Supplementary information

Mouse monoclonal anti-FUS	Santa Cruz Biotechnology
Rabbit monoclonal anti-SFPQ	abcam
Rabbit polyclonal anti-Tubulin β-3	BioLegend
Mouse monoclonal anti-Tubulin β-3	BioLegend
Mouse monoclonal anti-β-actin	Sigma
Rabbit polyclonal anti-LAMP1	abcam
Rabbit polyclonal anti-LC3B	Sigma
Chicken polyclonal anti-MAP2	abcam
Rabbit monoclonal anti-EEA1	abcam
Rabbit polyclonal anti-Tau	DAKO
Rabbit polyclonal anti-4R tau	Cosmo Bio
Mouse monoclonal anti-3R tau	Sigma
Mouse monoclonal anti-phospho-tau pS202/T205	Thermo Fisher Scientific
Rabbit monoclonal anti-phospho-tau S404	abcam
Rabbit polyclonal anti-eIF2alpha	Cell Signaling
Rabbit polyclonal anti-phospho-eIF2alpha	Cell Signaling
Rabbit polyclonal anti-Galectin 1	abcam

Supplementary table 1. Details of antibodies used in this study.

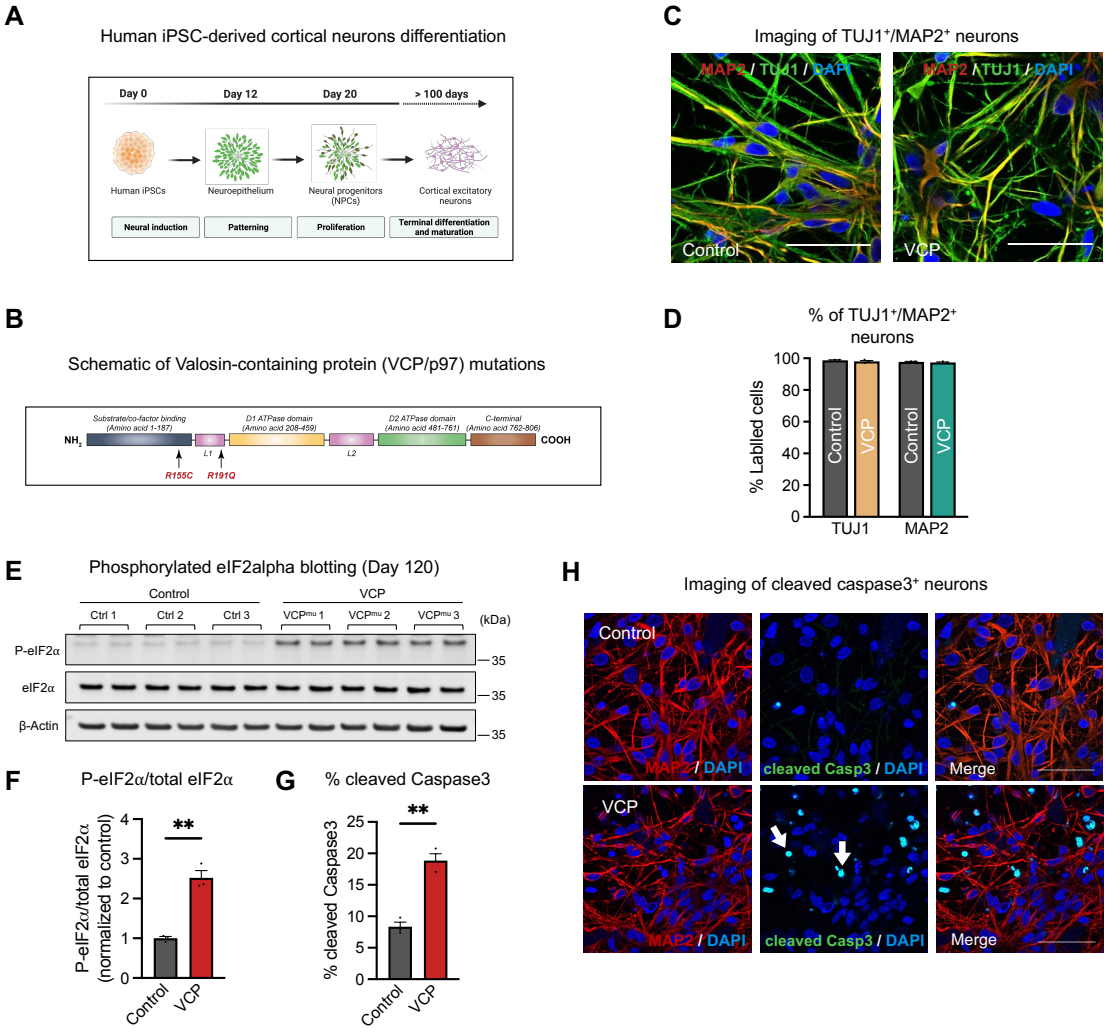


Figure S1. Increased apoptosis and ER stress in *VCP* mutant cortical neurons
(A) Schematic of hiPSC-derived cortical neurons differentiation.
(B) Schematic of the VCP mutations.
(C) Representative immunocytochemistry of neurons from control or carrying a *VCP* mutation (green, β3-tubulin; red, MAP2 and blue, DAPI). Scale bars, 50 µm.
