Expression of ALS-linked TDP-43 Mutant in Astrocytes Causes Non-cell-autonomous Motor Neuron Death in Rats

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(Supplementary Data)

Video S1: A GFAP-tTA$^{\#}$2/TRE-TDP43^{M337V} double transgenic rat was paralyzed in two hind legs at age of 70 days. A GFAP-tTA$^{\#}$2 single transgenic rat behaved normally at matched age.

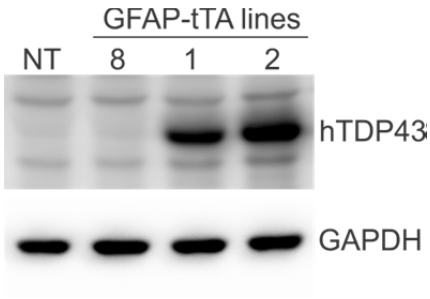

Figure S1: Establishment of GFAP-tTA transgenic rat lines. Immunoblotting reveals that two of the three GFAP-tTA transgenic rat lines (line-1 and -2, but not -8) express mutant human TDP-43 (hTDP-43) in GFAP-tTA/TRE-TDP43^{M337V} double transgenic background. Human TDP-43 is undetectable in nontransgenic control (NT). Each lane was loaded with 20 μg of total proteins extracted from rat spinal cord. Equal loading is confirmed by probing the same blotting membrane with an antibody against GAPDH.

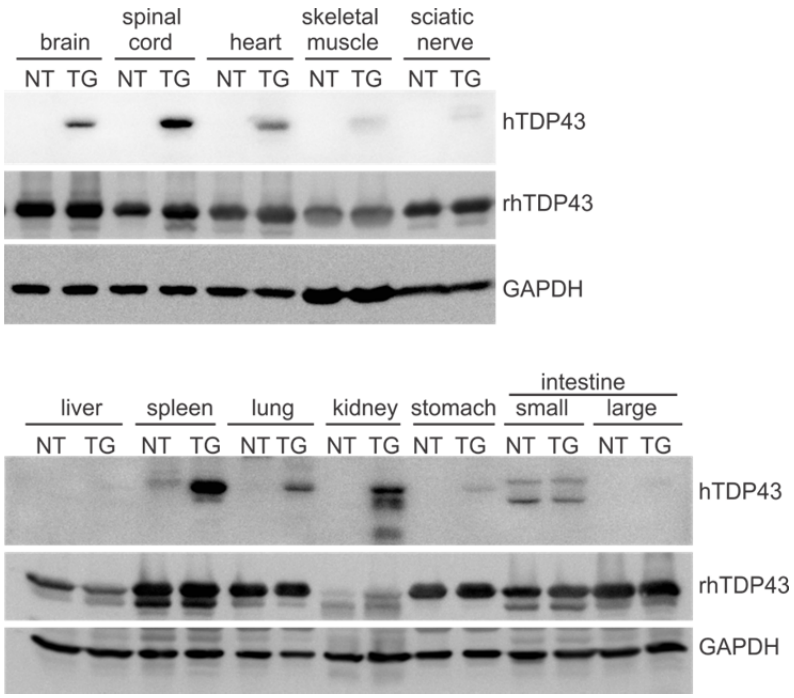

Figure S2: Expression of mutant human TDP-43 in GFAP-tTA$^{\#}$2 transgenic line. Immunoblotting revealed the profile of human TDP-43 (hTDP43) expression in GFAP-tTA/TRE-TDP43^{M337V} double transgenic rats (TG). While total TDP-43 (including rat and human TDP-43: rhTDP43) was detected in both the TG and nontransgenic (NT) rats, hTDP43 was detected only in the TG rats and not in the NT rats. The same blotting membranes were detected sequentially with antibodies recognizing hTDP43, rhTDP43, and GAPDH after stringent stripping. Each lane was loaded with 20 μg of total proteins.

