Supporting Online Material for SantaCruz et al

1) Materials and Methods

Animals. Briefly, our methods for generating rTg4510 mice utilized a system of responder and activator transgenes. Mice expressing the activator transgenes were derived from a generous gift of Dr. E. Kandel (*1*), and successively backcrossed at least five times onto a 129S6 background strain. Responder mice were maintained in the FVB/N strain. Mice were screened by PCR using the primer pairs 5'-GAT TAA CAG CGC ATT AGA GCT G-3' and 5'-GCA TAT GAT CAA TTC AAG GCC GAT AAG-3' for activator transgenes, and 5'-TGA ACC AGG ATG GCT GAG CC-3' and 5'-TTG TCA TCG CTT CCA GTC CCC G-3' for responder transgenes. Doxycycline 200 ppm in chow was administered to mice *ad libitum* to suppress transgene expression. All animals were housed and tested accordingly to standards established by the AAALAC and IACUC.

Transgene construct. The tau responder transgene construct is diagrammed in fig. S8. The tau_{P301L} cDNA encoding human 4-repeat tau lacking the amino-terminal sequences (4R0N) was modified such that the tau_{P301L} transgene (containing exons 1, 4-5, 7, 9-13, intron 13, and exon 14) driven by TRE was placed in the context of the mouse prion protein gene (*prnp*) transcribed but untranslated sequences, which were derived from the MoPrP.Xho expression vector, a generous gift of Dr. D. Borchelt (*2*). First, the *Sal* I fragment of a previously created tau_{P301L} transgene (starting 29 bases upstream of the start methionine and extending 1228 bases downstream of the stop codon) (*3*), including the whole coding sequence of tau plus the P301L mutation, was inserted into the unique *Xho* I site of MoPrP.Xho to generate prnp.tau_{P301L}. Next, the *Xba* I fragment of prnp.tau_{P301L},

including partial sequences of *prnp* introns 1 and 2, along with exons 2-3, and the tau_{P301L} open reading frame, was cloned into the unique *Xba* I site in the inducible expression vector pTRE (Clontech, Inc.), resulting in the plasmid, pTRE.prnp.tau_{P301L}. The resultant DNA was digested with *Xho* I and *Ngo*M IV enzymes, fractionated, and purified by electroelution followed by organic extraction. Purified fragments containing a modified tau transgene were introduced by microinjection into the pronuclei of donor FVB/N embryos, by standard techniques.

In situ hybridization. Sagittal cryostat brain sections (15μm) were fixed in 4% paraformaldehyde, dehydrated and hybridized with a human tau specific oligomer (5'-CTT TCA GGC CAG CGT CCG TGT CAC CCT CTT GGT C-3') 3'-end labelled with α^{35}S dATP. Sections were hybridized at 37°C overnight in buffer containing 4x SSC, 1x Denhardt's solution, 50% v/v de-ionised formamide, 10% w/v dextran sulphate, 200mg/μl herring sperm DNA and 0.03% β-mercaptoethanol. Control sections were hybridized in the presence of a 50-100 fold molar excess of unlabeled oligonucleotide. After hybridization, the sections were stringently washed (1x SSC at 50^{o}C), dehydrated and exposed to β max hyperfilm (Amersham) for 7-10 days.

Tau biochemistry. Tissue preparation for each hemibrain designated for biochemical studies involved removing olfactory bulbs, dissecting corticolimbic and subcortical structures from the brain stem and cerebellum, and quickly freezing tissue in isopentane for storage at 80°C. Frozen hemi-forebrains were thawed and gently mixed in a mortar with a few strokes of a pestle in 10 wet weight volumes of cold Tris-buffered saline (TBS) containing Protease Inhibitor Cocktail, PMSF, phenenthroline monohydrate, and Phosphatase Inhibitor Cocktails I and II (all Sigma), at a final dilution of 1:100. To estimate total levels of mouse and human tau, protein corrected (Bradford assay) brain extracts were diluted in reducing sample buffer, electrophoresed on 10% Tris-HCL gels (Biorad), then transferred onto 0.45 μm polyvinylidene difluoride membranes (Millipore). Briefly, blots were processed with primary antibody Tau-5 (Biosource), and visualized

using enhanced chemiluminescence reagents (Pierce) followed by exposure onto hyperfilm (Kodak). *Sarkosyl extractions.* 90µl of brain homogenate was ultracentrifuged at 150K *g* for 15 min at 4^{o}C. Supernatant (S1; soluble 1 fraction) was removed for analysis and the pellet was re-homogenized in 10mM Tris (pH7.4), 0.8M NaCl, 10% Sucrose, 1mM EGTA, 1mM PMSF and ultracentifuged as before. Supernatant was incubated with a final volume of 1% sarkosyl for 1hr at 37^{o}C before ultracentrifugation at 150K *g* for 30 min at 4^{o}C. Pellet was resuspended in 20µl of Tris-EDTA (10mM Tris, pH8.0; 1mM EDTA) and labelled P3 (sarkosyl insoluble tau). Equal loading for the S1 fraction was determined by protein assay with bicinchoninic acid (B9643; Sigma) and Copper (II) Sulfate (C2284, Sigma) against BSA standards. Equal loading for the P3 fraction was based on original brain weight (protein from 1.35mg of tissue was loaded for each sample). Standard Western protocol was utilized with samples run on 10% tris-glycine gels (EC6078 Box, Invitrogen). Blots were blocked in 5% non-fat milk in TBS-T, hybridized with primary antibodies (E1 human tau polyclonal, a generous gift of Dr. S. Yen, 1:10000, O/N; GAPDH monoclonal 1:3000 (Biodesign International), 1 hr.), followed by appropriate secondary antibody and visualization with Western Chemiluminescent ECL reagent (Pierce). Soluble and sarkosyl-insoluble tau species were electrophoresed beside 10ng recombinant 4R0N human tau run on the same gel for quantitation purposes. Soluble tau levels were adjusted for GAPDH levels to reflect equal loading. Band density from film exposed within linear range was measured using Image Quant 5.0 software (Molecular Dynamics).

Analysis of gene expression. *RNA extraction.* 20-40 mg anterior forebrain cortical tissue was dissected under stringent RNAse free conditions. RNA was extracted with the Trizol reagent according to the manufacturer's instruction (Invitrogen) and samples were equally rediluted to 1 µg/µl. The RNA integrity was assessed by microcapillary electrophoresis (RNA 6000 Nano Assay, Agilent Technologies) and samples showing degradation of the 18S and 28S ribosomal RNA bands were excluded.

