Supplementary Material for:

"An insoluble frontotemporal lobar degeneration-associated TDP-43 C-terminal fragment causes neurodegeneration and hippocampus pathology in transgenic mice"

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Human Molecular Genetics

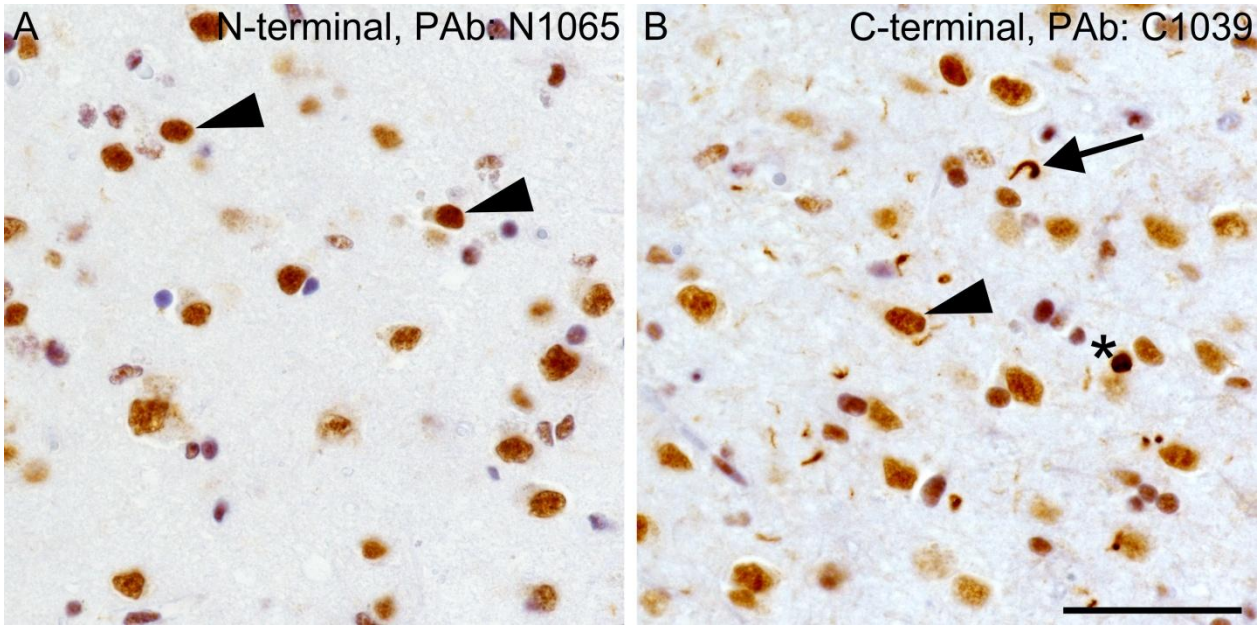

Figure S1. Pathology in FTLD-TDP brain is preferentially detected by C-terminal TDP-43 antibody. (A) IHC for TDP-43 using an N-terminal TDP-43-specific antibody (PAb: N1065) detects primarily normal, nuclear TDP-43 (arrowheads), while **(B)** IHC for TDP-43 using C-terminal TDP-43-specific antibody (PAb: C1039) detects normal nuclear TDP-43 (arrowhead) as well as pathological dystrophic neurites (arrow) and neuronal cytoplasmic inclusions (asterisk). These findings indicate that the FTLD-TDP pathology contains C-terminal TDP-43 CTFs. Scale bar: **A-B**, 50 µm.