(D) Analysis of TUJ1+ and MAP2+ neurons in control and *VCP* mutant neurons. (n = 3 control and 3 *VCP* mutant lines).
(E and F) Phosphorylate eIF2alpha levels were significantly higher in *VCP* mutant human cortical neurons. Representative western blots of phospho-eIF2alpha and total eIF2alpha are shown (E). Ratio of phospho-eIF2alpha to total eIF2alpha were calculated relative to control (F). (n = 3 control and 3 *VCP* mutant lines).
(G and H) A significant increase in the percentage of cleaved caspase 3+ cells in *VCP* mutant neurons compared to controls (G). Representative immunocytochemistry of hiPSC-derived neurons expressing cleaved caspase 3 (white arrows) (H) (red, MAP2; green, cleaved caspase 3; blue, DAPI). Scale bars, 50 µm. (n = 3 control and 3 *VCP* mutant lines).

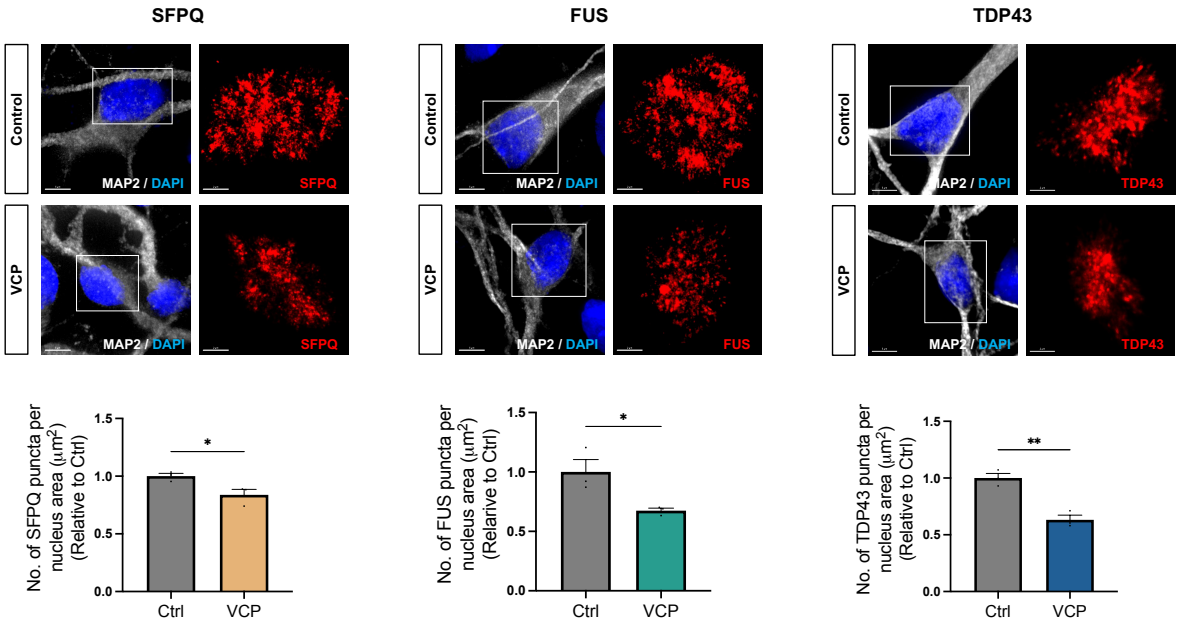


Figure S2. Nuclear loss of FUS, SFPQ and TDP-43 in *VCP* mutant cortical neuronal model of FTLD. Representative immunocytochemistry of hiPSC-derived cortical neurons expressing SFPQ (left), FUS (middle) and TDP43 (right) proteins (scale bars, 5 µm). A signification reduction in the no. of SFPQ (left), FUS (middle) and TDP43 (right) puncta per nuclear area. (n = 3 control and 3 *VCP* mutant lines).

