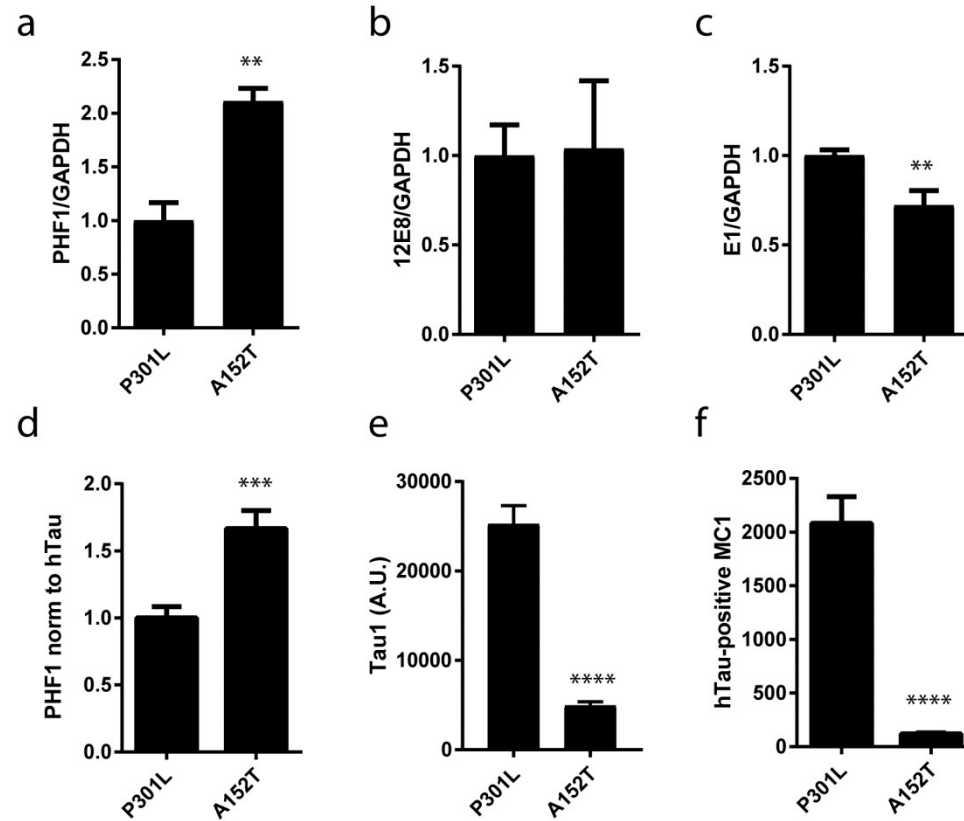
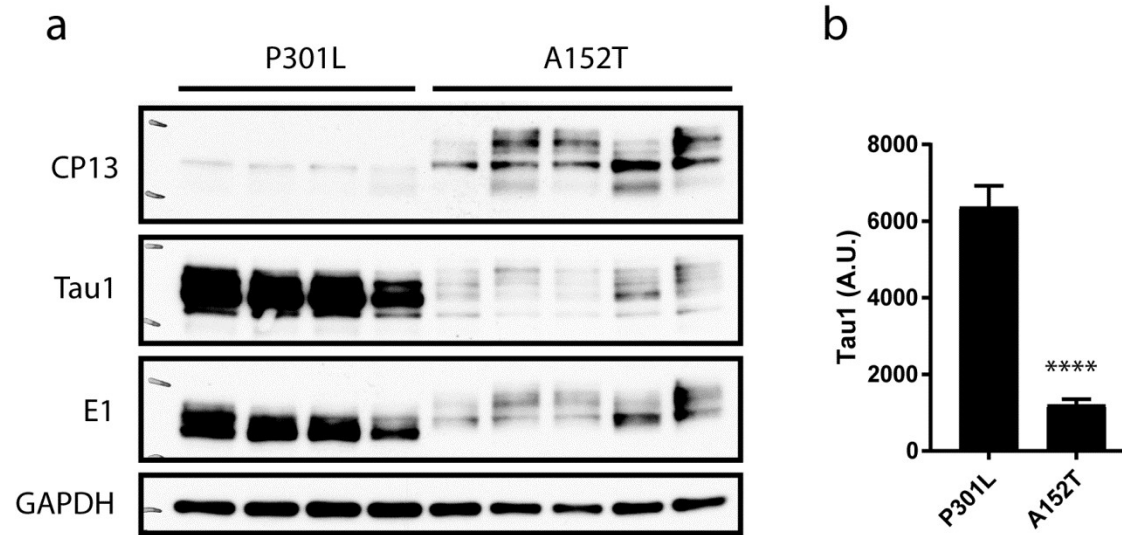