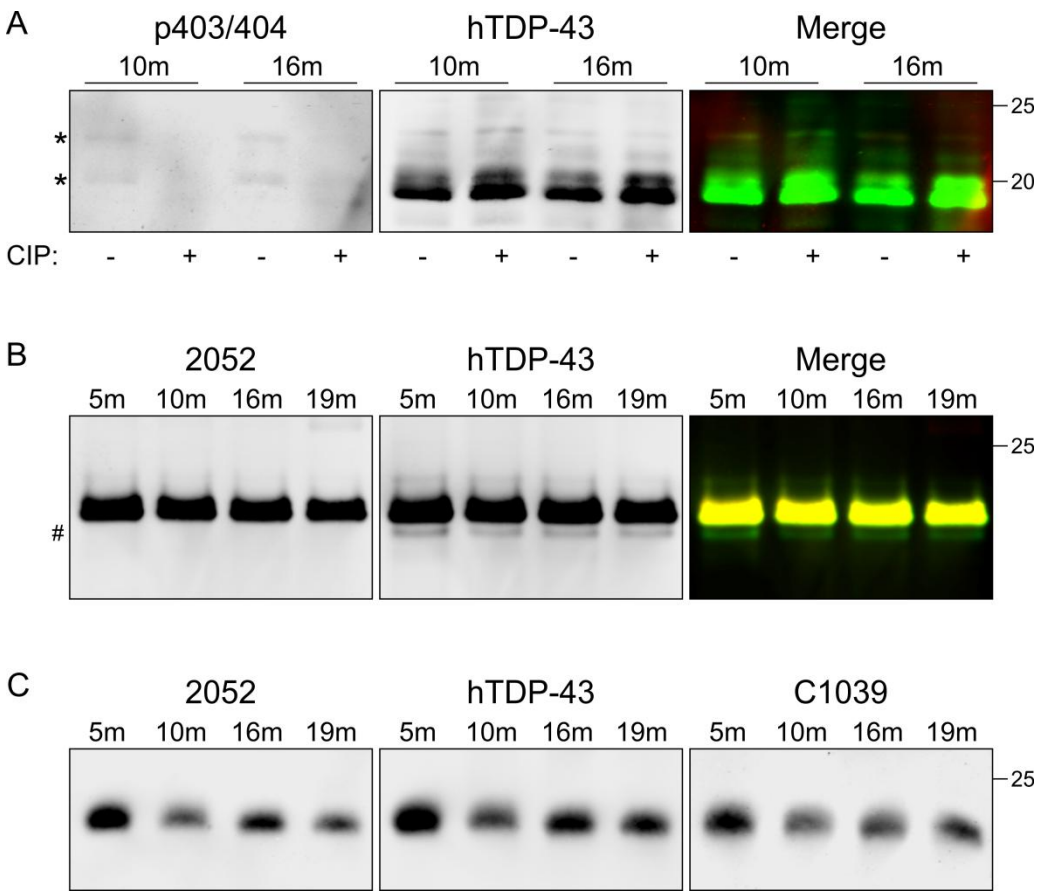

Figure S2. Minor 208 TDP-43 CTF bands are phosphorylated and C-terminally cleaved, but represent a single species under highly denaturing conditions. (A) Dephosphorylation of urea-soluble protein fractions from 208 TDP-43 mouse cortex with CIP and analyzed by SDS-PAGE IB. CIP treatment causes the loss of 2 phospho-S403/404-immunoreactive bands (indicated by asterisks) which co-locate with minor hTDP-43 (MAb: 5104) bands. **(B)** The lower minor band seen in urea-soluble protein fractions from 208 TDP-43 mouse cortex by electrophoresis using SDS-PAGE IB with MES buffer is detected using hTDP-43-specific antibody (MAb: 5104) but not 208-220 TDP-43-specific antibody (PAb: 2052, indicated by pound mark), with mice of ages as shown. **(C)** Only a single band is detected from urea-soluble protein fractions of 208 TDP-43 mouse cortex by 208-220 TDP-43-specific (PAb: 2052), hTDP-43-specific (MAb: 5104), and C-terminal TDP-43-specific (PAb: C1039) antibodies using 6M urea-gel IB, with mice of ages as shown. Approximate molecular weight markers in kDa are shown on the right.

