Supporting Information

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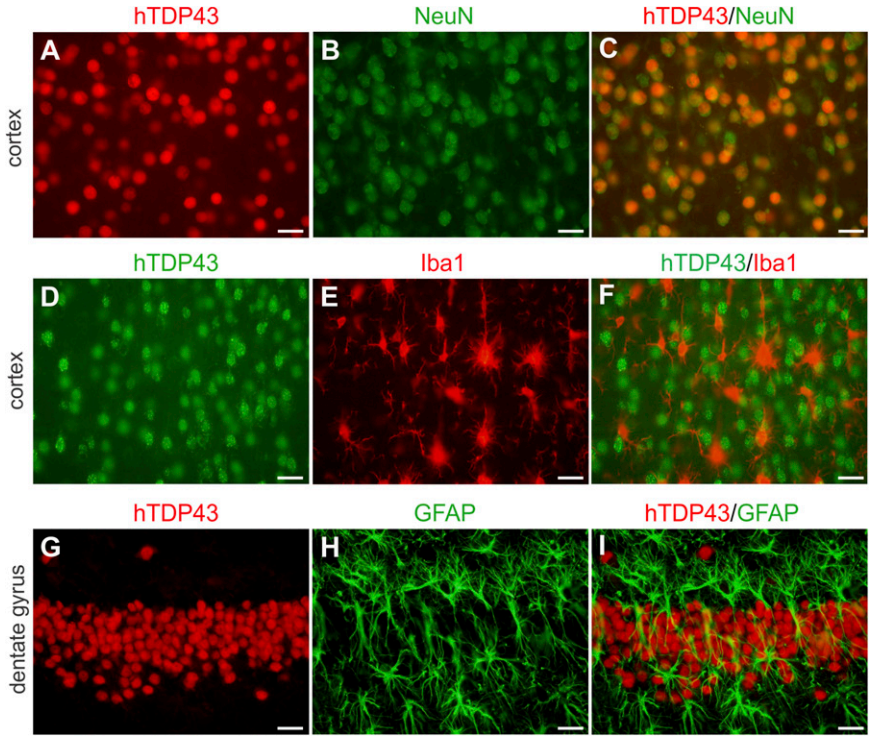

Fig. S1. Expression of mutant human TAR DNA-binding protein 43 (TDP-43) is restricted in neurons in the forebrain of transgenic rats. (*A–I*) Double-labeling fluorescence staining revealed that human TDP-43 with M337V substitution (hTDP43) colocalized with the neuronal marker NeuN (*A–C*) and did not colocalize with the microglial marker ionized calcium binding adaptor molecule 1 (Iba1) (*D–F*) or the astrocyte marker glial acid fibrillary protein (GFAP) (*G–I*). Tissues were taken from double-transgenic rats carrying both calcium/calmodulin-dependent protein kinase II α (Camk2α)-tetracycline responsive transactivator (tTA) and tetracycline-responsive element-TDP43^{M337V} transgenes. The transgenic rats were continuously given doxycycline (Dox) in drinking water during embryonic and postnatal development and were permanently deprived of Dox by the age of 35 d. Expression of human TAR DNA-binding protein 43 was examined in transgenic rats at the age of 55 d. (Scale bar: 20 μm.)