Next, the samples were DNA-se treated according to the manufacturer's instruction (R Q1 RNA-se free DNA-se, Promega), followed by RNA reprecipitation, by subjecting the samples to phenol:chloroform:IAA (pH 6.6 / 7.9, Ambion) (100% of starting volume) and 3M NaAc (Ambion) (10% of starting volume). After vortexing briefly, the samples were kept on ice (15 min.), followed by centrifugation at 15,000 g (15 min.) and transferring of the supernatant to a new tube. After adding 2.5x 100% EtOH and 1 µl glycogen (1:10, Ambion), the samples were vortexed and kept on ice (30 min.) before centrifugation at 15,000 g (30 min.). Finally, the resulting pellet was washed with 75% EtOH, recentrifuged and dissolved in H_20 to a final concentration of 500 ng/µl. *RT.* Reversed transcription (RT) was carried out on all RNA samples (Superscript II, Invitrogen). In brief, 2 µg of DNA-se treated RNA was mixed with random hexamers and 10 nmol of dNTPs. After denaturation at 65°C (5 min), the following reagents were added: 100 nmol of $MgCl_2$, 200 nmol of dithiothreitol (DTT), 40 U of RNAse Out Inhibitor and 2.0 µl of 10x RT-PCR buffer. After incubation in room temperature (2 min), 200 U of Superscript II reverse transcriptase (Invitrogen) was added, followed by incubation at 25°C (10 min) and 42°C (50 min.). Finally, the reaction was deactivated at 70°C (15 min). *Quantitative PCR.* Primers for quantitative PCR (qPCR) specific against the transgenic human tau construct (5'-CCC AAT CAC TGC CTA TAC CC-3' and 5'-CCA CGA GAA TGC GAA GGA-3'), mouse tau exon 7 (5'-AGC CCT AAG ACT CCT CCA-3' and 5'-TGC TGT AGC CGC TTC GTT CT-3') and GAPDH (5'-TGG TGA AGC AGG CAT CTG AG-3' and 5'-TGC TGT TGA AGT CGC AGG AG-3') were designed. The specificity of each primer pair was verified by microcapillary electrophoresis and sequencing. Duplicates of 25 µl reactions containing 12.5 µl SYBR green Mastermix (Applied Biotechnology), 400 nM (for transgenic tau) or 200 nM (for mouse tau and GAPDH) of each primer and 100 pg (for transgenic tau and GAPDH) or 1 ng (for mouse tau) of cDNA were used in the reaction. After initial denaturation at 95°C (6 min), 50 cycles at 95°C (30 sec), the primer-specific annealing temperature (58°C for Tg tau and GAPDH, 59°C for mouse tau) (30 sec) and 72°C (45 sec) were performed before a final incubation at 95°C (1 min). The

transgenic tau, mouse tau and GAPDH primer pairs were used to generate vector cDNA by using the TOPO TA Cloning® Kit according to the manufacturer's instructions (Invitrogen). The cloned vector cDNA was used to generate a standard curve ranging from attomolar to picomolar concentrations, allowing for calculation of molarity and number of mRNA molecules in the samples. Finally, the respective tau mRNA levels were normalized to murine tau and GAPDH mRNA levels, respectively.

Behavioral analysis. Spatial reference memory was measured using the Morris water-maze (*4*) tailored to more rapid learning in the 129FVBF1 background strain. Mice were handled 60 seconds per day for 10 days during the two weeks prior to the initiation of testing at 2.5 months. Pre-handling was designed to condition the mice to manipulations which would be experienced during introduction and removal from the testing pool and included a 20 second exposure to water at a depth of 1 cm. Mice were tested longitudinally from 2.5 to 9.5 months at approximately 6-week intervals. At each age tested mice received visible platform training for 3 days, 6 trials per day, and hidden platform training for 6 days, 4 trials per day. At 2.5 month, visible platform training preceded hidden platform training, while hidden platform preceded visible platform training at subsequent time points. The spatial cues and hidden platform location were changed at each age tested. Four probe trials of 30 seconds duration were performed 20 hours after 8, 12, 16, and 24 hidden training trials. The mean target quadrant occupancy of all four probes was calculated. All trials were monitored using a computerized tracking system (Noldus EthoVision 3.0) and performance measures were extracted using Wintrack (*5*). Statistical analysis consisted of analysis of variance (ANOVA) and repeated measures analysis of variance (RMANOVA). *Post hoc* comparisons were performed using unpaired *t*-tests or paired *t*-tests.

Motor assessment was conduced in a cohort of 26 mice (18 tauP301L negative and 8 tau_{P301L} positive) between 4 and 6 months of age. All mice were pre-handled 2 times daily (am and pm) for 3 consecutive days. All mice were administered a series of

behavioral tests focussing on their reactivity to sensory stimulation and evaluating their motor abilities and activity. First, *response to an auditory stimulus* was evaluated. A mouse was placed on an open arena in a quiet room and an experimenter delivered a clicking sound of a ball pen while holding a pen about 5 cm behind the head of a mouse. This sound elicited a Preyer reflex (backwards flick of pinnae) and backward head orientation in a normal mouse. Next a mouse was placed on a horizontally held large wire grid (rat cage top) which then was raised and inclined to a vertical position with an animal facing a floor. *Negative geotaxis response* (turning and climbing the grid) was recorded. Following, all mice were given a *grip test* which was administered in 2 consecutive days. The grip test measures the strength with which a mouse holds to a bar with its front paws while an experimenter pulls it gently backwards. During the test, we used a horizontally mounted 300g spring scale fitted with a grip bar (a toothpick). A mouse was held in a horizontal position and gently lowered towards the grip bar until it grasped it. It was then gently pulled backward until it released a grip. The force when an animal released the grip was recorded. During each day/session 3 trials were administered and the highest score was used for the analysis. Deficits in limb coordination and accuracy of limb placement as well as general motor coordination of the mice was evaluated in the *beam crossing test* in which a mouse has to walk across a narrow beam to reach a home cage. The difficulty to traverse a 120 cm beam was increased by placing on a beam a small (0.5×0.5 cm) obstacles spaced 10 cm from each other. The beam was suspended 50 cm off the ground (with the soft cloth stretched 40 cm below it to cushion a fall) and 2 home cages were available on each side of the beam. A mouse was placed in the middle of a beam and allowed to walk to one of the cages. A bright, 40W lamp was directed at the middle zone of the beam to facilitate walking towards a darker end with a home cage. All mice received two sessions, one per day, with 3 trials each. A 2.5 cm wide beam was used during the first session, and during the 2^{nd} session the task difficulty was increased by using a narrower, 1.9 cm wide beam. Maximum time allocated to each trial was 120 seconds. The time of active walking on a beam and number of falls during each trial were

recorded. Finally, the *open field test* was carried out in a 50 × 50 × 40 cm square box made of white plastic. The arena was divided into 10 × 10 cm (16 outer and 9 inner) squares. A 40 W lamp was suspended in the middle of the arena and an experimenter was sitting at one side of the apparatus recording on line the behavior of a mouse with the aid of computerized event recorder. The test was administered in 2 consecutive days with two trials per day. A mouse was placed near a wall of the arena and was allowed to explore it for 180 seconds. The open field test evaluates not only the locomotor exploratory activity of animals, but since an open and brightly lit arena is an aversive environment to rodents, a decreased exploration of the arena centre is usually interpreted as an indicator of increased anxiety-like behavior. The following behavioral measures were recorded: latency to enter central field of the apparatus, number of fields crossed in the periphery and centre, the total time of walking and pauses in the periphery and center, duration of rearing in the periphery and centre, leaning against a wall, and grooming. Additionally, at the end of each trial a number of faecal boli and urine drops were counted and recorded. Despite the simplicity of the protocol, the open field behavior is complex since most of the recorded behavioral measures are related. Therefore, in order to decrease the probability of obtaining a false positive results, the analysis of data was preceded by the examination of a correlation matrix of all variables and the redundant variables (like number of fields crossed which was highly positively correlated with the time of walking) were removed or some variables (like rearing in the periphery and wall leaning) were collapsed before the final analysis of open field data. RMANOVA with the genotype and cohort as between subjects, and days of testing as within subject (repeated measure) factors was used in the analysis of data.