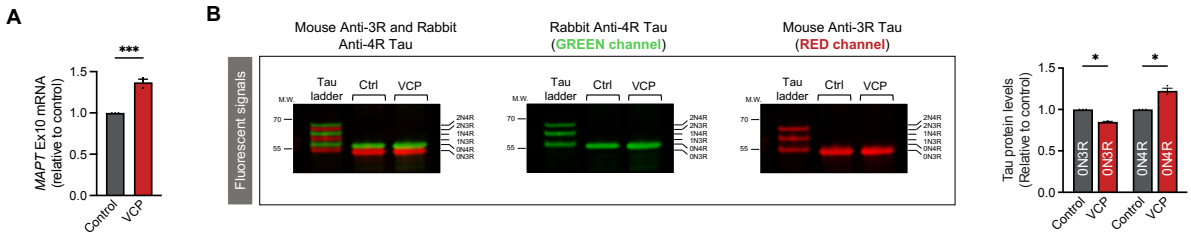


Figure S3. Increase in 4R tau levels in neurons carrying *VCP* mutations.
(A) RT-qPCR showing the relative abundance of *MAPT* mRNA containing the exon 10 (encoding for the additional microtubule-binding repeat).
(B) Tau isoforms with three (3R) or four (4R) microtubule-binding regions were detected by western blot analysis of dephosphorylated protein extracts from hiPSC-derived control and *VCP* mutant cortical neurons.

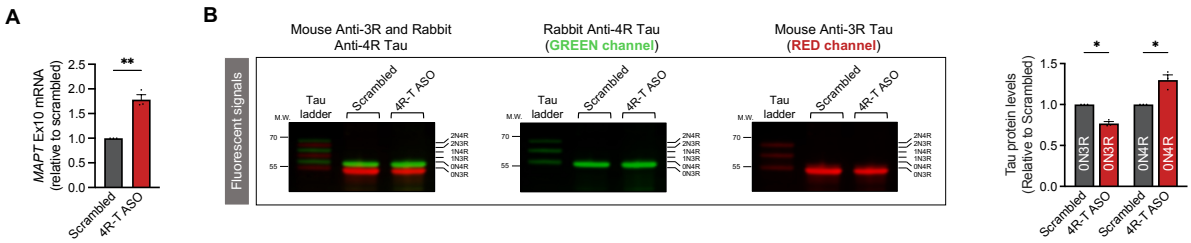
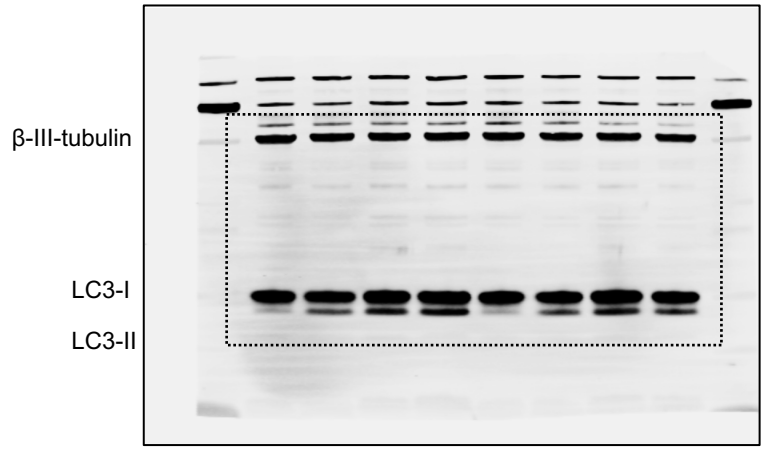


Figure S4. Increase in 4R tau levels in neurons treated with 3R to 4R *MAPT* splice-switching ASOs.