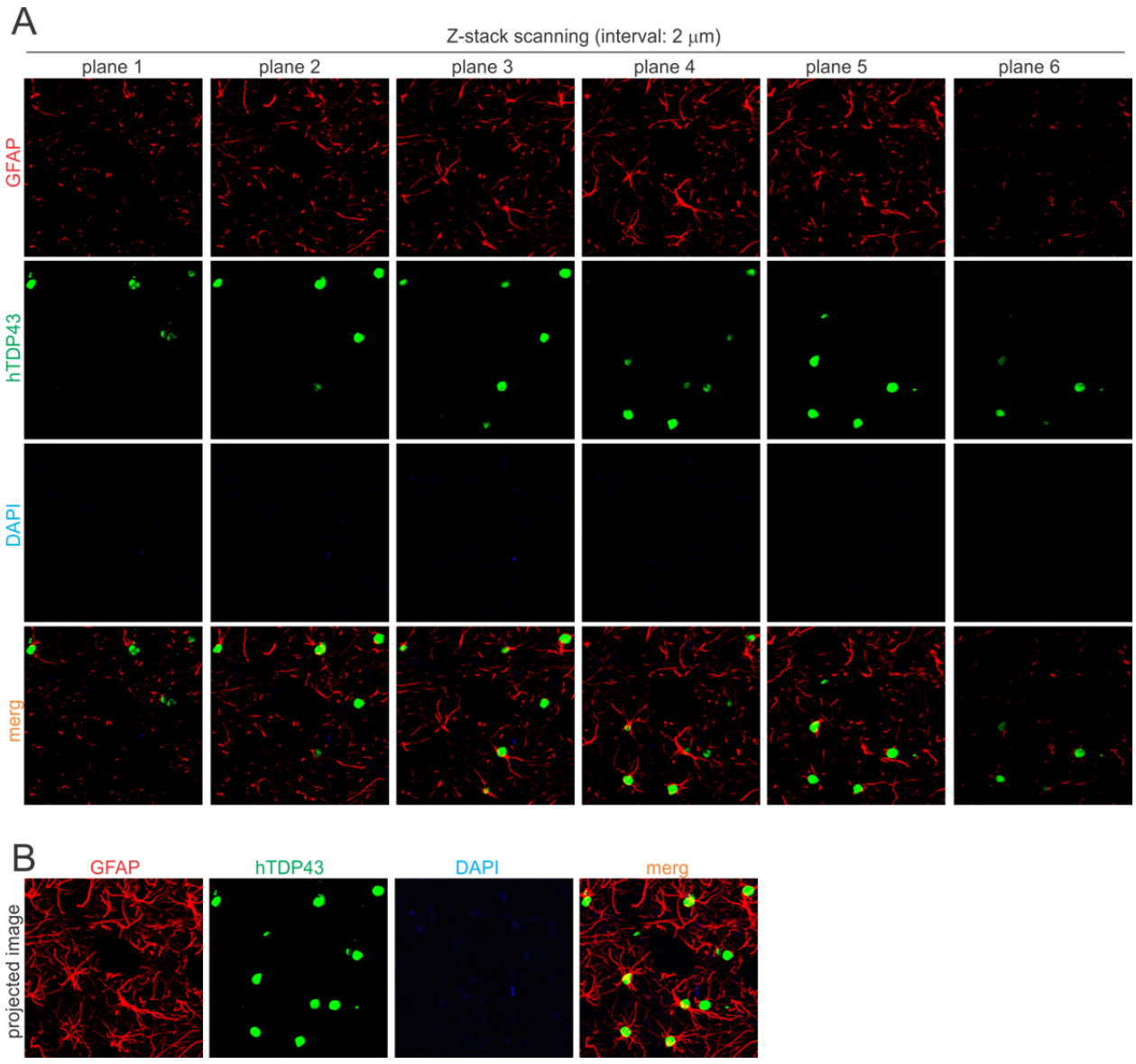

Figure S3: Localization of GFAP and human TDP-43 in astrocytes. (**A**) A series of Z-stack scanning with a confocal microscope shows the detailed localization of human TDP-43 (hTDP43) and the astrocyte marker GFAP in the ventral spinal cord horn of GFAP-tTA/TRE-TDP43^{M337V} double transgenic rats. (**B**) Images projected from the Z-stack scanning (shown in A) reconstruct hTDP43 localization related to the nuclei (stained with DAPI) and the processes (stained with GFAP) of astrocytes in rat's spinal cord. Panel B is also shown in Figure 1 (G-I).