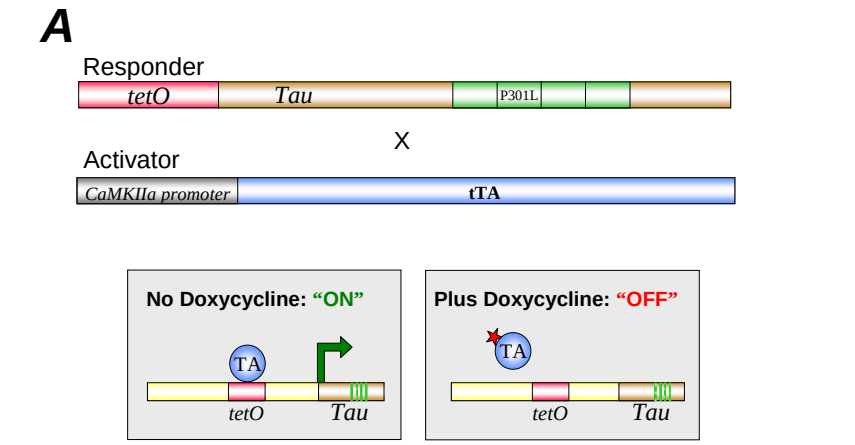
Histopathology. Formalin fixed hemibrains were processed and embedded in paraffin. Serial sections were cut at 5 microns, mounted onto CapGap slides and rehydrated according to standard protocols. Mounted slides were pretreated with a 6.0 pH citrate buffer in a Black & Decker steamer for 30 minutes with a 10 minute cool down.

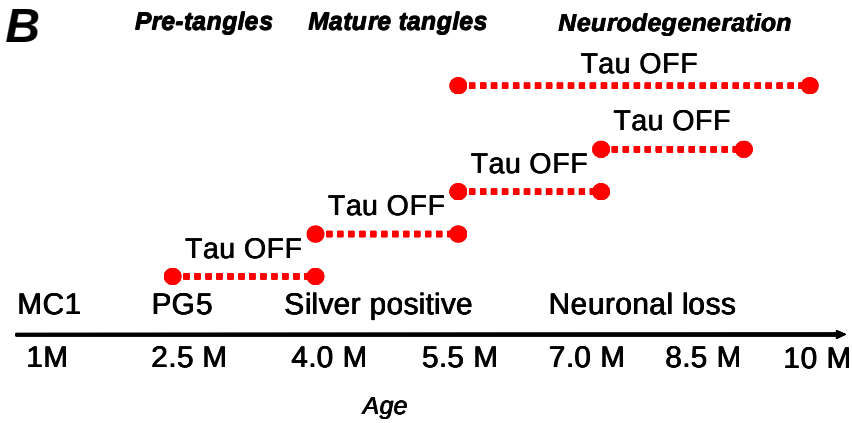
Standard 2 day immunostaining procedures using peroxidase-labeled streptavidin and DAB chromagen on an automated TechMate 500 capillary gap immunostainer (Ventana Medical Systems) were employed. Hematoxylin counterstaining was used to provide cytologic detail. Hematoxylin and eosin, Nissl, thioflavin S, Gallyas and modified Bielschowsky silver staining were performed using standard histologic techniques. Primary antibodies used included AT8 (p-202/205, Innogenetics Inc.; diluted 1:4000), AT100 (p-212/214, Innogenetics Inc.; diluted 1:1000), TG3 (p-231, generous gift of Dr. P. Davies; diluted 1:200), PG5 (p-409, Dr. P. Davies; diluted 1:200), PHF-1 (p-396/404, Dr. P. Davies; diluted 1:3000), CP-13 (p-202/204, Dr. P. Davies; diluted 1:2000) and MC1 (conformational epitope formed by tau amino acids 7-9 and 326-330, Dr. P. Davies; diluted 1:1000).

Stereology. Stereologic analysis to quantitate cell loss in a statistically "unbiased" fashion was performed using techniques previously used for other specific experimental paradigms including counting neurons in animal preparations (*6*). Paraffin-embedded hemispheres of rTg(tau_{P310L})4510 brain and control littermates were cut sagitally at 16 μm on a Leica RM2155 microtome. Every tenth section (selected on a systematic random basis) was immunostained with anti-PHF1 antibody (Dr. P. Davies) and horseradish peroxidase-conjugated secondary antibody to label phosphorylated tau then counterstained with cresyl violet. An image analysis system (CAST system) mounted on an Olympus BX51 upright fluorescence microscope, equipped with an integrated motorized stage (PRIOR Scientific), was used for these procedures. Briefly, neuronal nuclei and PHF-1 positive cell bodies were counted and densities calculated (cells/mm^3) using the optical dissector method with a counting frame of 21.8 μm x 21.8 μm placed in a systematic random fashion throughout the CA1 region of the hippocampus. CA1 volume was calculated to allow determination of neuron and PHF1-positive cell numbers in the region.

Tau filament number and length assays. Samples of sarkosyl insoluble tau were diluted another 20-fold with TBS, and 8 microliters of these samples were adsorbed onto