## SUPPLEMENTARY DATA



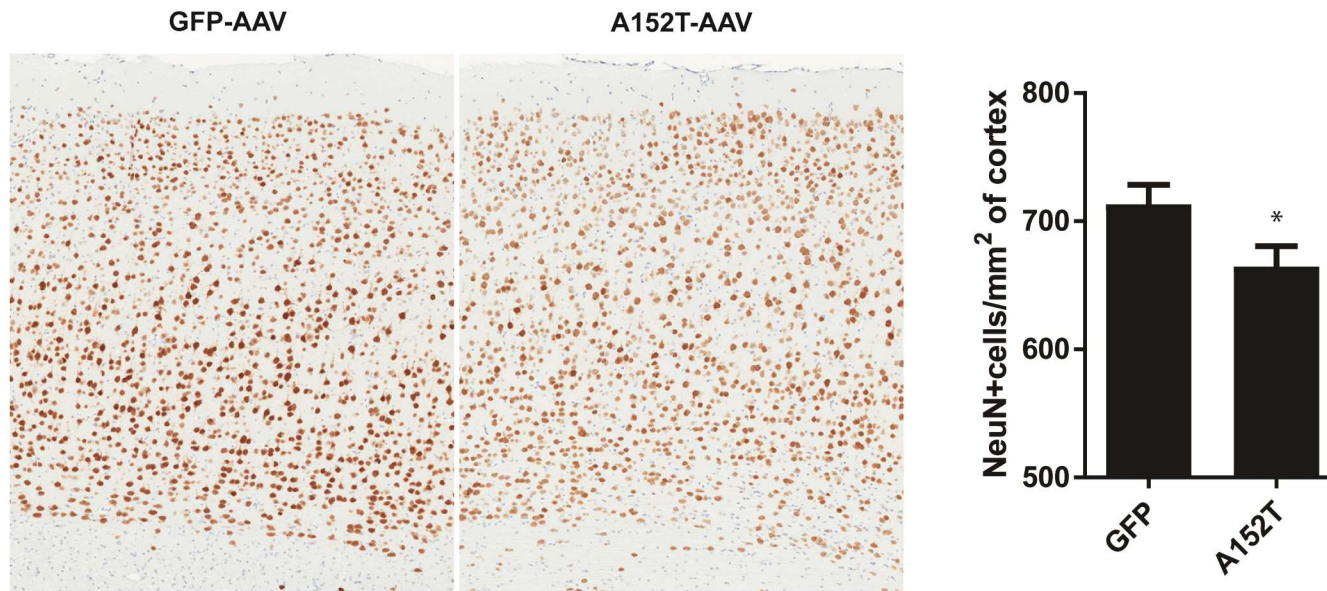
**Figure S1. Accumulation of soluble, hyperphosphorylated tau in A152T-expressing mice.** (a-c) Quantification of PHF1 (a;  $t=3.82$ ,  $p=0.002$ ), 12E8 (b;  $t=0.1$ ,  $p=0.9$ ) and E1 (c;  $t=3.048$ ,  $p=0.006$ ) levels in the S1 fraction by immunoblotting, with GAPDH used to control for protein loading. (d) Despite decreased total human tau protein expression, PHF1-positive human tau is significantly increased in Tau<sup>A152T</sup>-AAV mice by MSD immunoassay ( $t=4.061$ ,  $p=0.0006$ ). (e) Consistent with an increase in tau phosphorylation in Tau<sup>A152T</sup>-AAV mice, there is a significant reduction in Tau1 (unphosphorylated S195, 198, 199 and 202) levels by MSD immunoassay ( $t=10.01$ ,  $p<0.0001$ ). (f) Minimal MC1-positive human tau is detected in Tau<sup>A152T</sup>-AAV relative to Tau<sup>P301L</sup>-AAV mice ( $t=8.276$ ,  $p<0.0001$ ). \*\* $p<0.005$ , \*\*\* $p<0.001$ , \*\*\*\* $p<0.0001$

