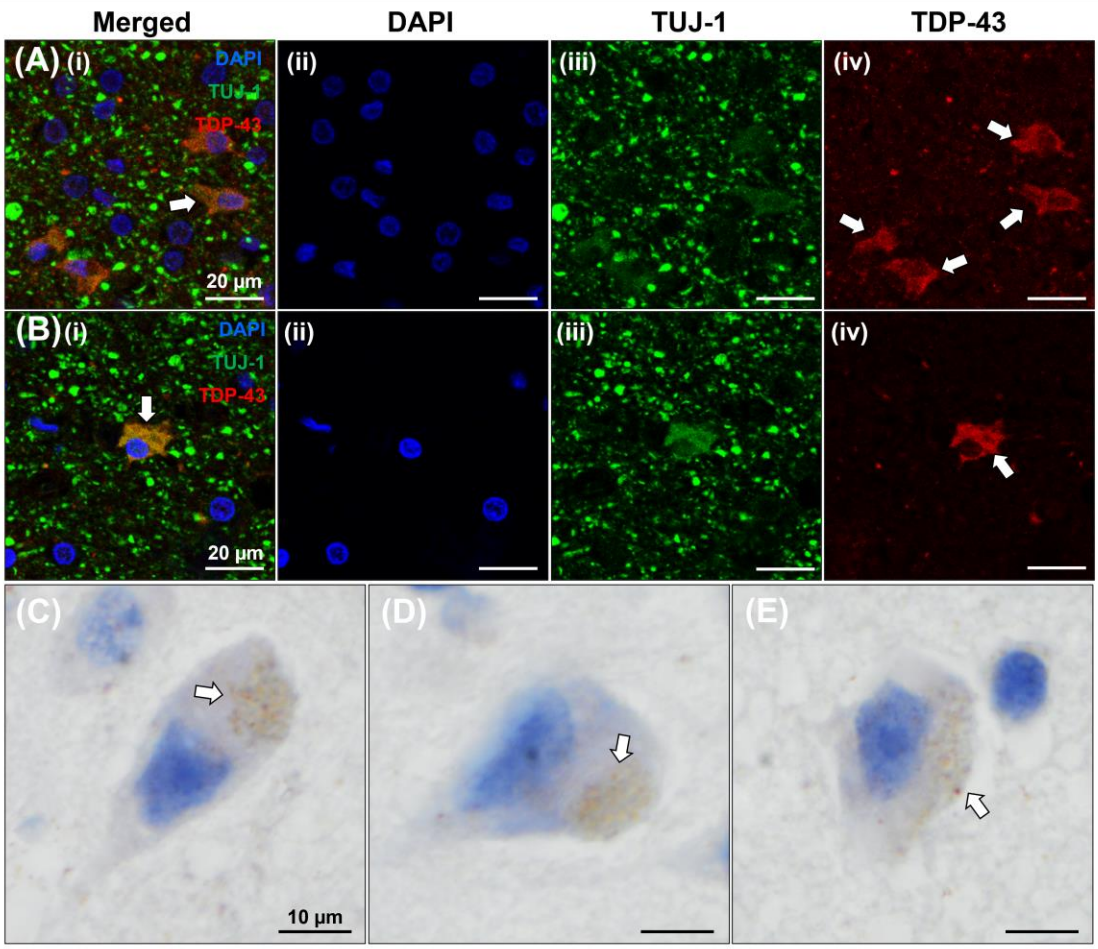


Figure S2. Confirmation of mislocalization of TDP-43 by immunofluorescence and IHC staining. (A, B) Punctate TDP-43 (red) deposits (white arrows) can be observed in the cytoplasm (TUJ-1, green) and, to a lesser extent, in the nucleus (A-iv B-iv) (DAPI, blue) of neurons in 10 µm-thick slide of the motor cortex from an end-of-life ARSACS patient. Images were acquired by immunofluorescence assay with a TDP-43-targeting antibody. Acquisitions were zoomed and cropped for clarity. **(C-E)** IHC staining revealed punctate aggregates of positively labeled pTDP-43 (white arrows) in the cytoplasm of neurons in the motor cortex from an ARSACS patient. Scale bars: 20 µm (A, B) and 10 µm (C-E). Magnification: 400X (A, B), 1000X (C-E).