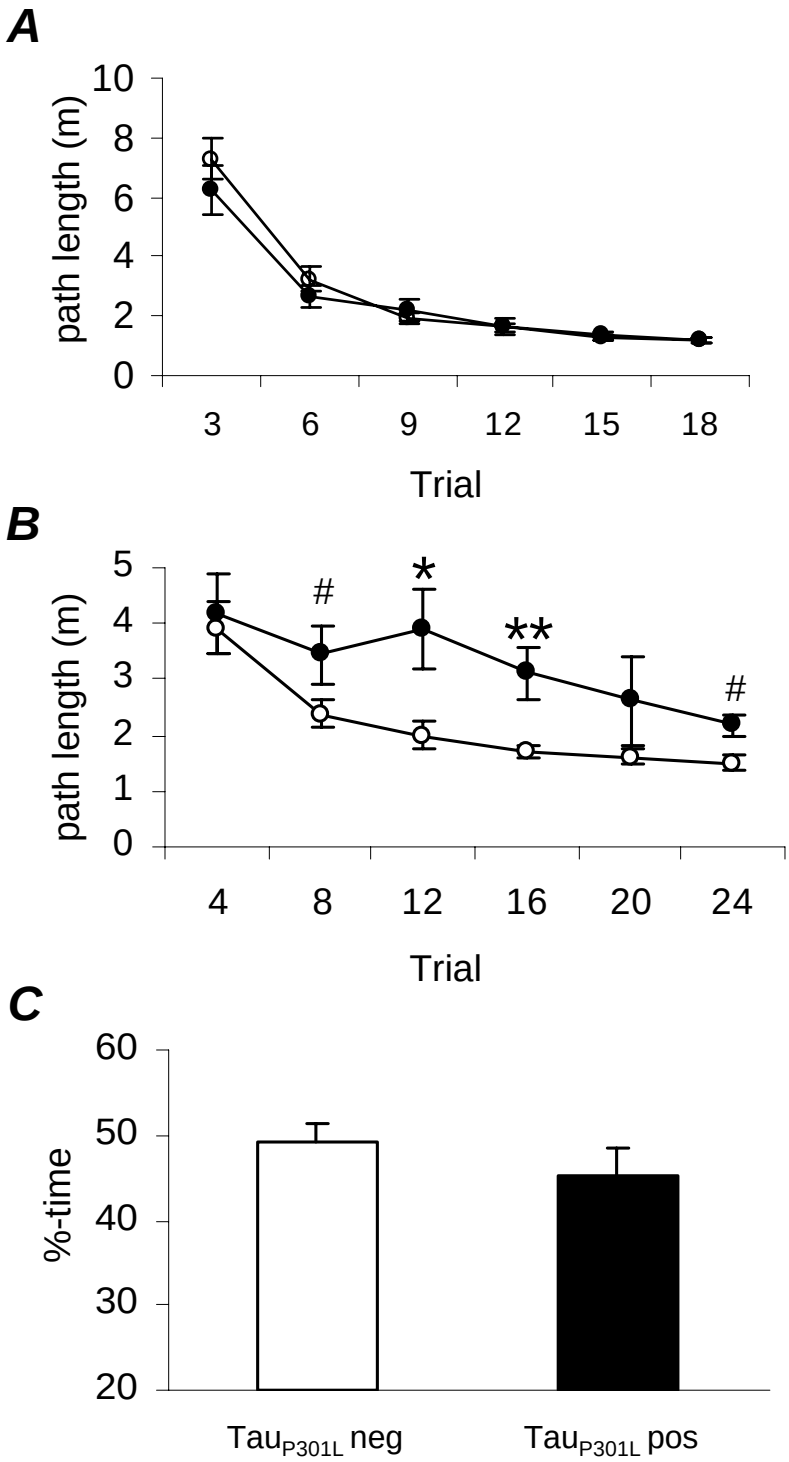
carbon-formvar grids (Electron Microscopy Sciences) for 45 seconds. To quantitate tau fibril number and length by electron microscopy using an EM 208S electron microscope (Phillips), samples were immunogold-labelled with the tau specific antibodies rabbit WKS44 (Dr. S. Yen; diluted 1/20) and mouse TAU46 (a generous gift of Dr. V. Lee; diluted 1/100) used with 5 and 10 nm gold-conjugated secondary antibodies (Amersham Pharmacia Biotech), respectively. These were also stained for 75 seconds with 2% uranyl acetate. Fields for quantitation with even staining were randomly chosen at low magnification (3500x) from preset positions, and fibrils with at least 4 gold particles were measured from at least 8 higher magnification electron micrographs (16,000x) of these areas using MCID software (Imaging Research Inc.). Filament lengths and numbers were determined for each field.





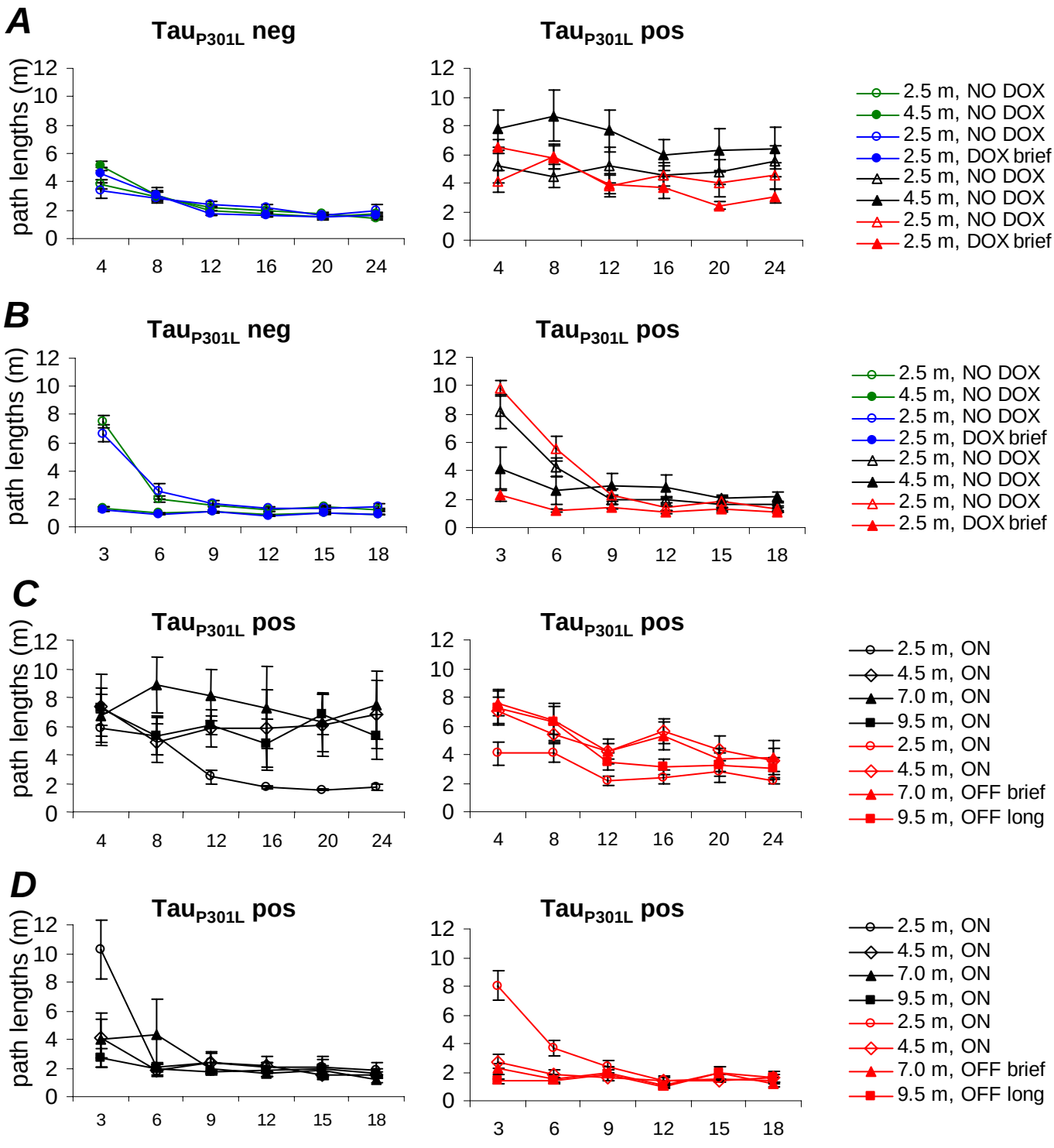
Supplementary Figure 1

Figure S1. A, The expression of tau_{P301L} is suppressed in the presence of doxycycline in mice transgenic for both responder and activator transgenes. **B,** Transgenic tau was suppressed in mice at different stages of pathology by feeding doxycycline in chow (200 ppm) for brief (6-8 weeks) or long (4-4.5 months) periods of time.