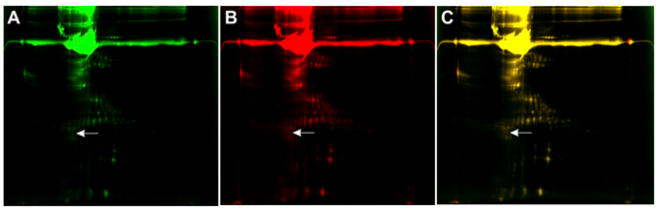

Fig. S2. Lipocalin 2 (lcn2) secretion is increased in the culture medium of brain slice from mutant TAR DNA-binding protein 43 (TDP-43) transgenic rats. (*A*–*C*) Two-dimensional gel and MS analyses revealed that the secretion of lcn2 from cultured brain slices was increased when mutant human TDP-43 was expressed in the neurons. The forebrains were dissected from P3 (postnatal day 3) male rats carrying Camk2α-tTA single or Camk2α-tTA/tetracycline-responsive element (TRE)-TDP43^{M337V} double transgenes. Cy2 (*A*, green) labeled the proteins secreted by the brain slices of Camk2α-tTA single-transgenic rats and Cy3 (*B*, red) labeled the proteins secreted by the brain slices of Camk2α-tTA/TRE-TDP43^{M337V} double-transgenic rats. (*C*) Merged data from (*A* and *B*). Rat's forebrains were cut into slices at 300-μm intervals. The brain slices were cultured for 4 d in the presence of doxycycline (Dox) to allow for recovery from slicing and then were cultured in the medium without Dox to allow for expression of mutant human TDP-43 transgene in neurons. By 8 d in culture, rat's brain slices were cultured in serum-free medium for 24 h and the culture medium was collected for analysis of secreted proteins by 2D gel and MS. Total proteins in the culture medium were precipitated and were labeled with cyanine dye (Cy) 2 or Cy3. Equal amounts of proteins from two cultures were mixed after fluorescence labeling and were analyzed on 2D gel. Protein spots with increased intensity in TDP-43 transgenic culture were dissected from the gel and further analyzed of composition by MS. (Arrows) The spot for the secretary protein lcn2.

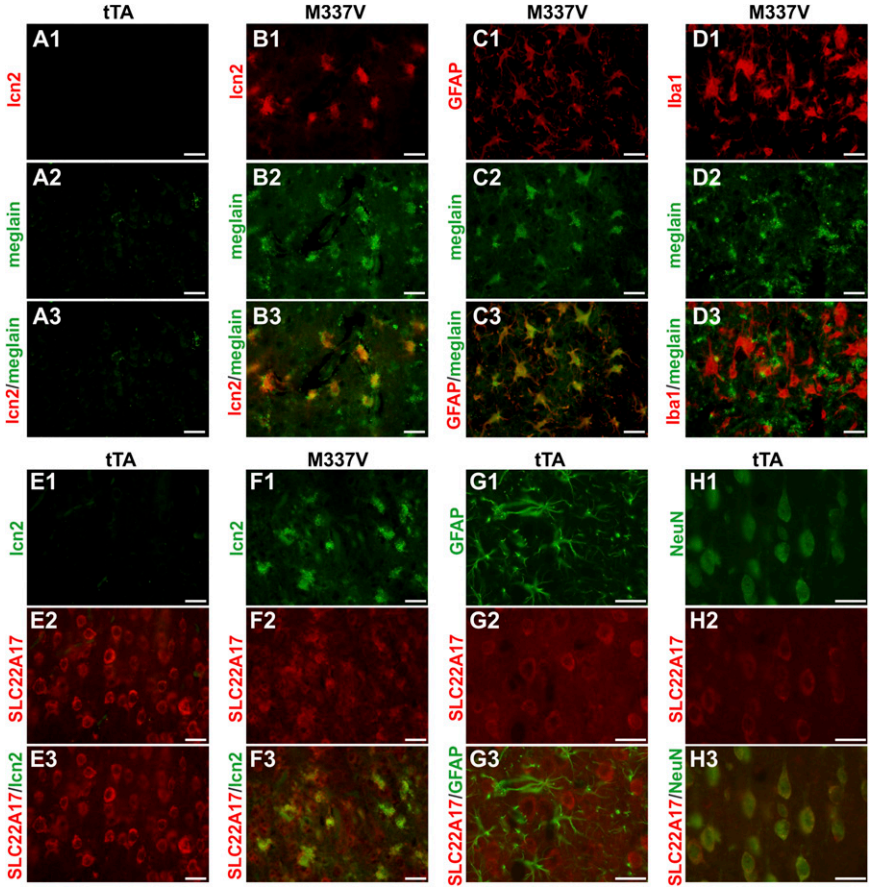

Fig. S3. Neurons and astrocytes express varying types of lipocalin 2 (lcn2) receptors. (*A*–*D*) Immunofluorescent staining revealed that megalin (a receptor of lcn2) was mainly expressed in reactive astrocytes in rat's brain. Coronal sections were cut from the forebrains of Camk2α-tetracycline-responsive tTA or Camk2α-tTA/tetracycline-responsive element–TAR DNA-binding protein 43 double (M337V)-transgenic rats at the age of 55 d. (*E*–*H*) Immunofluorescent staining revealed that solute carrier family 22 member 17 (SLC22A17) (another receptor of lcn2) was mainly expressed in normal neurons of WT rats (G1-G3 and H1-H3) but was also expressed in reactive astrocytes (F1-F3). (Scale bar: 40 μm.)