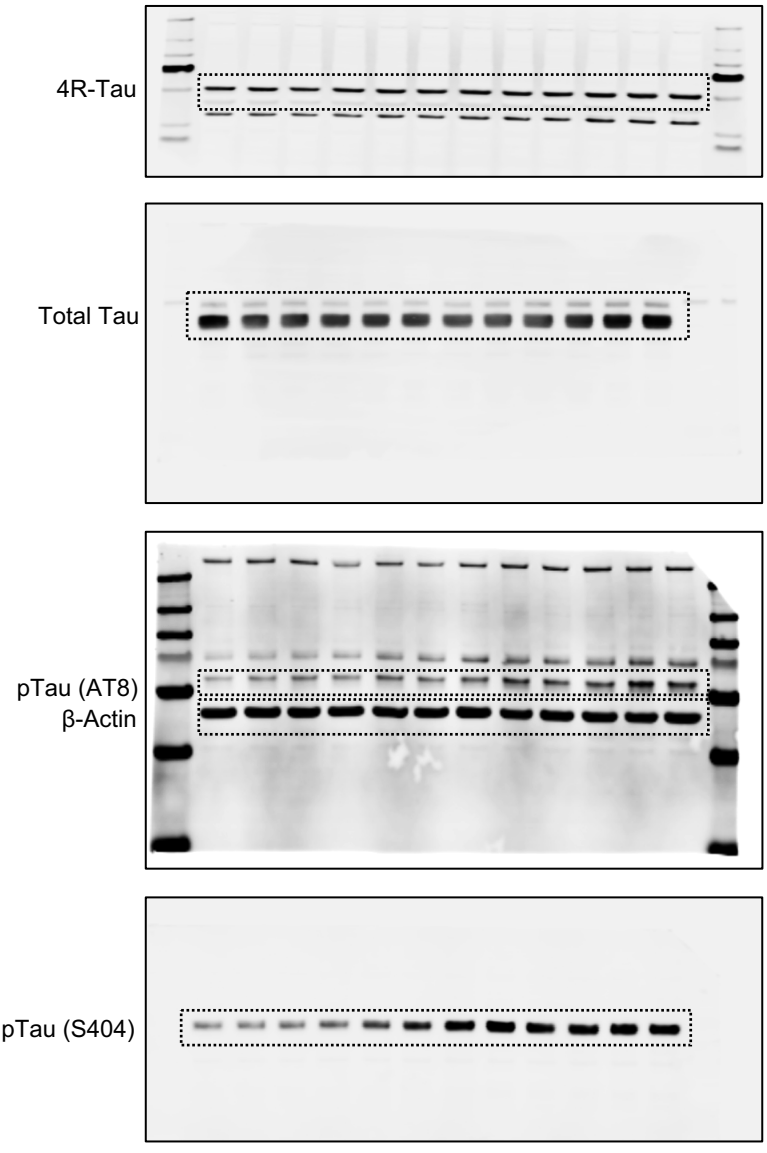
(A) RT-qPCR showing the relative abundance of *MAPT* mRNA containing the exon 10 (encoding for the additional microtubule-binding repeat).

(B) Tau isoforms with three (3R) or four (4R) microtubule-binding regions were detected by western blot analysis of dephosphorylated protein extracts from hiPSC-derived cortical neurons treated with scrambled ASOs or 4R-T ASOs.