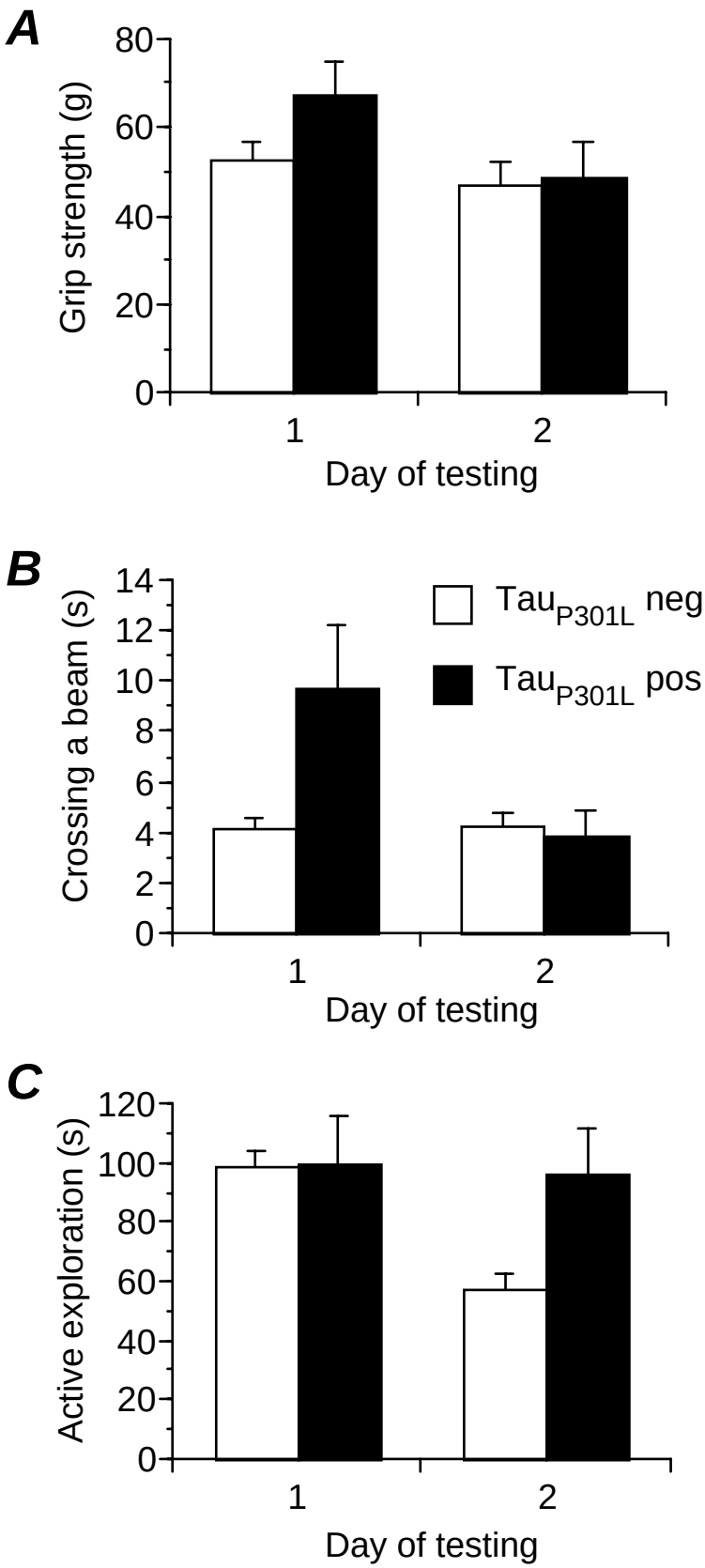
Supplementary Figure 2

Figure S2. Performance of 1.3-month rTg(tau_{P301L})4510 mice (n = 10, filled circles) and tau_{P301L} negative littermates (n = 31 consisting of 8 non-transgenic, 16 responder and 7 activator mice, open circles) in the Morris water maze. **A,** Path lengths (m = meters) in the cued (visible platform) phase of the Morris water maze showed no significant differences. **B**, Path lengths in the hidden platform phase of the Morris water maze showed a significant effect of tau_{P301L} expression (RMANOVA, $F(1,39) = 20.4$, $P<0.0001$) with increases in path lengths the tau_{P301L} positive mice. **C,** Mean target quadrant occupancy was not significantly different in tau_{P301L} positive mice compared to control littermates. $^{\#}P<0.05$, $*P<0.01$, $**P<0.001$.