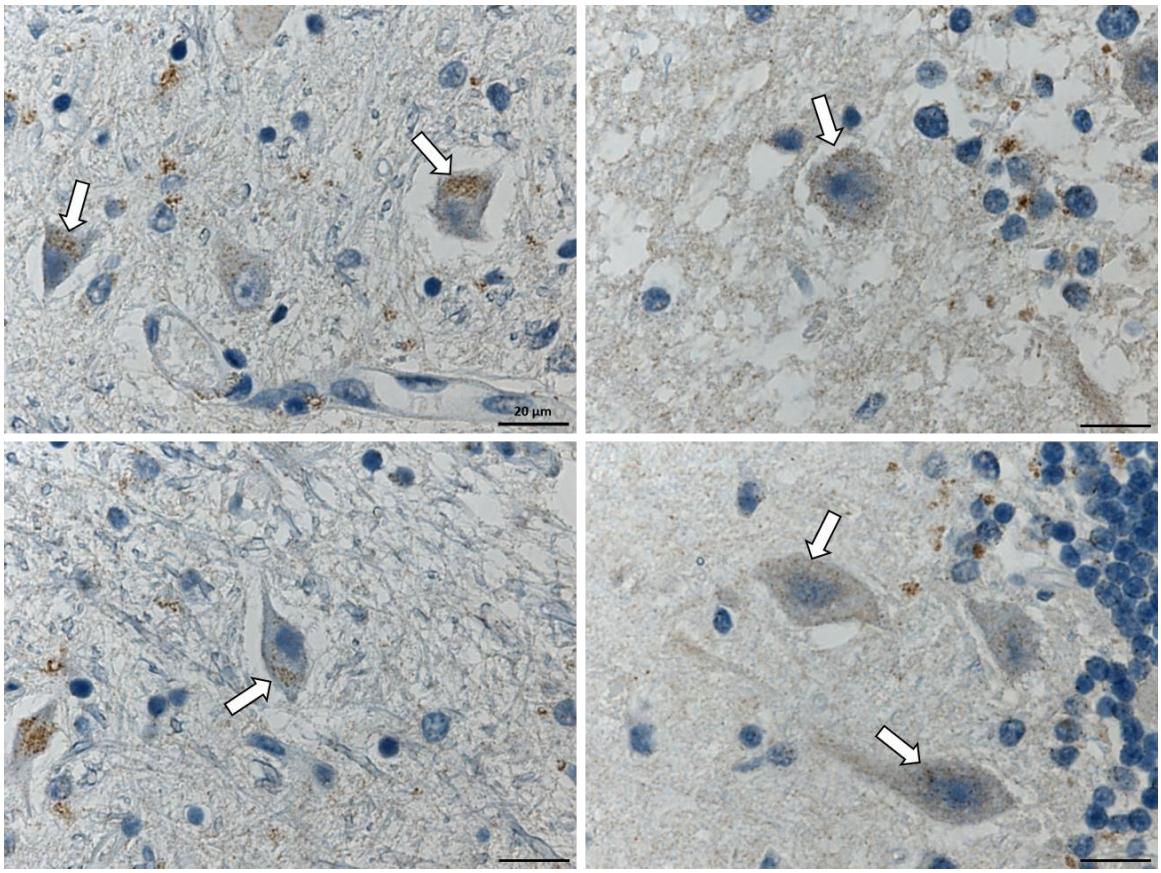


Figure S3. Additional observations of dot-like pTDP-43-positive staining in cerebellum from a female ARSACS patient (arrows). Immunohistochemistry was performed on cerebellum samples with anti-pTDP-43 antibody. Scale bars: 20 µm. Magnification: 400X.

