9. Supplementary Data

9.1. Supplementary Methods

9.1.1. Filter trap assay for fiber remodeling

Fibers of Aβ (2.5 μM) or tau (1 μM) were incubated with various concentrations of NPT088 or buffer, and then were applied to cellulose acetate membrane. Fibrillar protein amyloid aggregates are retained on the membrane while amorphous, non-fibrillar aggregates pass through the membrane. Membranes were blocked with 5% milk in PBS prior to detection of protein aggregates. Aβ protein aggregates were detected with 6E10 (1:80,000; Covance cat #: SIG-39300 or SIG-39320) and aggregates of the microtubule binding region (MTBR, amino acid residues 243-375 from the 2N4R tau isoform) of tau were detected with anti-myc (tau, 1:1,000). Primary antibodies were diluted in the blocking buffer. Antibody binding to protein aggregates was detected with HRP-tagged goat anti-mouse IgG (1:50,000; Jackson ImmunoResearch, Cat# 115-035-062).

9.1.2. In vitro tau seeding assay

Transduction protocols were described in details from Guo and Lee [56]. Briefly, Tau441-P301L was transfected in HEK-293 cells (ATCC CRL 1573) using Fugene 6 (Promega) one day prior to fibril transduction. Tau-K18P301L or MTBR pre-formed fibrils were sonicated and packaged into Bioporter reagent (Gelantis) prior adding to Tau-transfected cells. 24 hours post-transduction, cells were fixed in 4% PFA with 1% Triton-X-100 and stained with Tau 5, AT8, or MC1 antibodies. Goat anti mouse Alexafluor 488 secondary antibodies (Invitrogen) were used to visualize primary antibody binding. Images were captured by Zeiss AxioPlan2 upright microscope at 20X and 40 X magnifications.

9.1.3. Quantification of NPT088 in Tg2576 brain homogenates

9.1.3.1. Brain tissue preparation

Brain tissue (left frontal cortex) was homogenized in cold PBS using Pure M-Bio Grade beaded tubes and a Precellys024 Lysis Homogenizer (5,000 RPM twice for 20 sec, with a 5 sec interval between homogenization cycles). Homogenate was spun at 14,000 rpm for 5 min at 4 °C. Supernatant was removed to a new tube and used for all subsequent analyses. Protein content of brain lysate was determined using a Pierce BCA protein assay kit. Lysate was used at a 1:2 dilution.

9.1.3.2. NPT088 ELISA

NPT088 was quantified in brain lysates using a sandwich ELISA assay. MaxiSorp plates were coated with rabbit anti-M13 (Abcam: ab6188) at 1:1,000 (3.7 µg/mL, 0.37 µg/well) overnight in carbonate buffer (pH 9.6) at 4 °C. Plates were washed 3x with PBS containing 0.1% Tween-20 (PBST) and blocked with 1% milk in PBS for 2h at 37 °C followed by 1h at RT. After 3x washes with PBST, samples or standards were added to wells and incubated for 1h at 37 °C. After 3x washes with PBST, wells were incubated with HRP-labeled goat anti-Human IgG (heavy & light chains, Jackson ImmunoResearch: 709-035-149; 1:10,000) for 30 min at RT. After 3x washes with PBST, plates were developed at RT with TMB substrate. Reactions were stopped after the A450 of the highest standards was between 0.6 – 0.8. Levels of NPT088 were quantified from the absorbance read at 450 nm, minus the reference absorbance at 650 nm. Lysates were analyzed at a 1:2 dilution; no matrix interference was observed at a dilution of 1:2. Levels of NPT088 in brain are expressed relative to protein content of lysates.

9.1.4. Perls staining

The Perls reaction [70] was used to visualize hemosiderin. Sites of hemosiderin-positive material were counted in a series of sections from each brain at intervals of 960 µm representing all major brain regions from olfactory bulb through the brainstem. Sites were extremely rare, so discrete sites were counted without regard to size or intensity.