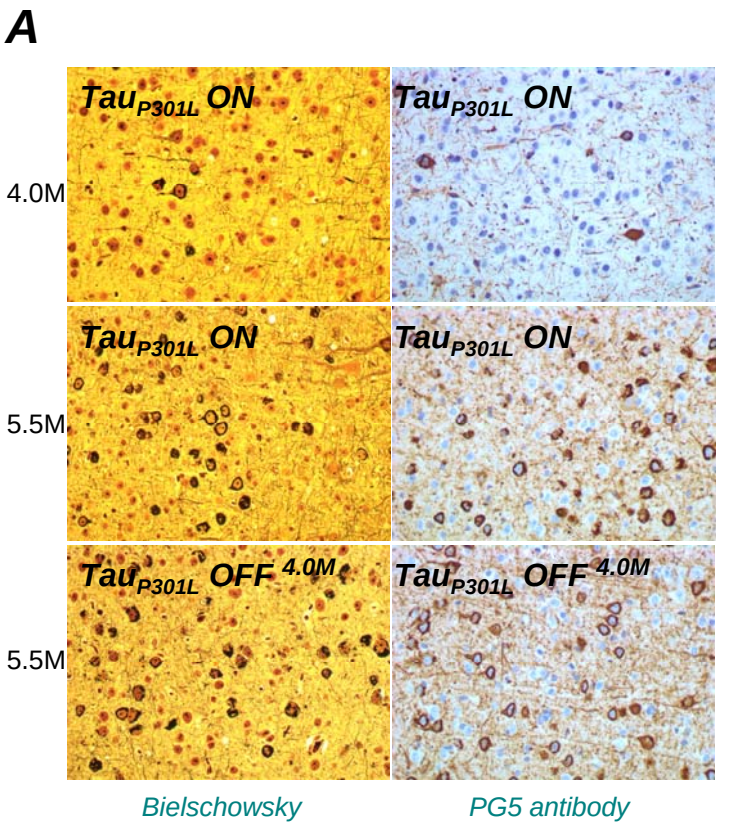
Supplementary Figure 3

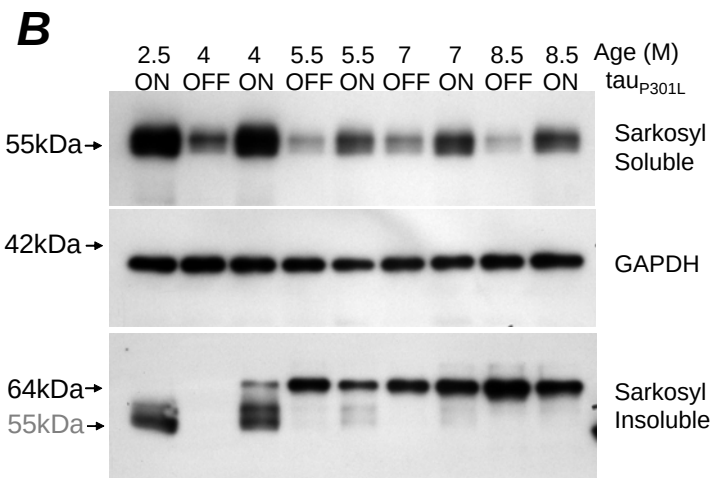
Figure S3. Spatial navigation in the hidden platform phase and performance in the cued platform phase of water maze testing of 4.5-month rTg(tau_{P301L})4510 mice fed doxycycline beginning at 2.5 months of age ($4.5M^{2.5OFF}$) and in $7M^{5.5OFF}$ and $9.5M^{5.5OFF}$ mice, compared to rTg(tau_{P301L})4510 mice without doxycycline and tau_{P301L} negative littermates. **A**, Path lengths to a hidden platform in the water maze were measured at 2.5 months prior to doxycycline and at 4.5 months after 6 weeks on doxycycline (tau_{P301L} positive, n = 10; tau_{P301L} negative, n = 13) or control feed (tau_{P301L} positive, n = 10; tau_{P301L} negative, n = 31). There was a significant age-at-testing by treatment by transgene interaction (RMANOVA, F(1,60) = 6.66, $P = 0.01$). Among the tau_{P301L} positive mice only, there was a significant age-at-testing by treatment interaction (RMANOVA, F(1,18) = 4.79, $P = 0.04$). There was no significant decrease in path lengths in mice on doxycycline (paired t(9) = 0.43, $P = 0.68$); the higher floor performance of tau_{P301L} positive mice shown in fig. S2 may contribute to the inability to detect significant improvement in this measure. Path lengths in mice without doxycycline were significantly longer (paired t(9) = 2.31, $P < 0.05$). **B,** Path lengths to a cued platform in the water maze measured at 2.5 months prior to doxycycline and at 4.5 months after 6 weeks on doxycycline or control feed showed no significant age-at-testing by treatment by transgene interaction during the final three trials of training (RMANOVA, F(1,60) = 1.93, $P = 0.17$). **C**, Path lengths to a hidden platform in the water maze in 15 mice tested at 2.5, 4.5, 7 and 9.5 months of age on doxycycline (n = 10) or control chow (n = 5) starting at 5.5 months. Path lengths of the 15 mice increased significantly between 2.5 and 4.5 months of age (paired t(14) = 4.46, P = 0.0005). Path lengths of mice on doxycycline at 7 or 9.5 months of age were not significantly shorter than those of 4.5-month mice. **D,** Path lengths during the final three training trials to a cued platform in the water maze in mice tested at 2.5, 4.5, 7 and 9.5 months of age, which were on doxycycline (n = 10) or control chow (n = 5) starting at 5.5 months, remained unchanged. **E**, No significant effects of doxycycline on tau_{P301L} negative littermates (n = 26) in the probe phase of the water maze, or in the cued or hidden platform phases of the water maze.



Supplementary Figure 4

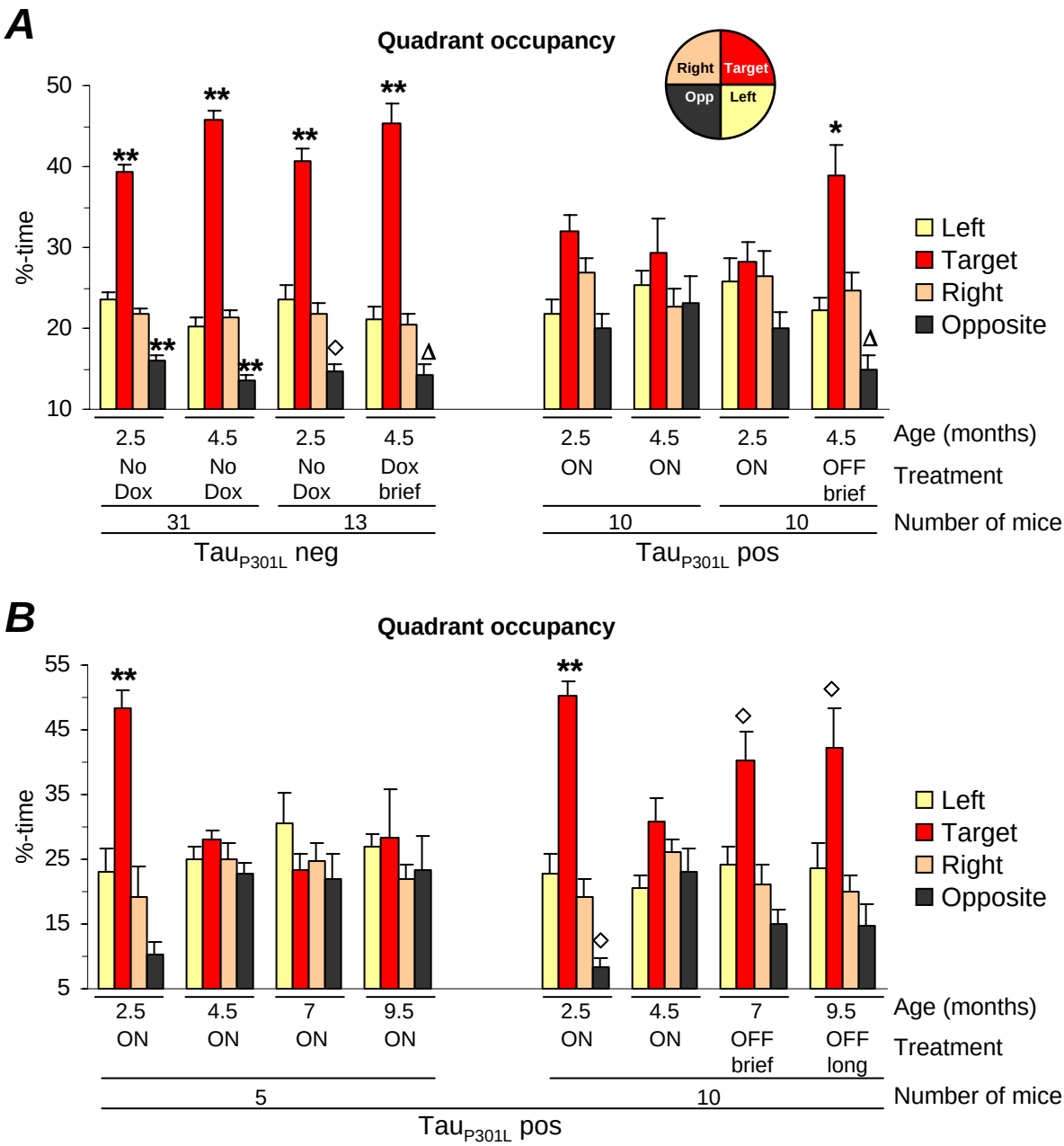
Figure S4. Tau_{P301L} positive mice (n = 8) and control littermates (n = 18 consisting of 10 non-transgenic and 8 activator mice) showed comparable locomotor propensity. The 4- to 6-month mice were tested in a battery of behavioral tests evaluating their motor characteristics. All mice responded to an auditory stimulus by backwards flick of pinnae, and showed a negative geotaxis response when placed on a vertically inclined wire mesh. **A,** Grip testing showed no differences in the grip strength between tau_{P301L} positive and negative mice ($F(1,24) = 2.8$, $P > 0.05$), or testing days ($F(1,24) = 3.1$, $P > 0.05$), and no significant factor interaction. **B,** In the beam crossing task the analysis of average time (s) of traversing the beam revealed significant genotype ($F(1,24) = 4.7$, $P = 0.04$), day ($F(1,24) = 12.5$, $P < 0.01$) and genotype by day interactions ($F(1,24) = 13.5$, $P < 0.001$). Tau_{P301L} positive mice walked on a beam longer only during the first day while traversing a wider, 2.5 cm beam. During the second day, when a more challenging task of traversing a narrower (1.9 cm) beam was administered, mice of both genotypes traversed the beam in a comparable time, and showed similar rates of falling. The tau_{P301L} positive mice exhibited longer pauses during walking on a beam which resulted in significantly longer latencies to complete the task, which may be attributed to compromised cognitive abilities rather than to physical inability to traverse the beam. **C**, The average locomotor activity of the mice in the open-field test revealed a genotype trend ($F(1,24) = 3.8$, $P = 0.06$), and a significant day effect ($F(1,24) = 9.5$, $P < 0.01$). Mice responded differently to repeated exploration of the open-field arena ($F(1,24) = 6.7$, $P < 0.05$; genotype by day interaction effect). Overall locomotor activity of tau_{P301L} positive and tau_{P301L} negative mice was comparable, decreasing on day 2 due to a habituation to the novelty of the arena. Tau_{P301L} positive mice show less reduction in their locomotor activity during day 2 (reflected by a significant genotype by day interaction), which likely contributed to a borderline significance between the genotypes. This lack of habituation over consecutive exploration sessions further points to compromised cognitive ability of the tau_{P301L} positive mice. The genotypes did not differ in their anxiety-like response to a novel environment and all mice had comparable latencies to enter and explore the central fields of the apparatus.