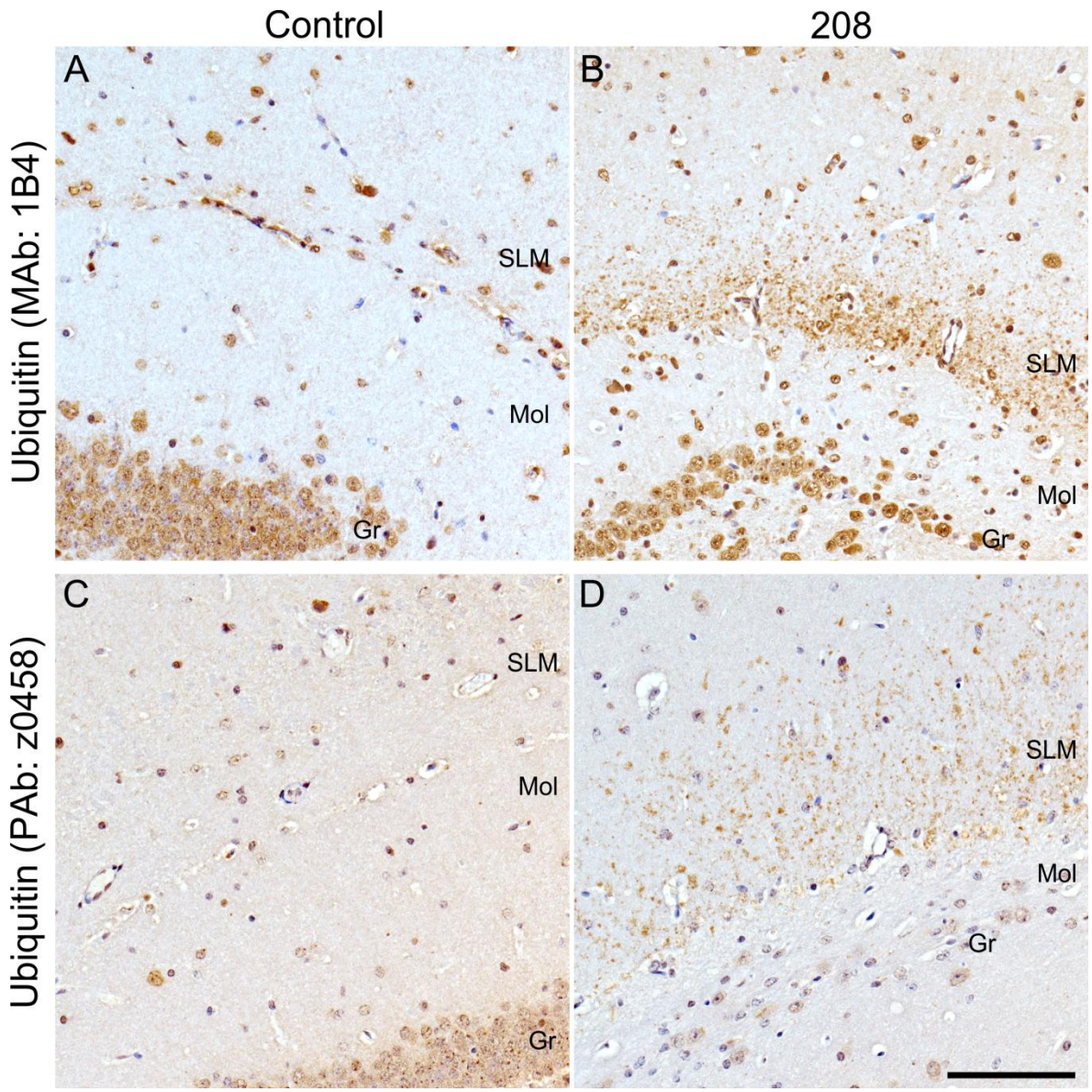

Figure S3. Ubiquitin accumulation in the SLM of 208 TDP-43 mouse hippocampus detected by additional antibodies. IHC for ubiquitin using antibodies **(A,B)** 1B4 or **(C,D)** z0458 detects punctate accumulation of ubiquitin in the SLM of the hippocampus in 208 TDP-43 mice, which is not detected in littermate controls. Images are shown for littermate control and 208 TDP-43 mice at 19 m off Dox. *SLM*, stratum lacunosum moleculare, *Mol*, molecular layer, *Gr*, granule cell layer. Scale bar: **A-D**, 100 µm.