9.1.5. Quantification of total and phospho-Tau in brain

9.1.5.1. Tissue homogenization and fractionation

Frozen brain tissue was thawed on ice, weighed and homogenized based on adapted protocols previously published [71, 72]. Brains were homogenized at a ratio of 1mL buffer: 150 mg tissue. Homogenization buffer consisted of: 10 mL high salt Tris-NaCl buffer (pH 8.0), 1 Complete ULTA tablet (Roche 05-892-970-001), 1 phosSTOP tablet (Roche 04-906-845), 0.5 mL 5M EDTA. Brains were sonicated on ice (10 sec, as above), divided 80:20 and both aliquots spun at 13,000xg for 30 min at 4 °C. The supernatant (HS=”high salt soluble”) was collected and the remaining pellet (NFT= “neurofibrillary tangle containing high salt insoluble fraction”) from the 20% aliquot was cleaned by a methanol/chloroform organic extraction step. The 80% HS aliquot pellet was washed, resuspended in 4x-volume of homogenization buffer (see above) with 1% Triton-X, and further homogenized on ice. The homogenate was spun at 45,000xg for 15 min at 4 °C. The supernatant (TX=”Triton-X soluble”) was collected and the resulting pellet was washed and further homogenized in 4x-volume RIPA+PPIs. Homogenate was spun at 45,000xg for 30min at 4 °C. The supernatant (R=”RIPA soluble”) was collected and the pellet was washed

and homogenized in 2% SDS (4x-volume). Homogenate was spun at 45,000xg for 30min at 4 °C. The supernatant (SDS="SDS soluble") was collected.

9.1.5.2. Western Blotting

Levels of total tau and several phospho-tau (p-tau) epitopes were quantified in brain lysates from the five homogenization steps above (HS, TX, RIPA, SDS and NFT) by western blot (WB). Antibodies used were as follows [epitope recognized]: Tau5 [human tau 210-241] (Fisher), AT8 [pSer202/pThr205] (Pierce), pSer422 [pSer422] (Abcam) and AT270 [pThr181] (Fisher). GAPDH (Cell Signaling) was used as a loading control. Samples were loaded by protein content (7µg) into 10% SDS-PAGE gels. Protein concentration was determined by BCA. Gel loading was randomized and balanced for genotype and treatment across multiple gels in a blinded fashion. Each gel was run with a molecular weight marker (Precision Plus), and a positive and negative quality control (QC) sample. QC(+) consisted of 5 pooled non-study rTg4510 mice and QC(-) was 5 pooled non-study WT mice. For a particular antibody, all gels were run and then transferred to nitrocellulose membrane (BioRad Mini transfer packs). Blots were washed in TBS+ 1% Tween (TTBS) and blocked for 60 min in TTBS+ 5% milk. After blocking, blots were incubated overnight at 4°C on a shaker in primary antibody diluted 1:1K with blocking buffer. The following day, blots were washed 6x5min in TTBS, incubated in an appropriate secondary antibody (anti-mouse IgG or anti-rabbit IgG, 1:10K) diluted in blocking buffer for 60 min at RT. Blots were washed 6x5min in TTBS prior to detection by ECL. Blots were exposed for 30 sec and visualized using a BioRad Chemi-Doc MP Imaging System. Tau and p-tau bands running at either 64kDa or 55kDa were quantified using BioRad ImageLabv4.1 software. Analysis of experiments with 2 treatment groups (i.e., **Supplemental Figure 7A-C**) were made using Student's t-Test with Welch's correction for unequal variances, while analysis of experiments with multiple treatment groups (i.e., **Supplemental Figure 7D-F**) was performed using a 1-way ANOVA and post hoc Dunnett's test.

9.2. Supplemental Figures

Supplemental Figure 1. Presence of hAPP in Aβ aggregates. Formic acid lysates from aged WT and Tg2576 mouse brain were resolved on 3-8% tris acetate gels under reducing (lithium dodecyl sulfate [LDS] sample buffer + reducing agent, heat at 70^{o}C for 10 minutes prior to loading) and non-reducing (Native Tris-Glycine sample buffer) conditions, transferred to nitrocellulose, and proteins detected using the anti-Aβ monoclonal antibody 6E10, which also recognizes hAPP. Under native conditions, formic acid lysates have high molecular weight Aβ that does not enter the gel, along with detectable levels of hAPP and Aβ. Under reducing conditions, no high molecular weight Aβ is detected and relatively higher levels of hAPP and Aβ are observed. These observations support the hypothesis that Aβ and hAPP are present in aggregates in the FA fraction.

Supplemental Figure 2. NPT088 remodels Abeta and Tau fibers. **A**) $A\beta_{42}$ fiber preparations (2.5 μM) were incubated with different concentrations of NPT088 and applied to a cellulose acetate membrane, which selectively retains fibrillar aggregates of amyloids. NPT088 remodels fibers in a concentration-dependent manner. **B**) Remodeling of Aβ42 fibers by NPT088 is a concentration- and time-dependent process. Maximal remodeling of fibers occurred after a 2 day incubation with NPT088 at all doses tested. Remodeling was assessed via cellulose acetate filter trap assay. N=3 for each time point. **C**) NPT088 remodeled fiber preparations of the tau MTBR (1 μM; amino acids 243-375 of the 2N4R isoform of tau) in a dose-dependent manner.