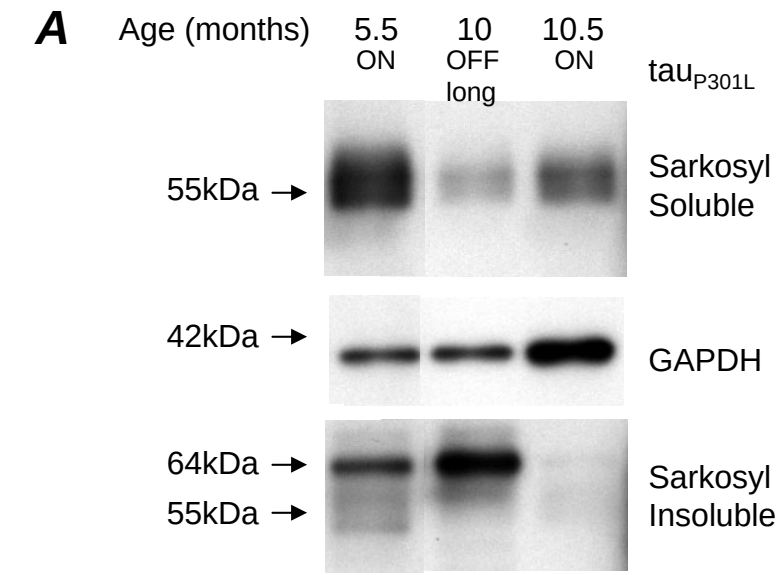
Supplementary Figure 5

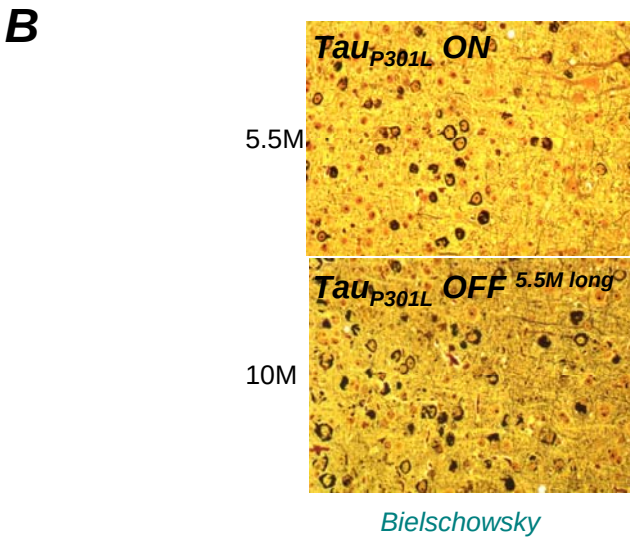
Figure S5. A, The histological appearance of representative cortical brain tissue sections shows a failure of tau_{P301L} suppression to halt the accumulation of tangles that are argyrophilic or that react with PG5 antibody in 5.5-month mice started on doxycycline at 4 months of age. **B,** Representative immunoblots of sarkosyl-soluble and sarkosyl-insoluble tau_{P301L} proteins in forebrain homogenates of mice given or not given doxycycline for 6 weeks. GAPDH was probed for loading consistency.