## SUPPLEMENTARY DATA



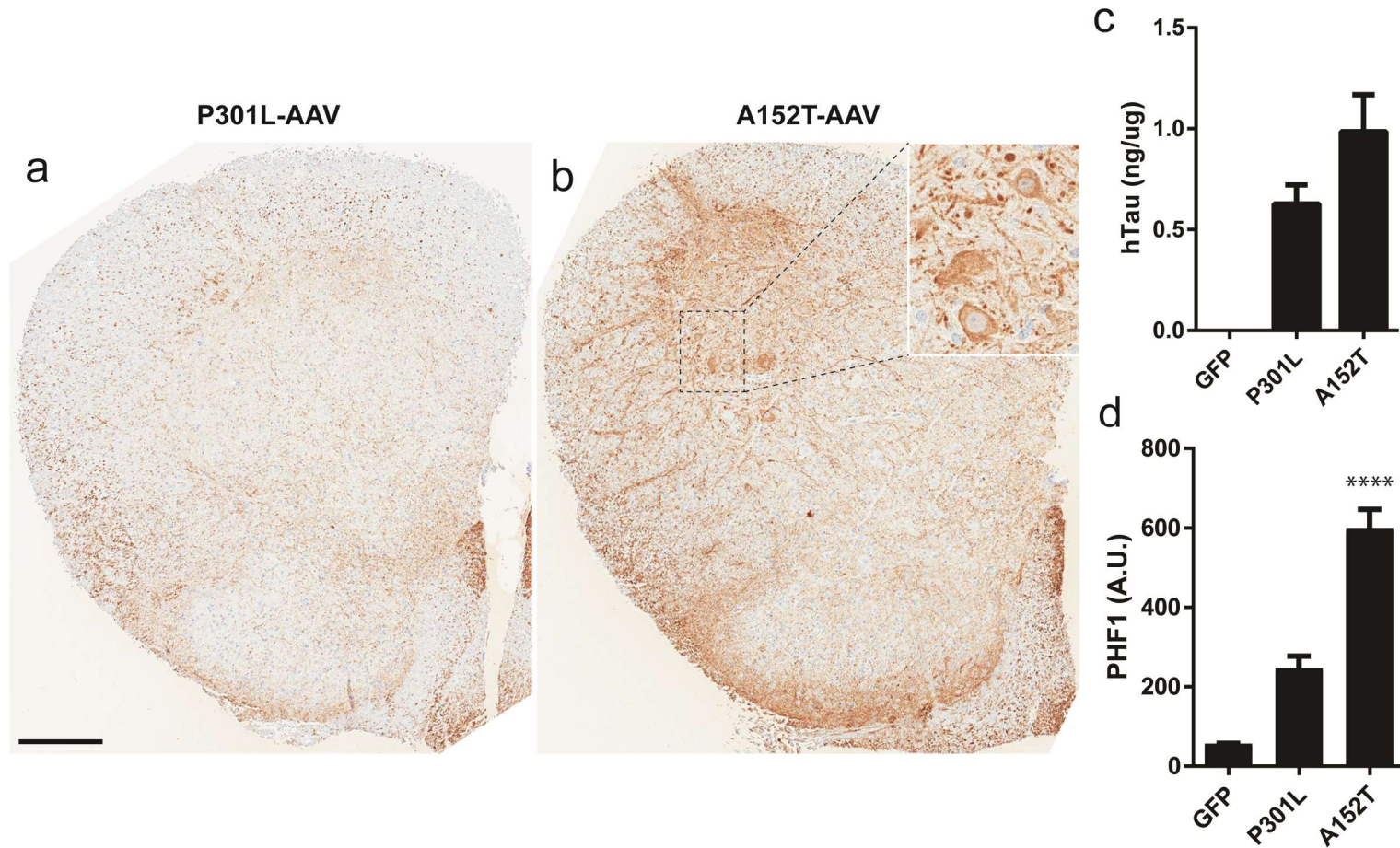
**Figure S2. Increased tau phosphorylation in the sarkosyl-soluble S2 fraction in Tau<sup>A152T</sup>-AAV mice.** (a) Biochemical fractionation followed by immunoblotting of the sarkosyl-soluble S2 fraction demonstrates accumulation of phosphorylated tau in Tau<sup>A152T</sup>-AAV mice, revealed by elevated CP13 (pS202) and reduced Tau1 (unphosphorylated S195/198/199/202). E1 levels are also lower in Tau<sup>A152T</sup>-AAV mice, consistent with reduced human tau protein in the S2 fraction, and shifted to a higher molecular weight indicative of hyperphosphorylation. (b) Tau1 levels in the S2 fraction measured by MSD immunoassay are also significantly lower in Tau<sup>A152T</sup>-AAV compared to Tau<sup>P301L</sup>-AAV mice ( $t=9.431$ ,  $p<0.0001$ ). \*\*\*\* $p<0.0001$

