Supporting Information

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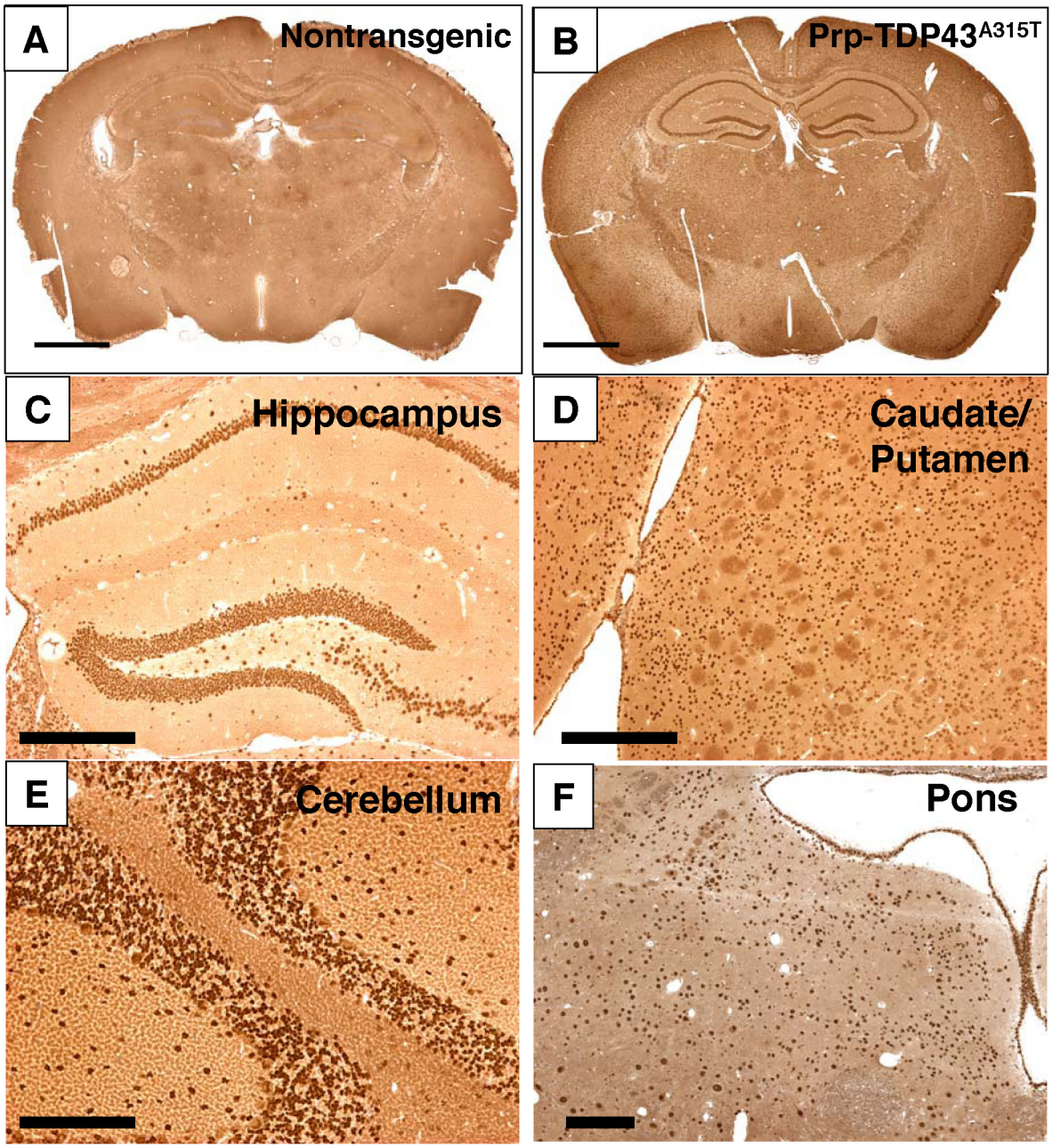


Fig. S1. Expression of the TDP43^{A315T} transgene in neurons and glia is present throughout the nervous system in Prp-TDP43^{A315T} mice. Immunohistochemistry with anti-Flag antibody, which is inserted at the N terminus of the human TDP43^{A315T} transgene. No staining was seen in nontransgenic littermate mouse brain (*A*), whereas nuclear staining was seen in neurons and non-neuronal cells in all brain regions in Prp-TDP43^{A315T} mice (*B–F*). This included all areas of hippocampus (*C*), caudate/putamen (*D*), cerebellum (*E*), and brainstem structures including the pons (*F*). (Scale bars for *A* and *B*, 1.25 mm; scale bars for *C–F*, 200 μm.)