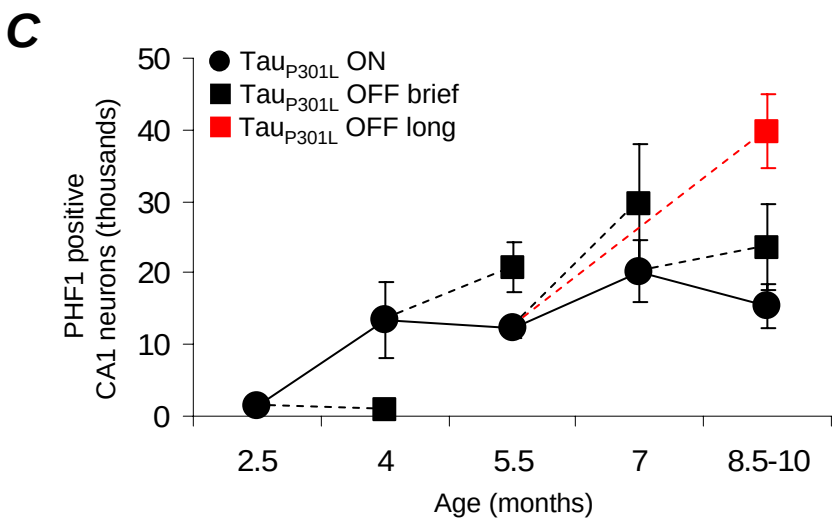


Supplementary Figure 6

Figure S6. Quadrant occupancy during probe trials (means of four trials) of the water maze in rTg(tau_{P301L})4510 mice fed doxycycline beginning at 2.5 months of age ($4.5M^{2.5OFF}$) and at 5.5 months of age ($7M^{5.5OFF}$ and $9.5M^{5.5OFF}$), compared to rTg(tau_{P301L})4510 that had not received doxycycline and to tau_{P301L} negative littermates. **A,** Percentage time spent in each of four quadrants at 2.5 months in mice comprising the bottom performing half of a 2.5-month cohort, and at 4.5 months after 6 weeks on doxycycline or control chow. Tau_{P301L} positive mice displayed a significant positive search bias for the target quadrant only after tau was suppressed. Tau_{P301L} negative mice which were performing in the bottom half of a 2.5-month cohort displayed a positive search bias for the target quadrant at 2.5 months of age and also at 4.5 months after receiving doxycycline or control chow. **B,** Percentage time spent in each of four quadrants was determined at four ages in tau_{P301L} positive mice which were in the top performing half of their cohort at 2.5 months of age. Doxycycline or control chow was administered at 5.5 months. There was a loss of a significant search bias for the target quadrant when mice were tested at 4.5 months of age. Mice receiving doxycycline starting at 5.5 months displayed a significant search bias for the target quadrant when subsequently tested at 7 and 9.5 months of age, while mice receiving control chow continued to display no search bias for the target quadrant. Differences between percentage time spent in the target and opposite quadrants compared with all other quadrants was determined using an ANOVA with Fisher's PLSD *post hoc* analysis (Δ $P<0.05$; $\diamondsuit P<0.01$; $*P<0.001$; $**P<0.0001$).

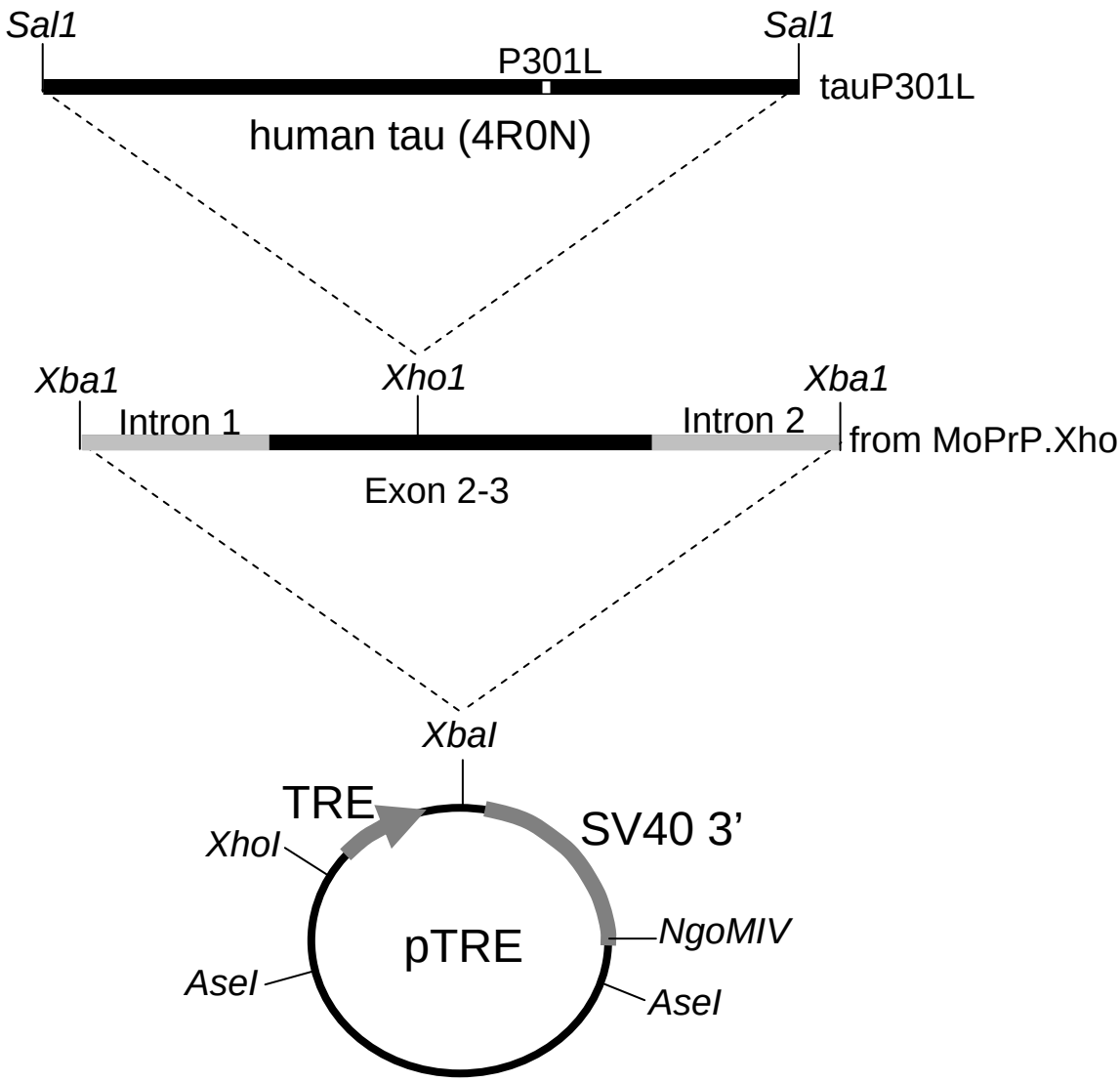






Supplementary Figure 7

Figure S7. A, Representative immunoblots of sarkosyl-soluble and sarkosyl-insoluble tau_{P301L} proteins in forebrain homogenates of a 5.5-month rTg(tau_{P301L})4510 mouse, a 10-month rTg(tau_{P301L})4510 mouse on doxycycline beginning at 5.5 months of age, and a 10.5-month rTg(tau_{P301L})4510 mouse. GAPDH was probed for loading consistency. The 64kDa sarkosyl-insoluble tau species in the 10-month mouse on doxycycline is more abundant than in the 5.5-month mouse continuously expressing tau_{P301L}. The inhibition of transgenic tau expression for over 4 months significantly diminished the expression of soluble tau, but failed to suppress the accumulation of insoluble tau. The reduction in both sarkosyl-soluble and sarkosyl-insoluble tau in 10.5-month mice continuously expressing tau_{P301L} reflects the loss of neurons. **B**, Representative photographs of histological sections from a 5.5-month rTg(tau_{P301L})4510 mouse and a 10-month rTg(tau_{P301L})4510 mouse on doxycycline beginning at 5.5 months of age, showing progression of pathological changes detected by Bielschowsky staining. **C,** The average number of PHF-1 positive CA1 neurons in one hemisphere of three 10-month rTg(tau_{P301L})4510 mice on doxycycline beginning at 5.5 months (red square) is significantly higher than in three 5.5-month rTg(tau_{P301L})4510 mice continuously expressing transgenic tau.



Supplementary Figure 8

Figure S8. The tau_{P301L} responder transgene construct.

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