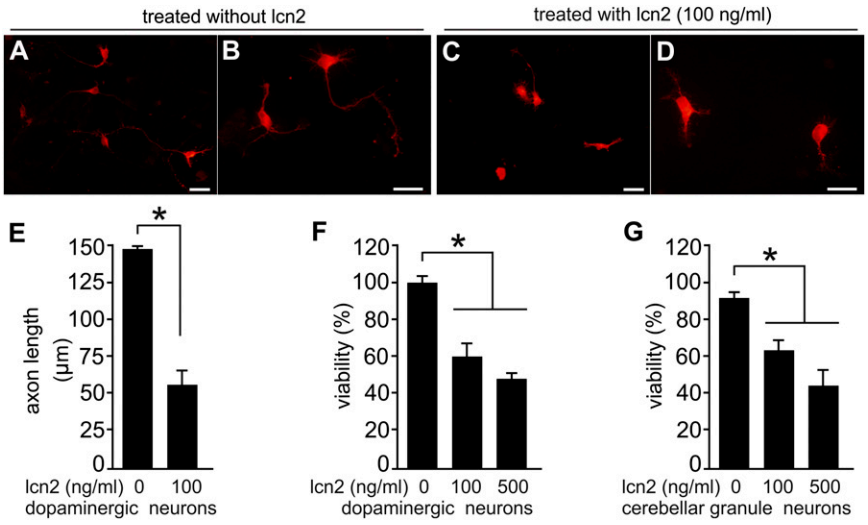

Fig. S4. Lipocalin 2 (lcn2) is toxic to dopaminergic and cerebellar granule neurons. (*A–C*) Representative photos show dopaminergic neurons that were isolated from the midbrain of rat embryos at embryonic day 14 and were stained with an antibody to tyrosine hydroxylase (TH). (Scale bar: *A* and *C*, 40 μm; *B* and *D*, 30 μm.) (*E* and *F*) TH-positive neurons and their axon length were quantitated after incubation with or without lcn2. Fifty TH-positive neurons were measured of axon length (*E*) and seven wells of midbrain culture were examined of survival TH-positive neurons (*F*). Data are means ± SEM (*E*, $n = 50$; *F*, $n = 7$). (*G*) lcn2 killed cerebellar granule neurons dose dependently. Primary granule cells were isolated from postnatal rats and were grown for 5 d before lcn2 treatment. Data are mean ± SEM ($n = 7$). *$P < 0.05$.