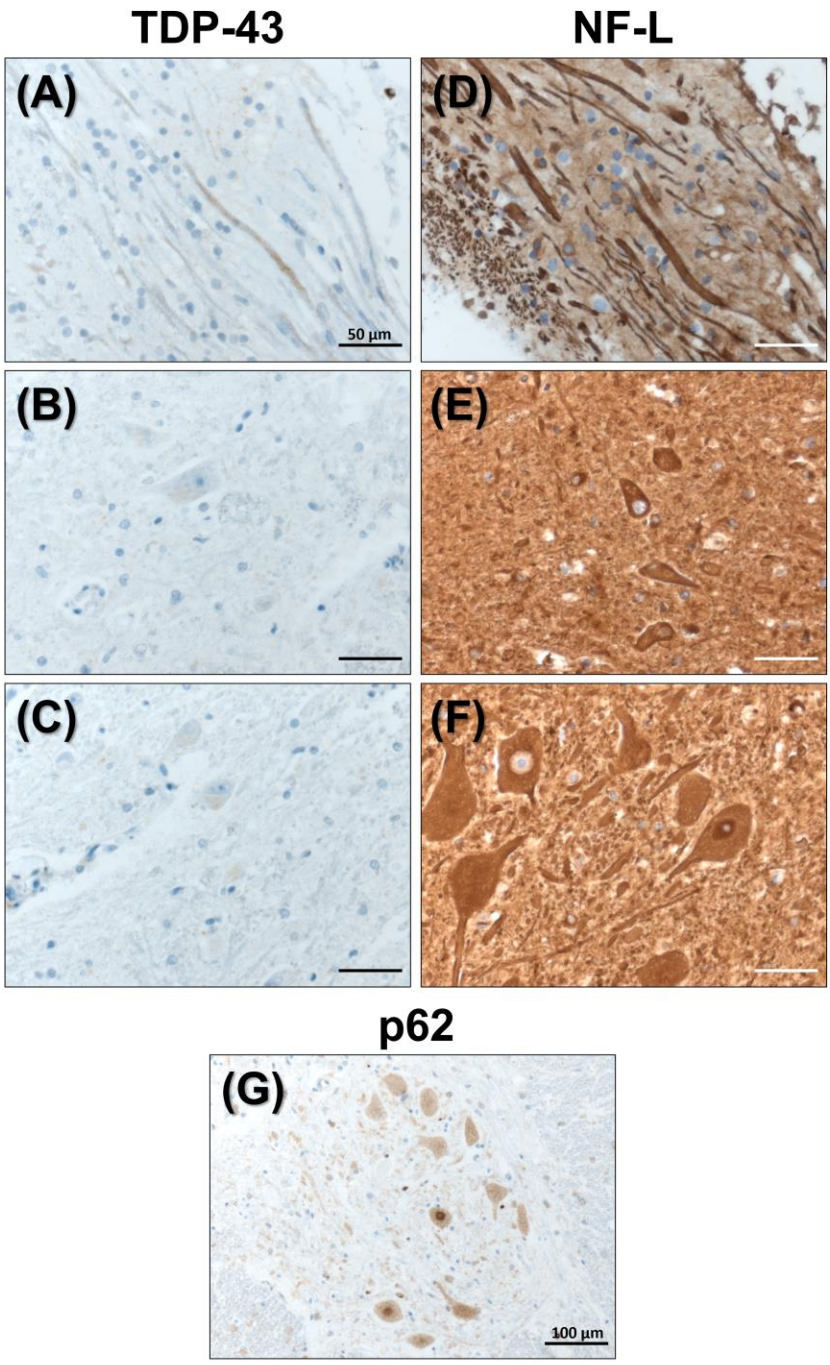


Figure S4. Immunohistochemistry staining using different antibodies in spinal cord of an ARSACS patient. (A-C) IHC staining revealed aggregates of positively labeled pTDP-43 (dot-like deposits; arrows) in the cytoplasm of neurons in a 5 µm cross section of the dorsal spinal cord of the ARSACS patient. **(D-F)** Increased NF-L expression was detected in 5 µm cross sections of the dorsal spinal cord of the ARSACS patient. **(G)** Positively labeled p62 patient in the dorsal spinal cord of an ARSACS patient. Scale bars: 50 µm (A-F) and 100 µm (G). Magnification : 200X (A-F) and x100 (G).