## SUPPLEMENTARY DATA



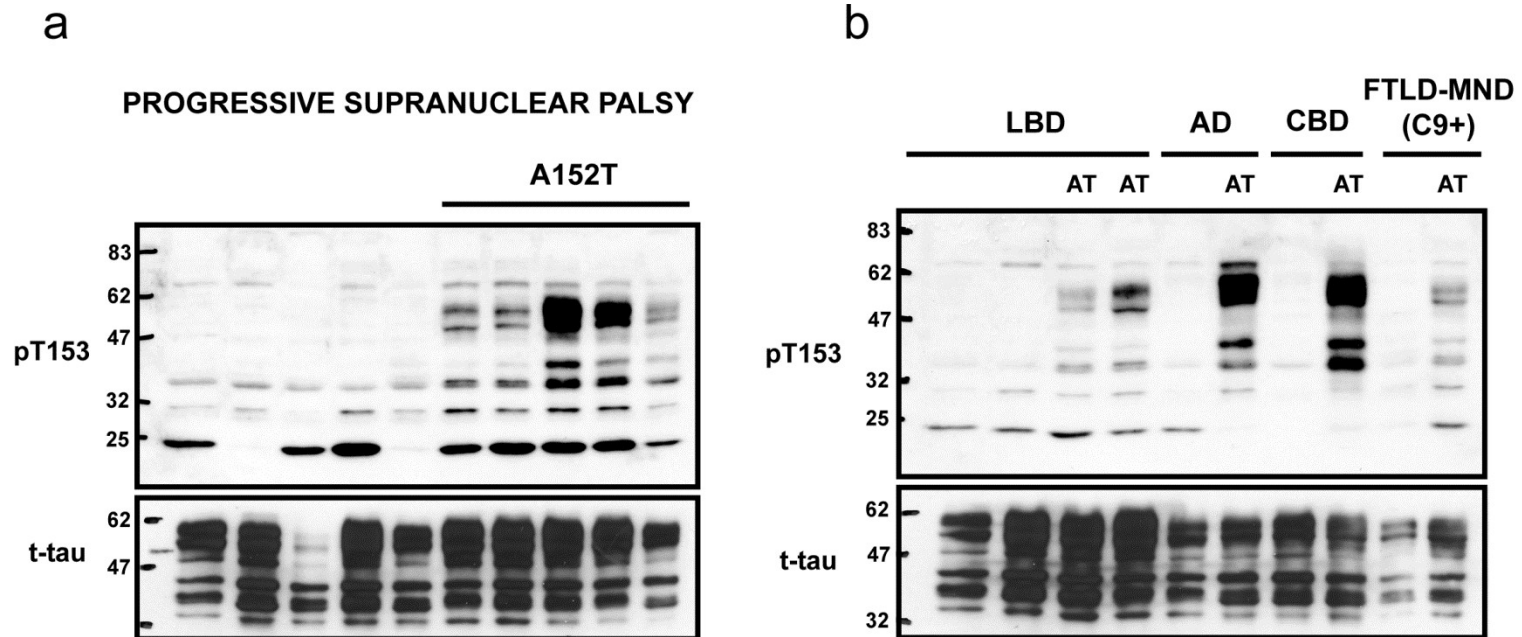
**Figure S3. Neurodegeneration observed in Tau<sup>A152T</sup>-AAV mice.** Representative images of immunohistochemical analysis of NeuN in the cortex of GFP-AAV and Tau<sup>A152T</sup>-AAV mice. The total number of NeuN-positive nuclei in the cortex was calculated, and divided by the total area to assess cortical neuron density, which was significantly reduced in Tau<sup>A152T</sup>-AAV mice ( $t=2.183$ ,  $p=0.04$ ). \* $p<0.05$

## SUPPLEMENTARY DATA



**Figure S4. Human tau expression is detected in the spinal cord of tau-AAV injected mice.** (a-b) Immunohistochemistry was used to evaluate human tau expression in spinal cord from Tau<sup>P301L</sup>-AAV and Tau<sup>A152T</sup>-AAV injected animals using E1 (human-tau specific antibody). (c) Total human tau levels were evaluated in spinal cord lysates by MSD immunoassay. Values are reported in ng/ug protein, based on a standard curve generated from full-length recombinant human tau. (d) Tau phosphorylated on the PHF1 epitope (pS396/404) in spinal cord lysates were measured by MSD immunoassay ( $F=70.54$ ,  $p<0.0001$ ). Scale bar 200 $\mu$ m. \*\*\*\*  $p<0.0001$

## SUPPLEMENTARY DATA



**Figure S5. Accumulation of soluble pT153 is specific to A152T carriers. (a-b)** Biochemical fractionation followed by immunoblotting of the soluble S1 fraction from frontal cortex reveals significant accumulation of pT153 in A152T carriers relative to noncarriers, independent of disease classification. LBD=Lewy body dementia; AD=Alzheimer's disease; CBD=corticobasal degeneration; FTLD-MND (C9+)=frontotemporal lobar degeneration with motor neuron disease associated with C9ORF72 mutation