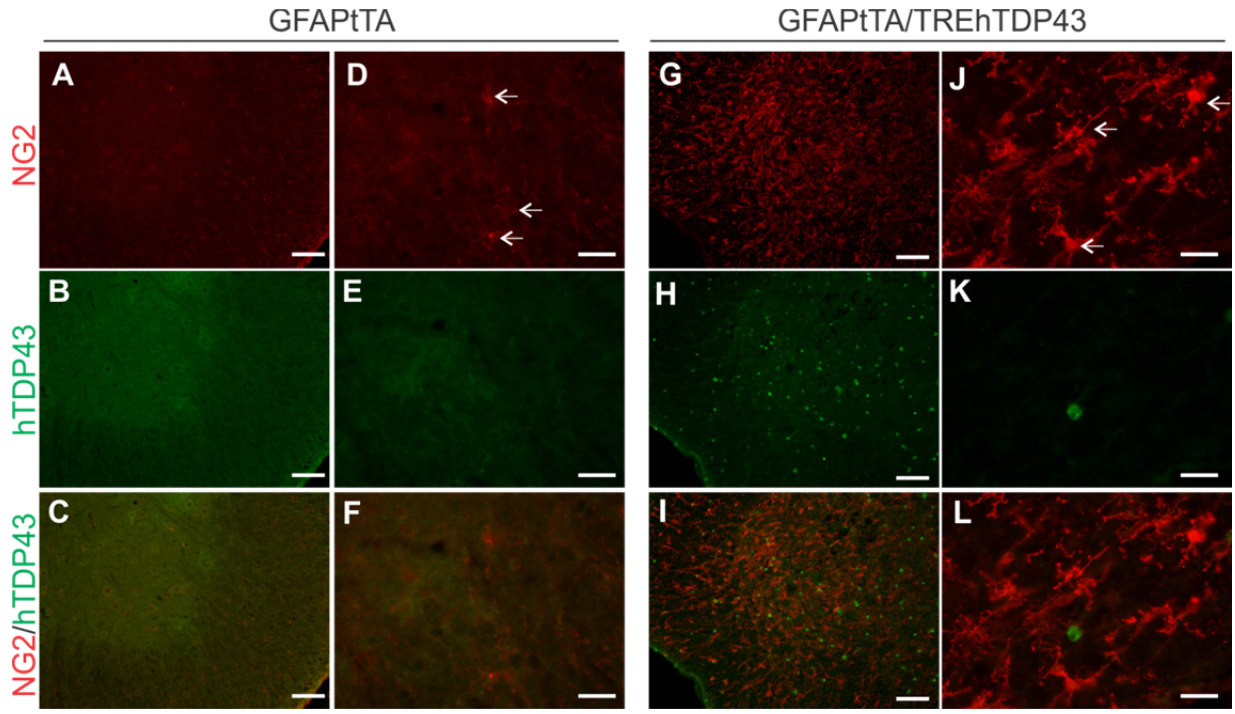

Figure S4: Localization of NG2 and human TDP-43 in glial cells. (**A-L**) Double-labeling fluorescence staining reveals the localization of mutant human TDP-43 (hTDP43) and the glial marker NG2 in rat's spinal cords. NG2 expression was upregulated in the GFAP-tTA/TRE-TDP43 double transgenic rats. Arrows point to the cells stained for NG2 but not for hTDP43. Scale bars: 100 μm (A-C, G-I, and M) and 30 μm (D-F and J-L).

