

## **Supplemental Experimental Procedures**

**Biochemistry:** Mouse brain tissue for biochemical studies was rapidly dissected and quickly frozen in isopentane for storage at  $-80^{\circ}\text{C}$ . To generate forebrain lysates, olfactory bulbs, corticolimbic and subcortical brain stem structures, and the cerebellum were removed. Frozen hemi-forebrains were thawed and mixed gently in a mortar with a few strokes of a pestle in 10 wet weight volumes of ice-cold Tris-buffered saline containing phosphatase inhibitors [phenylmethylsulfonyl fluoride, phenanthroline monohydrate and phosphatase inhibitor cocktails I and II; 1:100, Sigma] and protease inhibitor cocktail [1:100; Sigma]). To estimate total levels of human and mouse tau, protein-corrected (BCA assay) brain extracts were processed and visualized using western blot analyses with primary antibodies Tau-5, Tau-13, and  $\alpha$ -tubulin.

## **Supplemental Figure Legends**

**S1. Characterization of rTgWT mice.** (A) Responder mice encoding human four-repeat tau, lacking both N-terminal inserts (4R0N) were crossed with activator mice expressing the forebrain-specific neuronal CaMKII promoter. (B) Representative immunoblot showing soluble human tau expression in rTgWT and rTgP301L mice aged 3 months. Human- and mouse-specific tau species were detected with the Tau-5 antibody. Equal loading was ensured after probing for  $\alpha$ -tubulin. (C) Quantification of human tau in immunoblots showed overlapping intensities from the two mouse lines at multiple dilutions in the linear range of detection. (D) Biochemical analysis indicated high expression of WT tau limited to the forebrain. (E) Expression of WT tau is first seen at P7 as indicated by western blot analysis of forebrains using Tau-13, a human-specific tau antibody. For Tau-5 and Tau-13, 3  $\mu\text{g}$  of rTgWT forebrain extract were loaded and compared to 30  $\mu\text{g}$  of TgNeg lysate. For  $\alpha$ -tubulin, 3  $\mu\text{g}$  of protein were loaded for both groups and run in parallel. Olf, olfactory bulb; Ctx, cortex; Hpp, hippocampus; Cbm, cerebellum; BnSt, brainstem; Spn, spinal cord.

**S2. Unprocessed images of cultured rat neurons co-transfected with DsRed and GFP-WT htau or GFP-P301L htau.** (A) Images consist of six pairs of serial planes ( $z$ -step = 0.5  $\mu\text{m}$  from bottom to top) that were closest to the focus of a dendrite of interest in a 3 week-old cultured rat neuron, which was co-transfected with DsRed (to label total dendritic spines) and GFP-WT htau. White arrows denote DsRed-labeled spines that do not contain GFP-WT htau. (B) Images consist of six pairs of serial planes ( $z$ -step = 0.5  $\mu\text{m}$  from bottom to top) that were closest to the focus of a dendrite in a 3 week-old cultured rat neuron co-transfected with DsRed and GFP-P301L htau. White arrows denote DsRed-labeled spines that contain GFP-P301L htau. Images have not been subjected to deconvolution processing and  $z$ -planes have not been averaged. These stacks of images clearly demonstrate the drastic difference between the dendritic spine distributions of GFP-WT htau and GFP-P301L tau.