Online Resource Figure Captions

Figure 1. Characterization of SETX expression in PrP transgenic control mice.
(a) Human SETX transgene expression levels from whole brain RNAs were determined by Taqman qRT-PCR analysis for two month-old SETX-wt mice (lines #1406, #1408, #1415, #1416, and #1420). Expression level is shown relative to endogenous murine *Setx*, and was normalized to *Gapdh*. n = 3 mice / genotype, n = 3 technical replicates.
(b) Mouse endogenous *Setx* expression levels from whole brain RNAs were determined by Taqman qRT-PCR analysis for two month-old PrP-SETX-wt transgenic mice (lines #1406, #1408, #1415, #1416, and #1420) and for two month-old PrP-SETX-R2136H transgenic mice (lines #1917 and #1920). Expression level is shown relative to endogenous murine *Setx* in non-transgenic control mice, and was normalized to *Gapdh*. n = 3 mice / genotype, n = 3 technical replicates.

Figure 2. Strategy for generating SETX L389S knock-in mice.
A targeting construct was generated by PCR amplification from murine BAC clone DNA. The right arm was cloned into a holding vector, and we employed site-directed mutagenesis to introduce the L389S substitution (asterisk). Both targeting arms were then cloned into a standard targeting vector backbone (4317G9). Random insertion was selected against, based upon retention and expression of the Diptheria Toxin (DT) gene product. Following electroporation into embryonic stem (ES) cells, we screened for recombinants (red dashed lines) based upon presence of the neomycin resistance cassette (cross-hatched box). We confirmed three properly targeted clones by long-range PCR and Southern blot analysis. Injection of one clone into 3.5 day-old mouse blastocysts yielded chimeric mice, based upon coat color (~45% to 95%), which were bred to produce two F_1 offspring with the properly integrated recombination cassette. F_1 positive mice were bred with CMV-Cre transgenic mice to permit deletion of the neomycin resistance gene to produce F_2 mice, as shown. The location of the PCR primers for genotyping of knock-in mice is given by the red and blue arrows.

Figure 3. SETX protein level are tightly regulated.

(a) HeLa cells were treated as indicated, and then immunoblot analysis with an anti-Flag or anti-beta-actin antibody was performed. Flag-SETX transfected cells express full-length SETX protein (~303 kDa). For control purposes, we did not transfect cells (non-transfected), or we transfected cells with an expression construct containing a N-terminal truncation fragment of SETX (control transfection) that yields a 75 kDa protein that is not shown here.
(b) HeLa cells were treated as indicated, and then immunoblot analysis with an anti-SETX or anti-beta-actin antibody was performed. Note comparable SETX band intensities and molecular mass for non-transfected cells, control siRNA-transfected cells, and Flag-SETX transfected cells.

Figure 4. SETX ALS4 mice display neurological abnormalities.
(a) Scoring of mice of the indicated age and genotype (n = 8 mice / genotype) for the four components of the neurological dysfunction screening examination. $^{**}P < .01$, $^{***}P < .001$; ANOVA with post-hoc Bonferroni test.
(b) We measured the average latency to fall from the accelerating rotarod for mice of the indicated age and genotype (n = 8 mice / genotype, 3 runs / day averaged over 4 days). The same cohorts of mice were used for the entire experiment. $^{**}P < .01$; ANOVA with post-hoc Tukey test. Error bars = s.e.m.

Figure 5. SETX ALS4 mice do not exhibit loss of motor neurons or innervated neuromuscular junctions.
(a) Representative confocal imaging of non-transgenic control lumbar cord section co-stained for choline acetyltransferase (ChAT) and TDP-43 to indicate motor neurons (yellow) in the anterior horn region of the spinal cord.
(b) Quantification of motor neuron numbers for 10 month-old mice of the indicated genotypes (n = 3 mice / genotype; n = 5 sections / mouse). $P = 0.46$; ANOVA.
(c) Quantification of innervated neuromuscular junctions for 10 month-old mice of the indicated genotypes (n = 3 mice / genotype; n = 100 NMJs / mouse). $P = 0.14$; ANOVA.

Figure 6. Motor neurons from SETX ALS4 mice display enhanced stress granule formation.

Primary E13 spinal cord motor neurons were prepared from mice of the indicated genotypes, treated with 0.5 μM sodium arsenite or untreated, and then immunostained for β-III tubulin and FMRP (stress granule marker). Note the increased number of stress granules observed in SETX-R12136H motor neurons. Scale bar = 50 μm