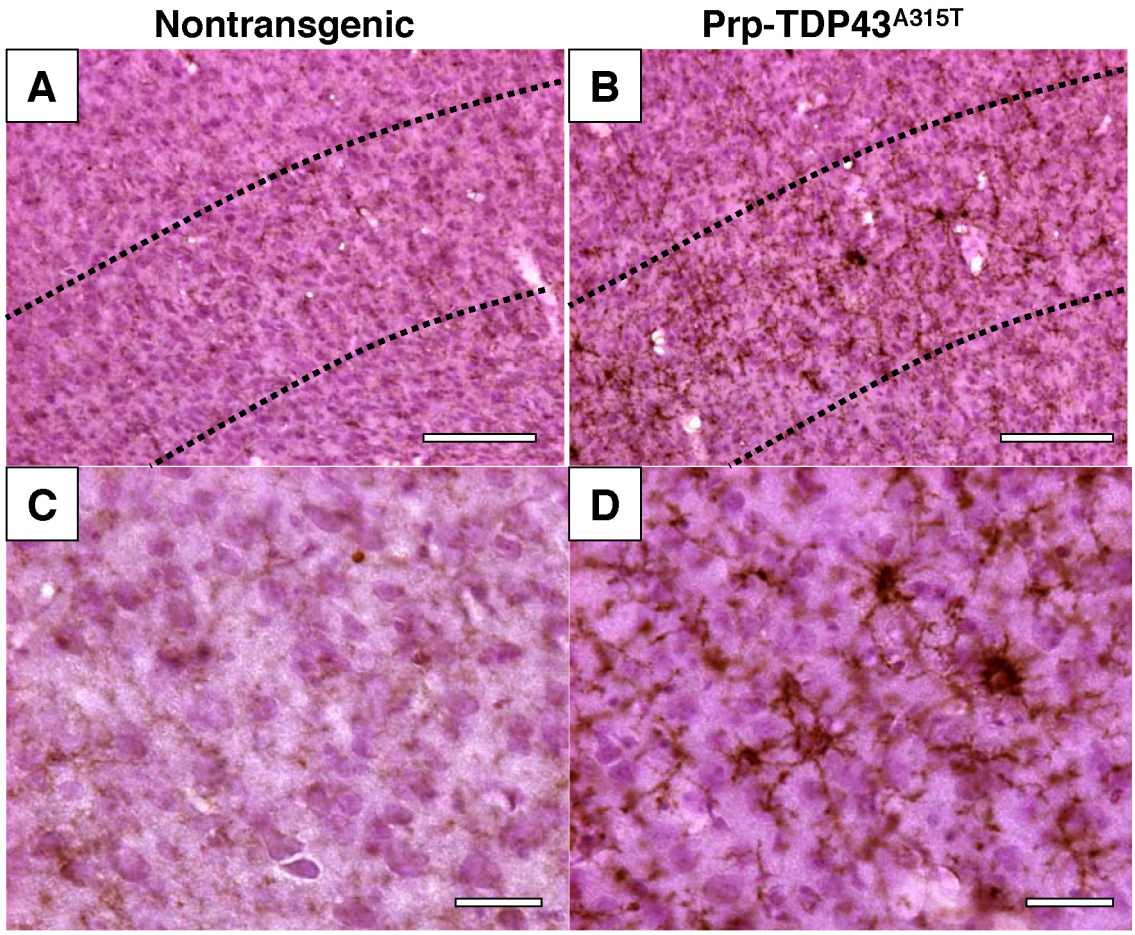


Fig. S2. Microglial activation in cortex of Prp-TDP43^{A315T} mice. CD11b immunostain of motor cortex from nontransgenic (*A* and *C*) and Prp-TDP43^{A315T} mice (*B* and *D*). Activated microglia were seen predominantly in layer 5 of cortex (demarcated by dashed lines in *A* and *B*). Higher magnification images (*C* and *D*) show large stellate activated microglia staining with CD11b selectively in Prp-TDP43^{A315T} cortex. (Scale bar for *A* and *B*, 100 μm; scale bar for *C* and *D*, 25 μm.)