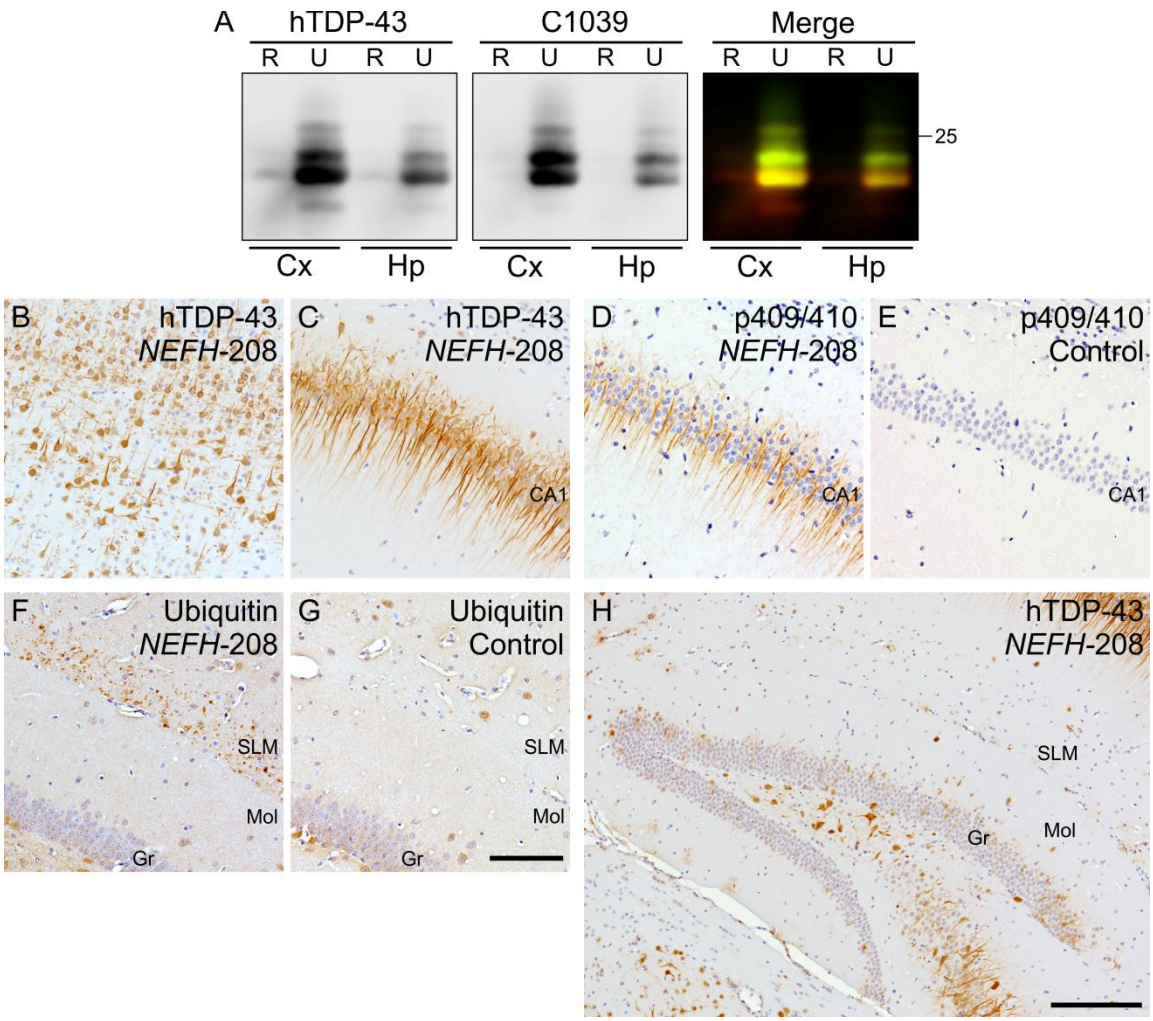

Figure S4. The *NEFH* promoter drives 208 TDP-43 CTF expression in cortex and hippocampus, with phospho-TDP-43 in CA1 and ubiquitin in SLM but with little DG expression and no overt neurodegeneration. (A) Expression in the cortex (Cx) and hippocampus (Hp) shows predominantly urea (U)-soluble rather than RIPA (R)-soluble 208 TDP-43 CTF at 5 m off Dox, detected using human TDP-43-specific antibody (MAb: 241) and C-terminal TDP-43-specific antibody (PAb: C1039). Approximate molecular weight marker in kDa is shown on the right. **(B,C)** 208 TDP-43 CTF was widely expressed in cortex and hippocampus, detected using human TDP-43-specific antibody (MAb: 5104). **(D)** Phospho-409/410-TDP-43 was detected in the CA1 region of the hippocampus in *NEFH*-208 TDP-43 mice, but not in **(E)** littermate control. **(F)** Ubiquitin (MAb: 1510) accumulation