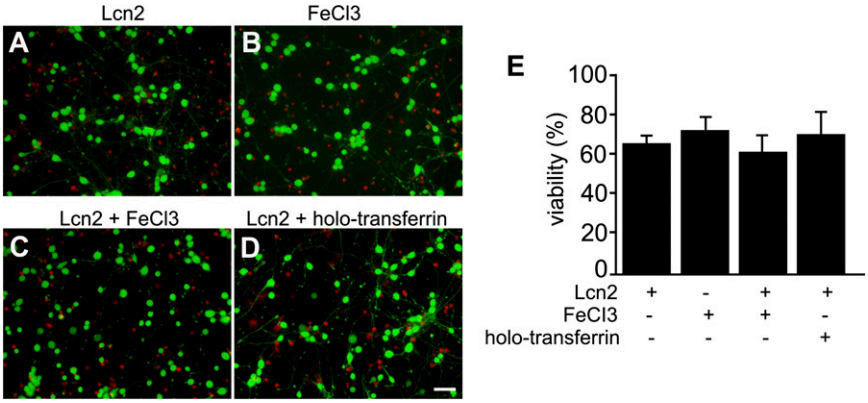

Fig. S5. Effects of iron and transferrin on lcn2-mediated neurotoxicity. (*A–D*) Graphs show that rat cortical neurons were treated with varying combination of lipocalin 2 (lcn2) with iron ($FeCl_3$: 50 μM) or holo-transferrin (iron-loaded: 100 μg/mL). Cortical neurons were initially cultured for 6 d and then were treated with lcn2 combination (10 μg/mL) for four more days. (Scale bar: 40 μm.) (*E*) Cell viability was determined with Live/Dead viability/cytotoxicity kit (L3224; Invitrogen). Data are mean ± SEM ($n = 8$).

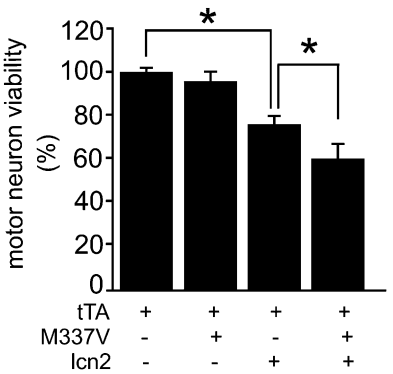

Fig. S6. Mutant TDP-43 overexpression sensitizes motor neurons to lcn2 toxicity. Primary motor neurons were isolated from rat embryos carrying ChAT-tetracycline-responsive transactivator (tTA, line 10) and TRE-TAR DNA-binding protein 43 (TDP-43^{M337V}) (M337V) transgenes. Primary spinal neurons were cultured for 10 d and then were treated with or without lipocalin 2 (lcn2; 10 ng/mL) for four more days. By 6 d in culture, neurons were deprived of Doxycycline to induce mutant TDP-43 expression. After lcn2 treatment, cholinergic neurons were detected by immunostaining for ChAT and were quantitated under microscope. Data are mean ± SEM ($n = 7$). *$P < 0.05$.

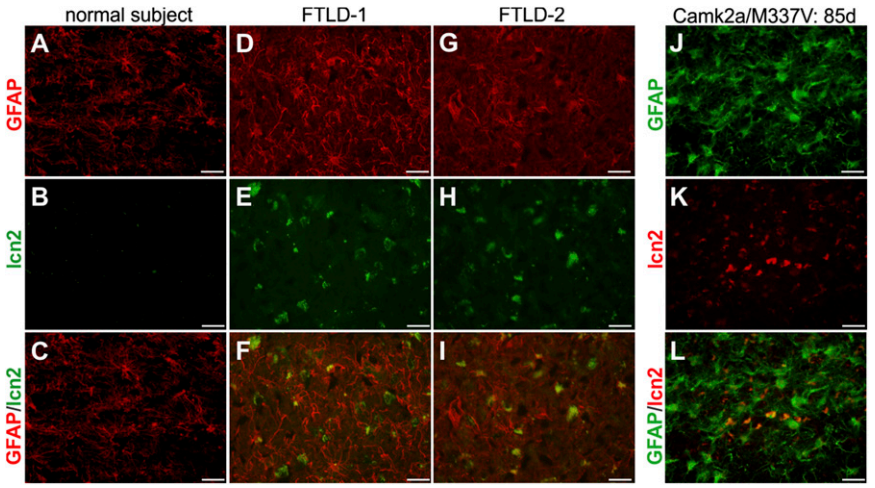

Fig. S7. lcn2 is up-regulated in the brain of frontotemporal lobar degeneration (FTLD) patients. (*A–I*) Immunofluorescent staining revealed increased lcn2 in FTLD patient brains (*D–I*) compared with healthy control subject brains (*A–C*). Frontal cortex was examined in two FTLD patients (FTLD-1 and -2) and two control subjects without neurological diseases. (*J–L*) Immunofluorescence staining revealed the distribution of up-regulated lcn2 in the frontal cortex of end-stage TAR DNA-binding protein 43 transgenic rats (Camk2α/M337V). Similar patterns of lcn2 up-regulation were detected in FTLD patients and end-stage TDP-43 transgenic rats. (Scale bars: 30 μm.)