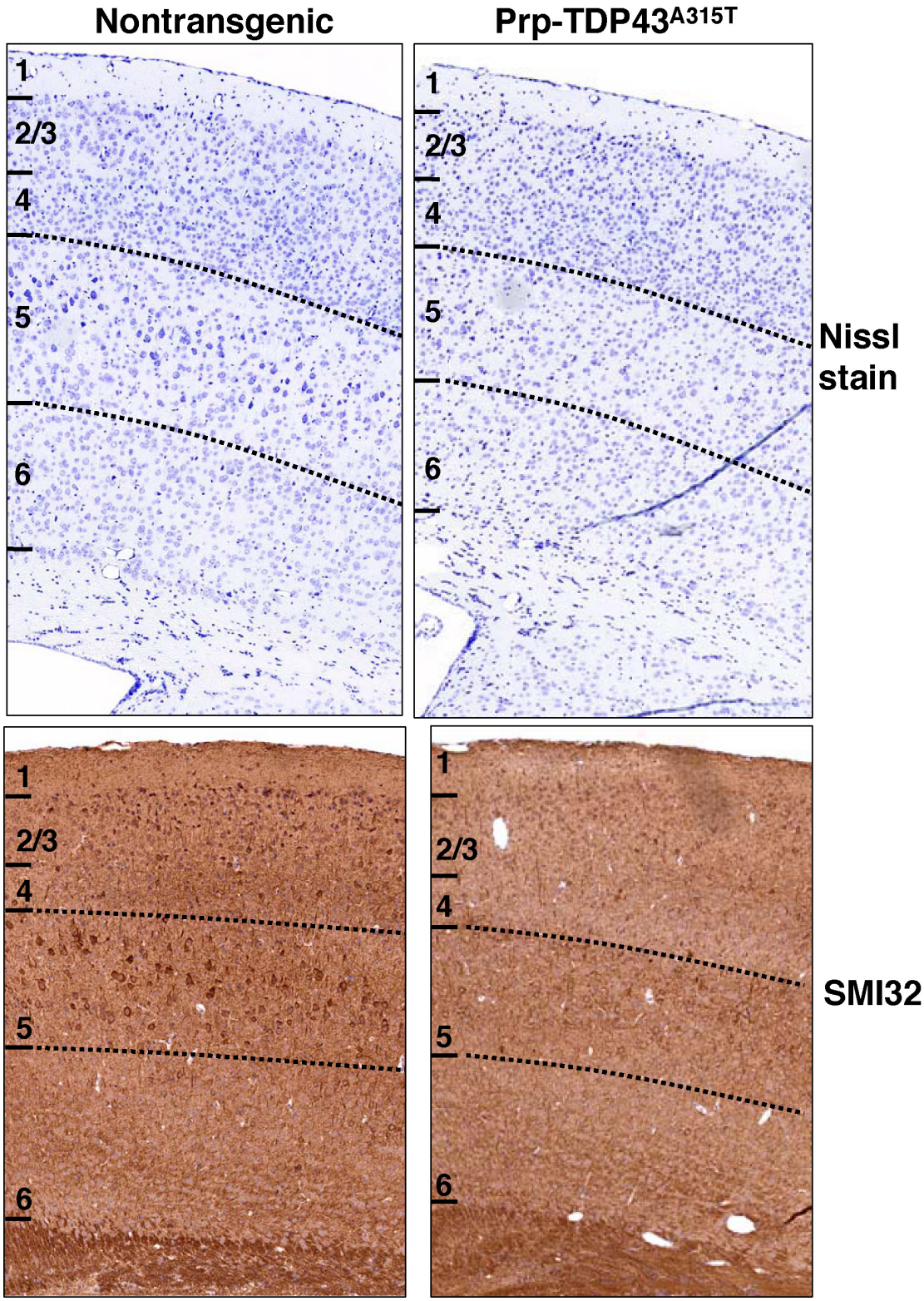


Fig. S3. Neuronal loss in cortex of Prp-TDP43^{A315T} mice. Brain sections from motor cortex are shown with Nissl stain (top panels) and SMI32 immunostaining (bottom panels) from a nontransgenic control and Prp-TDP43^{A315T} mouse. In nontransgenic littermates, numerous neurons with dark Nissl stain (Nissl bodies, corresponding to rough endoplasmic