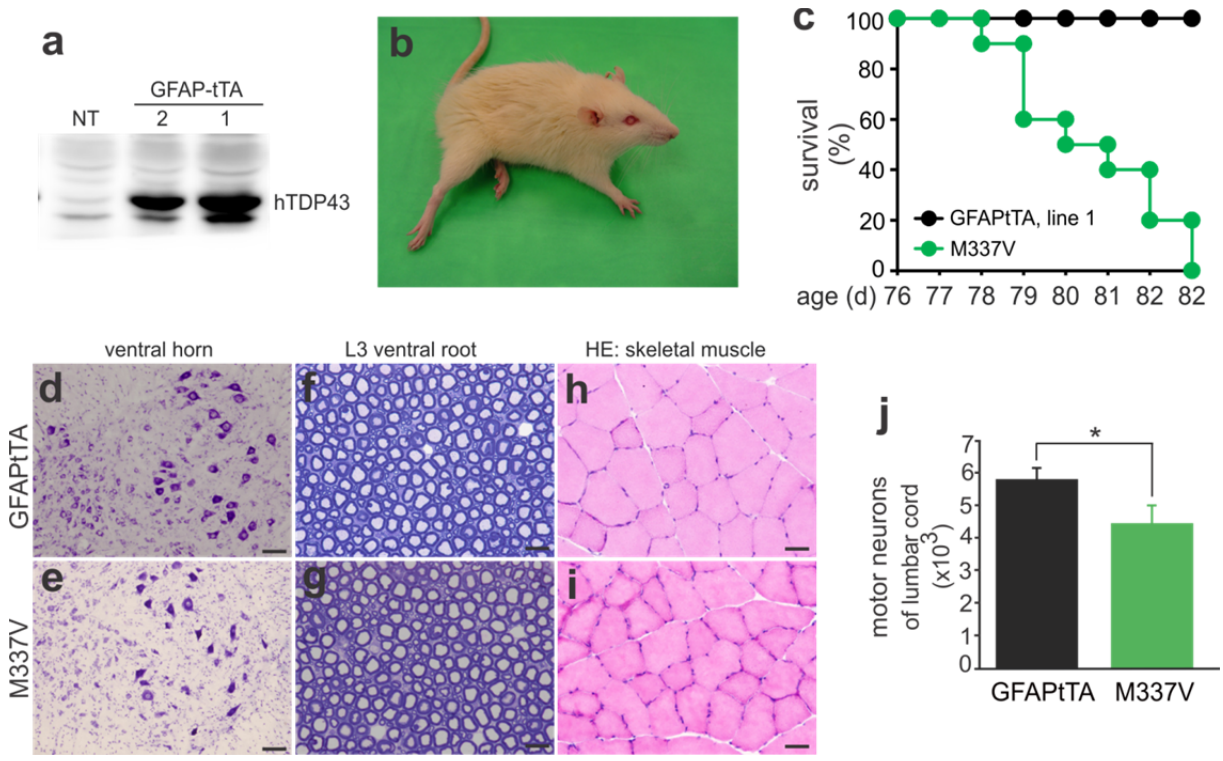

Figure S5: Astrocytic TDP-43^{M337V} expression causes motor neuron death and paralysis in GFAP-tTA$^{\#}$1 transgenic line. (**A**) Immunoblotting detected the expression of mutant human TDP-43 (hTDP43) in the medial septum of GFAP-tTA/TRE-TDP43^{M337V} double transgenic rats (GFAP-tTA line #1 and #2), but detected no hTDP43 expression in a nontransgenic rat (NT). Transgenic rats were deprived of Dox at 40 days of age to allow for hTDP43 expression in the astrocytes. (**B**) A representative photo showing that a mutant rat was paralyzed at the age of 80 days. (**C**) A graph shows the probability of mortality in GFAP-tTA$^{\#}$1 single (GFAPtTA: line-1) and GFAP-tTA/TRE-TDP43^{M337V} double (M337V) transgenic rats. Mutant rats were counted death when they were unable to right themselves within 30 seconds after placing on their sides. Fifteen rats of each genotype were assessed of mortality. (**D, E**) Cresyl violet staining reveals motor neurons in the ventral horn of rat's spinal cord. M337V rats were