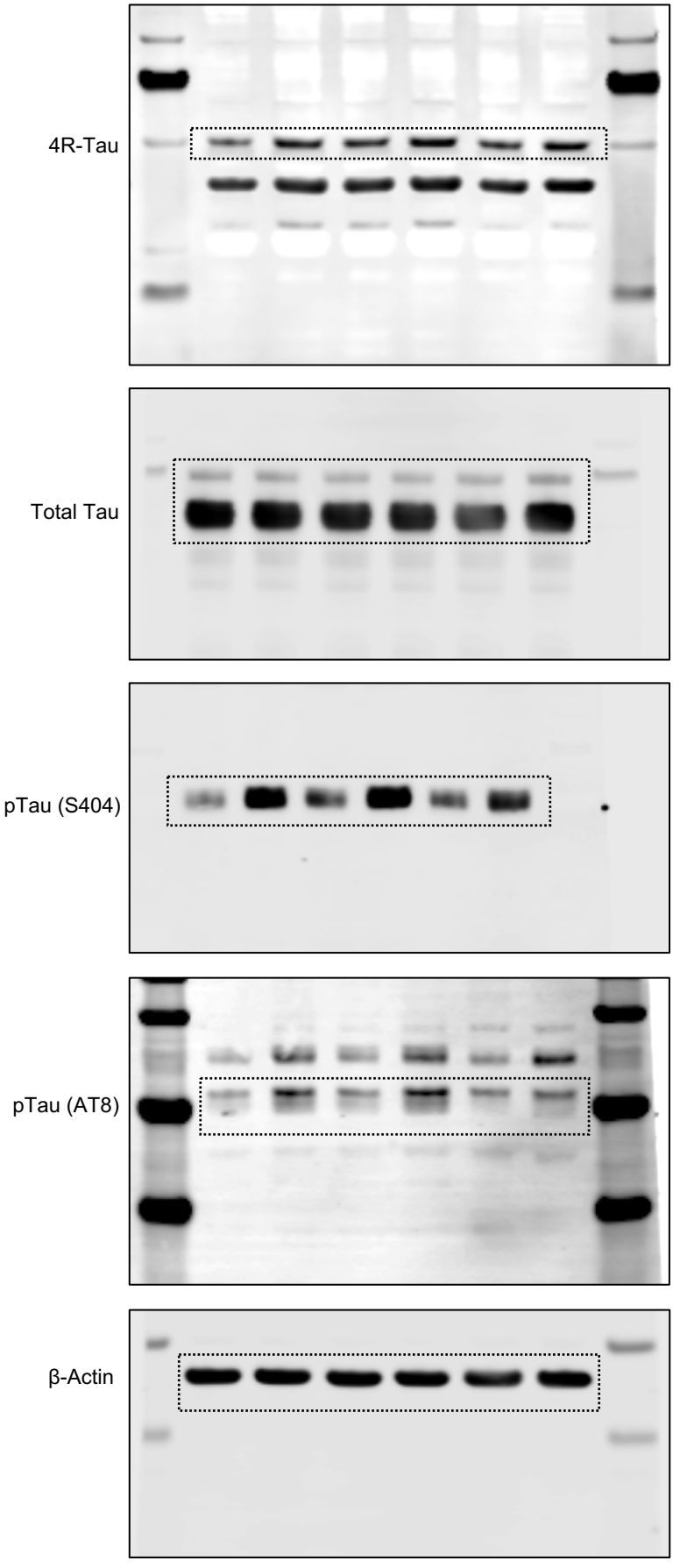
Original western blot for **Figure 1H**



Original western blots for **Figure 3A**



Original western blots for **Figure 4B**



Original western blots for **Figure 4E**

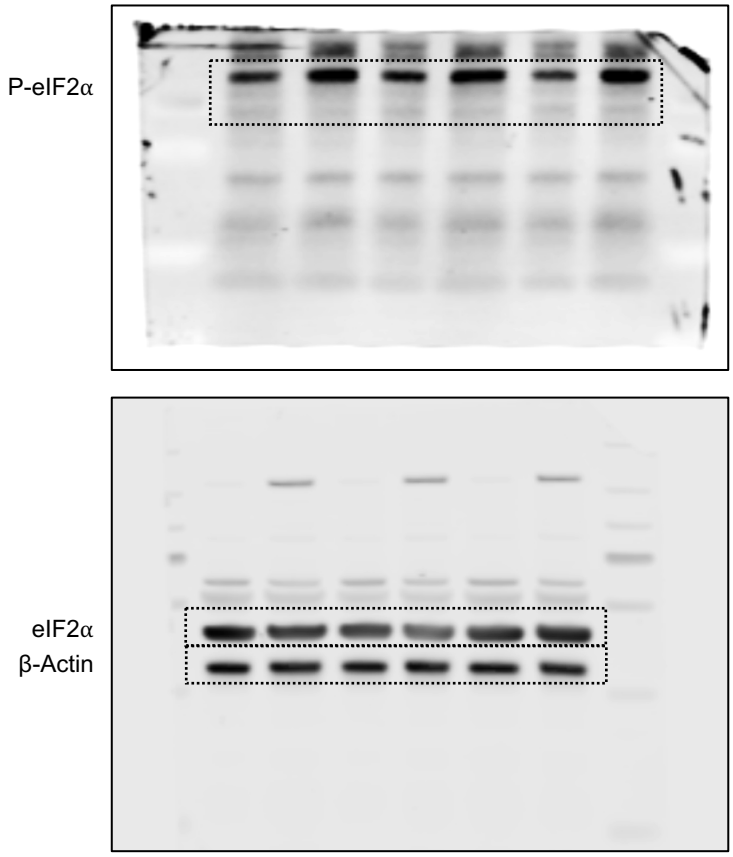


Figure S5. Full western immunoblots. Blots for each figure are indicated. Dashed lines indicate bands used as representative images.