reticulum) are seen in layer 5, but are absent in end stage Prp-TDP43 ^{A315T} transgenic mice. A similar decrease was seen in the number of neurons visualized by immunohistochemistry with SMI32, a monoclonal antibody which recognizes unphosphorylated neurofilament, and preferentially labels layer 5 pyramidal neurons.

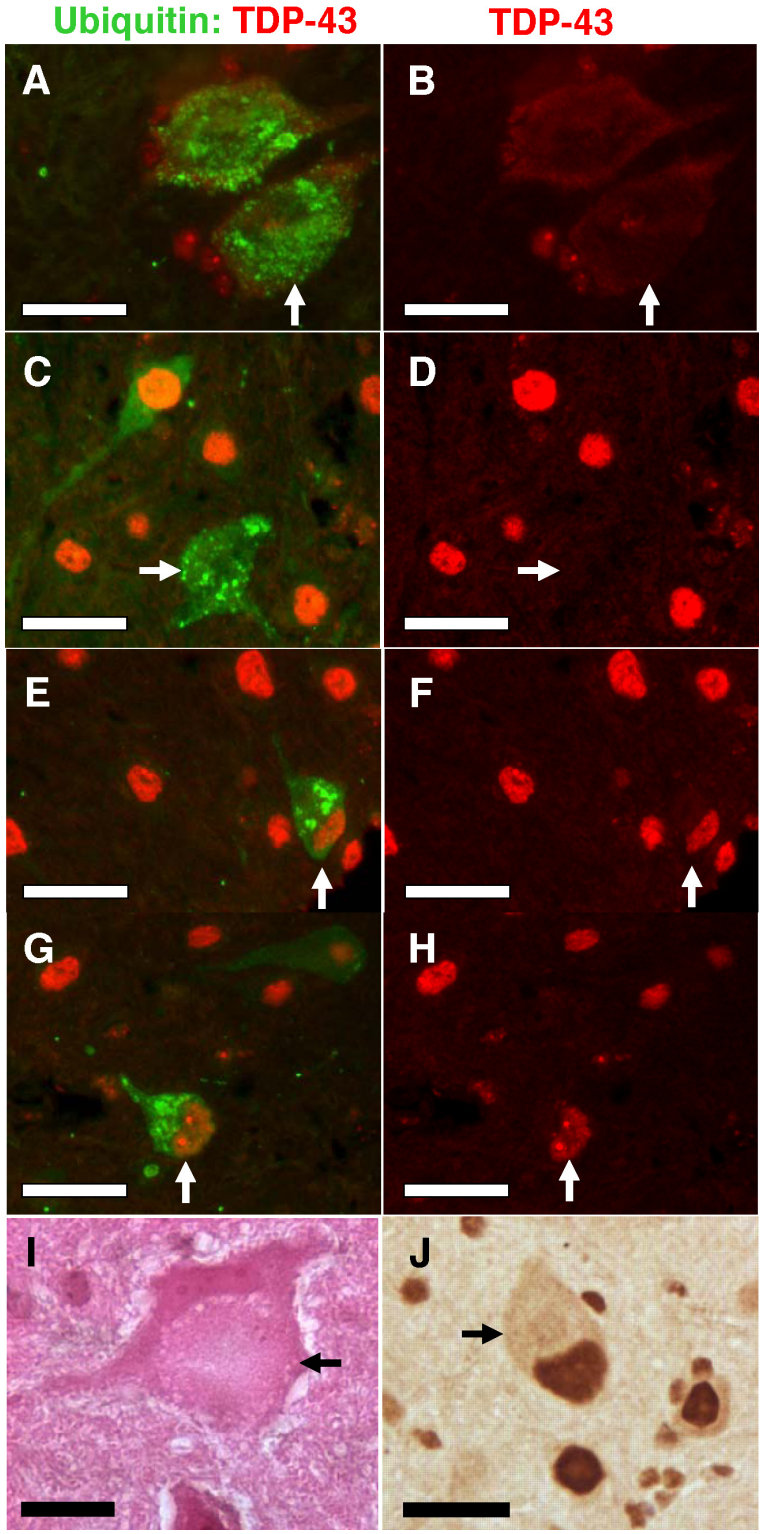
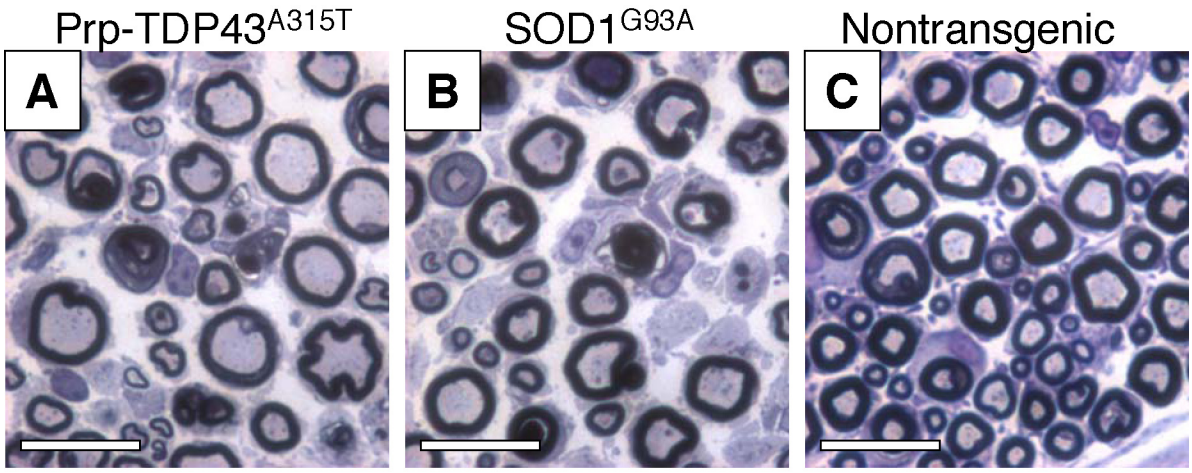