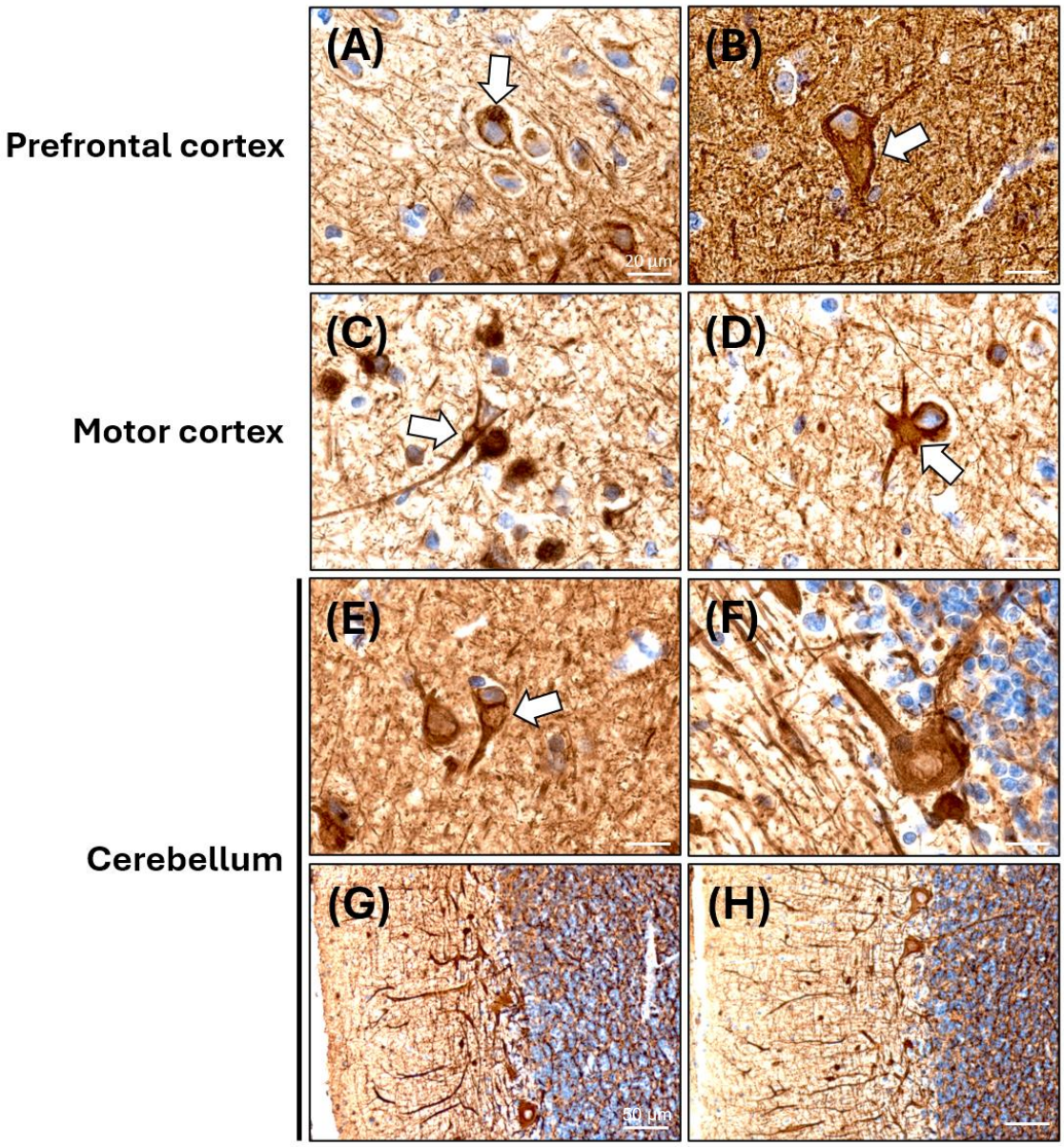


Figure S5. IHC staining reveals neurofilaments abnormalities and Purkinje cells loss in the brain of an ARSACS patients. (A-F) Intense NF-L expression was detected in different ARSACS brain regions, along with the presence of abnormal, clear vacuole-like structures within neurons (arrows). **(G, H)** Diffuse loss of Purkinje neurons and their dendritic arborization was observed in the cerebellum. Each column represents a different region of the same tissue section. Scale bars: 20 µm (A-F) and 50µm (G, H). Magnification: 400X (A-F) and 100X (G, H).