killed at paralysis stages and the control GFAPtTA rats were killed at matched ages. (**F, G**) Toluidine blue staining reveals the structure of motor axons in mutant rats at paralysis stages. (**H, I**) H&E staining reveals the structure of skeletal muscles. Scale bars: 100 μm (D, E), 20 μm (F, G) and 40 μm (H, I). (**J**) Stereological cell counting reveals that motor neurons in the L3-L5 lumbar cords were significantly lost in M337V rats at paralysis stages. Data are means ± SEM (n = 6). * $p < 0.05$.

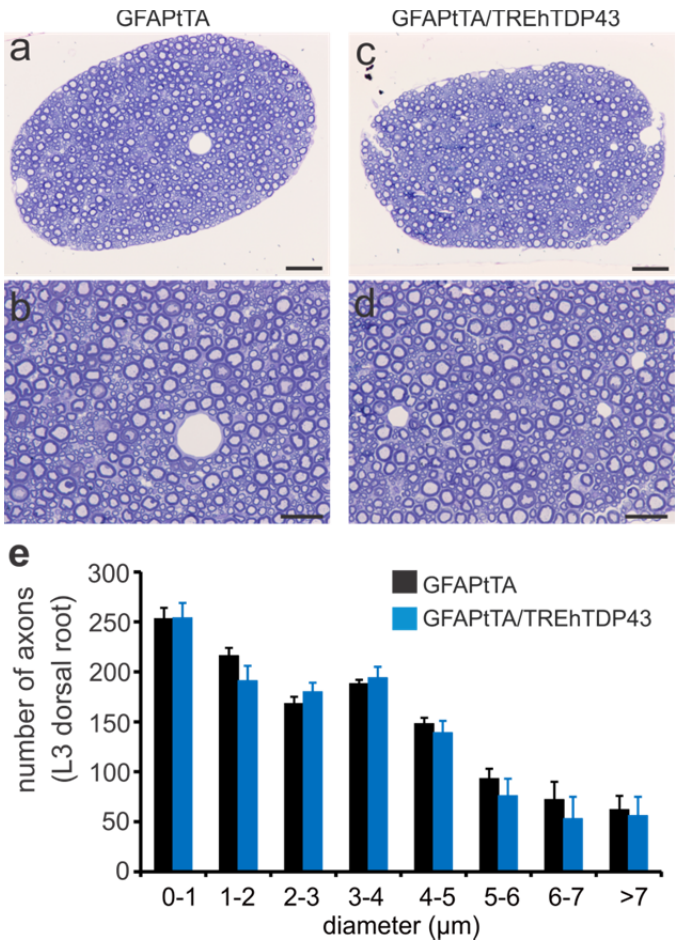

Figure S6: Effects of astrocytic TDP43^{M337V} expression on sensory neuron axons in the dorsal roots. (**A-D**) Toluidine blue staining revealed the structure of ventral roots in the GFAP-tTA$^{\#}$2/TRE-TDP43^{M337V} double transgenic rats with paralysis and in the GFAP-tTA single transgenic rats at matched ages. Scale bars: 50 μm (A, C) and 30 μm (B, D). (**E**) The distribution of sensory axons is determined with Image J on the cross sections of L3 dorsal roots. Data are means ± SD (n = 3 rats).