Fig. S4. Ubiquitin aggregate pathology in spinal cord ventral horn neurons. Overlay images of double immunofluorescence labeling of ubiquitin (green) and TDP-43 (red) (*A*, *C*, *E*, and *G*), or TDP-43 alone (*B*, *D*, *F*, and *H*). Some large ventral horn neurons with ubiquitin positive aggregates showed loss of nuclear TDP-43 staining, with occasional increase in diffuse cytoplasmic TDP-43 staining (*B*), but more commonly no cytoplasmic TDP-43 was detected (*D*). Other cells with ubiquitin aggregates appeared to have peripherally displaced nuclei with retained nuclear TDP-43 staining (*F* and *H*). White arrows point out the same neuron in both columns. (Scale bars, 20 μm.) (*I*) H&E stain of a large ventral horn neuron in the spinal cord showing an eccentrically displaced nucleus, with a large amorphous aggregate in the cell body (arrow). (Scale bar, 10 μm.) (*J*) TDP-43 immunostaining showing ventral horn neurons with a TDP-43 positive eccentric nucleus, and a TDP-43 negative aggregate (arrow) displacing it. (Scale bar, 20 μm.)



D

	Number of Axons	S.D.	p-value
Femoral Motor (n=4)			
Nontransgenic	563	19.9	<0.001
Prp-TDP43^{A315T}	472	15.7	
Femoral Sensory (n=3)			
Nontransgenic	698	59.6	N.S.
Prp-TDP43^{A315T}	663	47.7	

Fig. S5. Axon degeneration and loss in femoral motor nerves from Prp-TDP43^{A315T} mice. (*A–C*) Toluidine blue-stained plastic sections of femoral motor nerves from Prp-TDP43^{A315T} (*A*), SOD1^{G93A} (*B*), and a non-transgenic mouse (*C*) showing degenerating axonal profiles in Prp-TDP43^{A315T} mice. (Scale bar, 10 μm.) (*D*) Quantitation of intact motor axons showed significant loss of femoral motor axons in Prp-TDP43^{A315T} mice compared to non-transgenic controls.