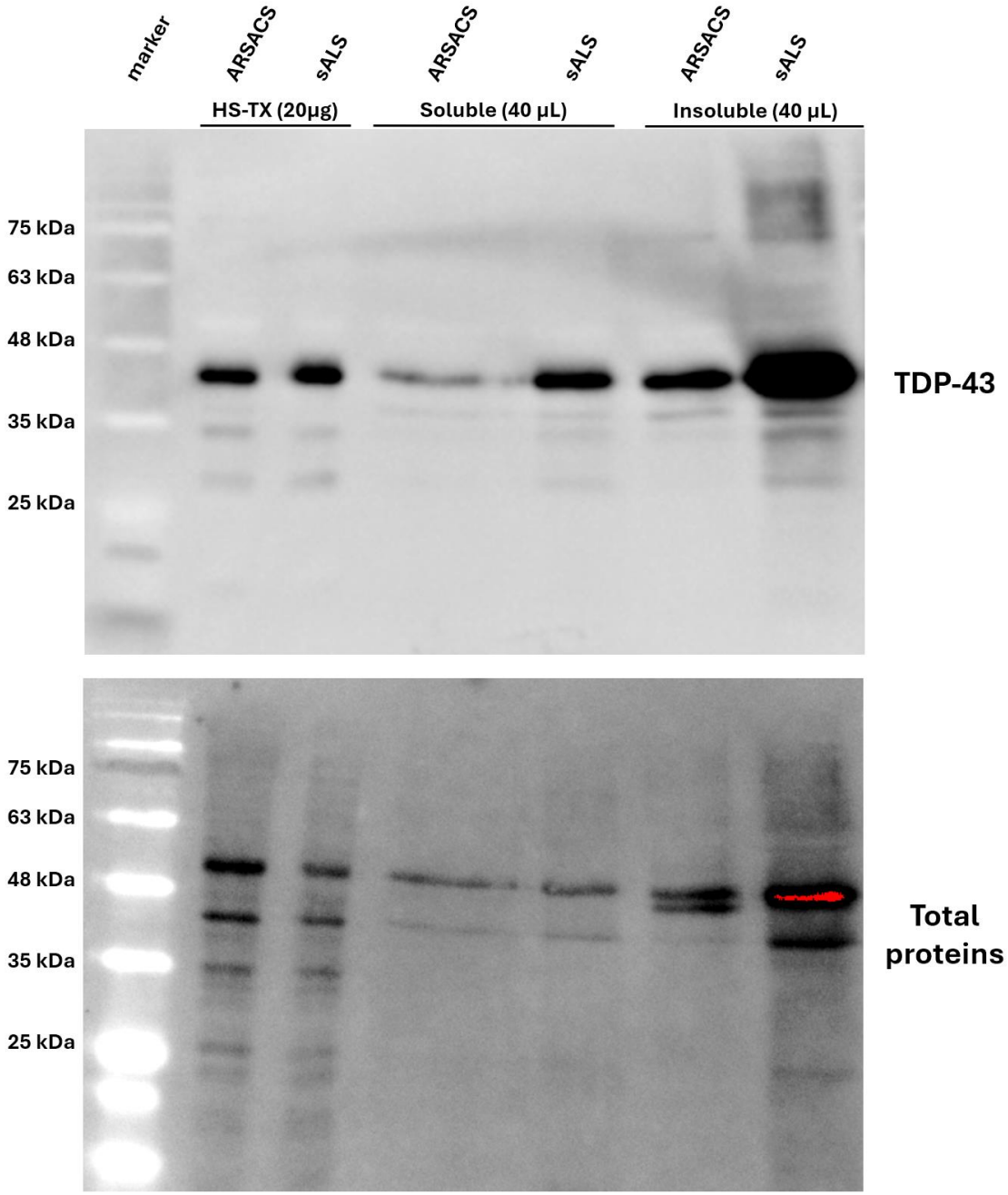


Figure S6. Biochemical assessment of TDP-43 aggregation by sarkosyl fractionation and Western blotting. Western blot showing TDP-43 levels in the high-salt buffer with Triton-X (HS-TX), the sarkosyl- soluble and insoluble fractions extracted from frozen brain cortex. Samples include an ARSACS patient and a sporadic ALS (sALS) patient. Total proteins in samples are displayed in the lower panel, with the red stains indicating signal saturation.