was detected in the SLM of the hippocampus in *NEFH*-208 TDP-43 mice, but not in **(G)** littermate controls. **(H)** 208 TDP-43 CTF (MAb: 5104) was detected in only a small minority of DG cells, and the dramatic DG neuron loss found in all *CAMK*-208 TDP-43 mice at 19 m (see Fig. 3) was not seen in any *NEFH*-208 TDP-43 mice at the same time point. Images shown are at 19 m off Dox, representative of *n*=3. Scale bars: **B-G**, 100 µm; **H**, 200 µm.

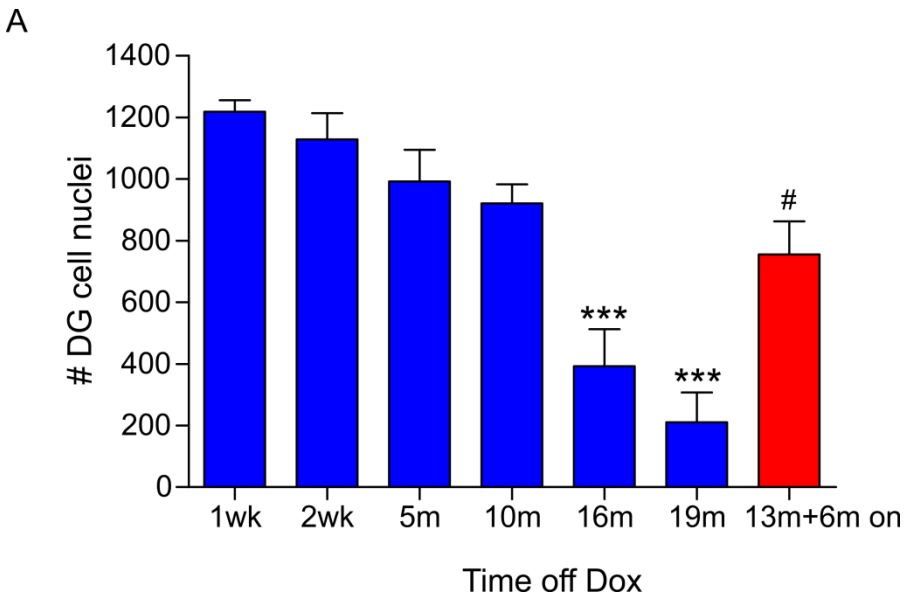

Figure S5. Prevention of further neuron loss in the DG of 208 TDP-43 mice returned to Dox at 13 m. (A) Quantification of DG cell nuclei numbers in 208 TDP-43 mice at 1 wk, 2 wk, 5 m, 10 m, 16 m or 19 m off Dox and at 13 m off Dox + 6 m on Dox, *n*=3-4 per group. ***p<0.001 versus 1 wk, and #p<0.05 versus 19 m, by one-way ANOVA with Bonferonni's post test. See also Fig. 3 and Fig. 6.