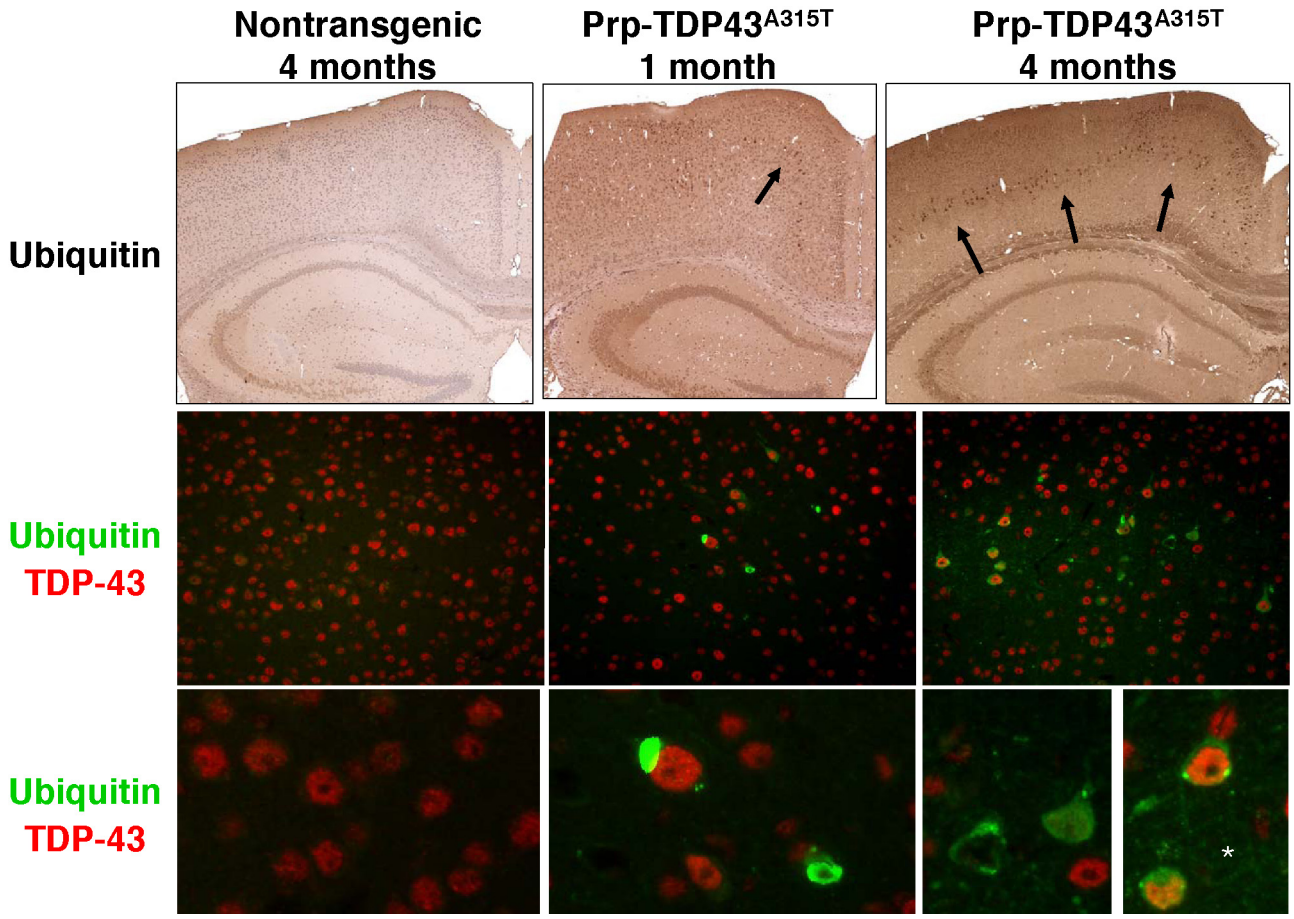
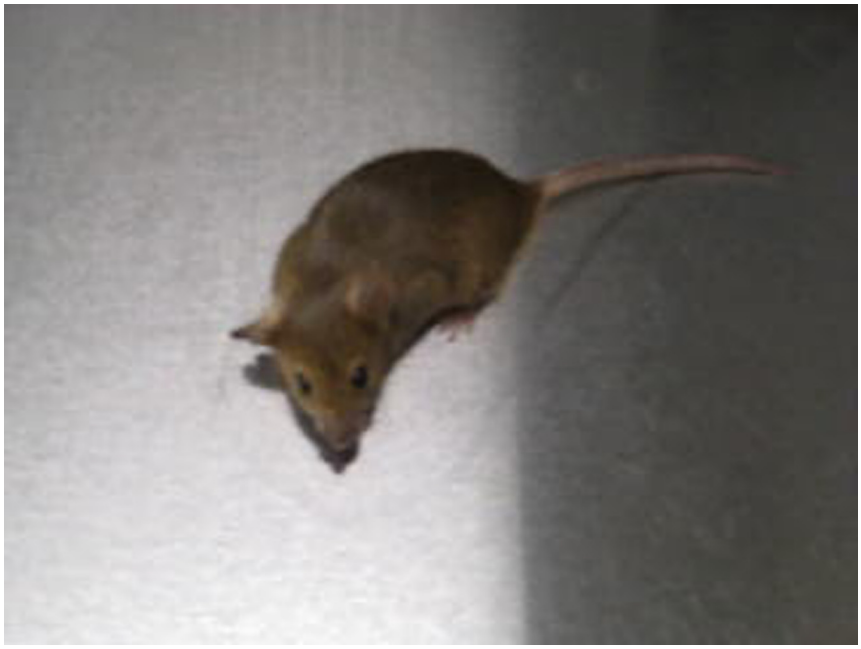