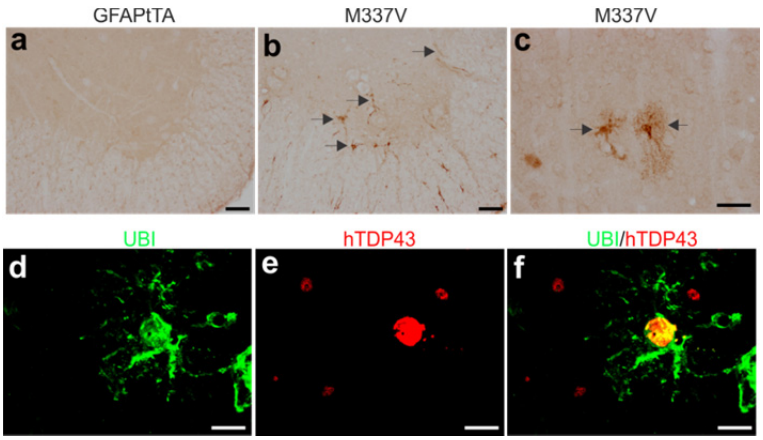

Figure S7: Astrocytic TDP43^{M337V} overexpression leads to ubiquitin accumulation in astrocytes. (**A-C**) Immunohistochemistry reveals that ubiquitin was accumulated in the GFAP-tTA$^{\#}$2/TRE-TDP43^{M337V} double transgenic rats (M337V) at paralysis stages, but not in the GFAP-tTA single transgenic rats at matched ages. (**D-F**) Double-labeling fluorescence staining reveals that ubiquitin (UBI) was accumulated in the cells expressing mutant human TDP-43 (hTDP43). Scale bars: 100 μm (A, B), 40 μm (C) and 25 μm (D-F).

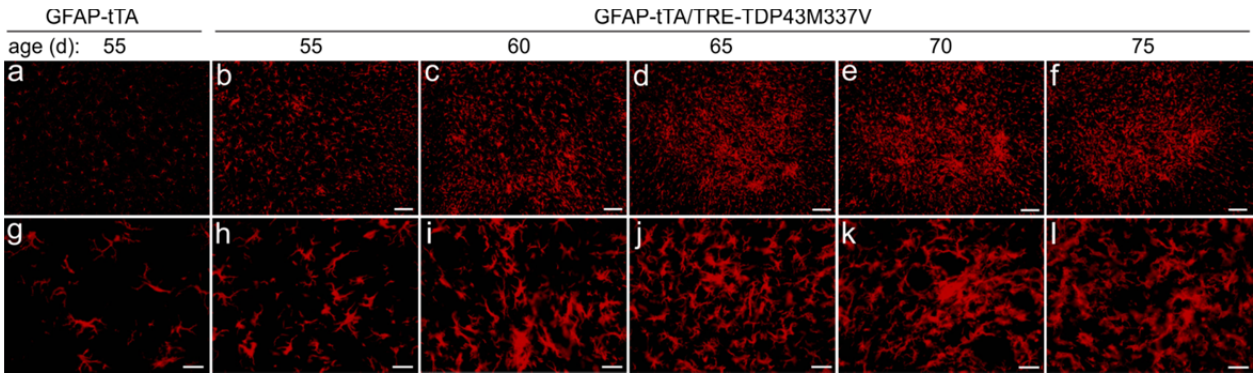

Figure S8: Astrocytic TDP43^{M337V} expression leads to microglia activation. (**A-L**) Micrographs of low (A-F) and high (G-L) magnification show that microglia was gradually activated in the GFAP-tTA$^{\#}$2/TRE-TDP43^{M337V} double transgenic rats compared to GFAP-tTA$^{\#}$2 transgenic rats. The ventral horns of lumbar spinal cords were examined of the microglia marker Iba-1. Rats were deprived of Dox at 40 days of age to allow for TDP43^{M337V} transgene expression. Scale bars: 100 μm (A-F) and 30 μm (G-L).