Fig. S6. Time course of ubiquitin aggregate pathology in Prp-TDP43^{A315T} mice. Top panels: ubiquitin immunohistochemistry of brain sections from Prp-TDP43^{A315T} mice at 1 month of age (presymptomatic) and at 4 months (late stage). At 1 month of age rare neurons are seen (here in retrosplenial cortex) with ubiquitin aggregate pathology, in contrast to the widespread pathology in layer 5 seen in late stage animals. Middle and bottom panels: double immunofluorescence for ubiquitin (green) and TDP-43 (red). Although ubiquitin aggregates could occasionally be found in 1-month-old animals, no loss of nuclear TDP-43 staining was seen. In contrast, ubiquitin aggregate pathology was widespread in cortex in late stage animals, with more frequent loss of nuclear TDP-43 (arrows). Note also the increase in ubiquitin staining seen in neuronal processes throughout the neuropil of layer 5 (asterisk in bottom right panel).



Movie S1. Four-month-old Prp-TDP43^{A315T} mouse, showing typical early appearance of gait difficulty.

Movie S1 (AVI)

Movie S2. Characteristic ''swimming'' gait seen in a late stage 5-month-old Prp-TDP43^{A315T} mouse. The mouse is unable to stand with its abdomen off the ground, and slides along the surface using its limbs in a ''flipper'' like fashion to ineffectively propel itself.

Movie S2 (AVI)

Table S1. Regional distribution of ubiquitin aggregate pathology in the nervous system of late stage Prp-TDP43^{A315T} mice

Region	Ubiquitin cytoplasmic inclusions
Cortex (predominantly layer V)	
Orbital, frontal association, motor, cingulate, insular, sensory, retrosplenial, visual, auditory	+++
Pyriform, ectorhinal, entorhinal	-
Hippocampus	
Polymorphic dentate gyrus, subiculum	+++
Granular cell layer (CA1, CA2, CA3)	-
Basal ganglia	
Caudate/putamen, thalamus	-
Cerebellum	
Deep nuclei	+
Purkinje cells, molecular layer, granular cells	-
Brainstem	
Midbrain: red nucleus	+
Pons: vestibular nuclei	+
Medulla: hypoglossal nucleus, reticular nuclei	+
Spinal cord	
Ventral horn	++
Dorsal horn	-
Dorsal root ganglia	-