Supplemental Figure 3. Tau fibers remodeled by NPT088 are transmission-competent. Tau441-P301L was transfected into HEK-293 cells (ATCC CRL1573) one day prior to fibril transduction. Tau-K18P301L (**B**) or MTBR (**C**) pre-formed fibrils (PFFs) were sonicated, packaged into Bioporter reagent and transduced into HEK cells. Cells were fixed in 4% PFA with 1% TritonX-100 one day post-transduction and stained with Tau5, AT8, or MC1. Upper panel shows formation of insoluble tau inclusions in only K18P301L-(B), and MTBR(C)-PFF-transduced cells. Lower panels show tau inclusions in higher magnification which are insoluble (**D**,**G**) (1% TX extraction followed by Tau 5 staining), phosphorylated (**E**, **H**) (AT8) and misfolded (**F**, **I**) (MC1).

Supplemental Figure 4. Levels of NPT088 in aged Tg2576 mouse brain after a single IP injection. Aged (19-20 mo) male Tg2576 mice received a single, IP injection of NPT088 (10 mg/kg). Brain tissue was harvested 12h, 1d, 3d and 14d after injection after thorough, transcardial perfusion to eliminate vascular NPT088. Levels of NPT088 peaked in frontal cortex 1d after IP injection at 4.7 ng NPT088 per mg protein (±1.3 ng/mL SEM). N=3 mice per time point.

Supplemental Figure 5. Systemic administration of NPT088 did not increase microhemorrhage in aged Tg2576 mice. IP administration of NPT088 over 14 weeks had no effect on microhemorrhage levels as assessed using Perls staining. ($p = 0.2$). Data were analyzed using a Kruskal-Wallis test due to differences in variance across groups.

Supplemental Figure 6. Normalization of cognition in rTg4510 mice was not associated with a change in locomotor activity. **A)** Treatment with NPT088 did not affect distance traveled during performance of novel object recognition by rTg4510 mice ($F_{2,10}$ = 0.2, P=0.8). **B)** There was no significant correlation between novel object recognition and distance traveled during performance of the task (R=0.3, P=0.3).

Supplemental Figure 7. Systemically administered NPT088 reduces levels of p-tau in rTg4510 mice. Levels of p-tau were assessed in various biochemical fractions isolated from brain tissue of rTg4510 mice (3.5 mos) that received 14 weeks of weekly IP treatment with NPT088 or PBS. **A**) Significant decreases in levels of AT8 p-tau were observed in the high-salt insoluble, NFT-enriched fraction from cortex ($t=2.4$, $df=4.2$, $P=0.04$). Representative western blots shown above summary quantification. N= 5 (Tg-PBS), 4 (Tg-NPT088). Asterisk indicates significant difference from rTg4510-PBS. **B**) Significant decreases in levels of pSer422 Tau were observed in the Triton-X soluble fraction from cortex ($t=2.0$, $df=8$, $P=0.04$). Representative western blots shown above summary quantification. N= 5 (Tg-PBS), 5 (Tg-NPT088). Asterisk indicates significant difference from rTg4510-PBS. **C**) Decreases in levels of AT270 p-tau were observed in the Triton-X soluble fraction from cortex ($t=1.7$, $df=5$, $P=0.08$). Representative western blots shown above summary quantification. N= 4 (Tg-PBS), 3 (Tg-NPT088). **D**) A significant decrease in levels of AT8 p-tau were observed in the high-salt insoluble, NFT-enriched fraction from cortex at all doses of NPT088 tested ($F_{3,20} = 4.2$, $P=0.02$). N= 8 (rTg4510-PBS), 5 (rTg4510-0.2 mg/kg), 5 (rTg4510-2 mg/kg), 6 (rTg4510-20 mg/kg). **E**) A significant decrease in levels of pSer422 Tau were observed in the Triton-X soluble fraction from cortex after treatment with 20 mg/kg NPT088 ($q_{20} = 1.8$, $P=0.05$). N= 8 (rTg4510-PBS), 5 (rTg4510-0.2 mg/kg), 5 (rTg4510-2 mg/kg), 6 (rTg4510-20 mg/kg). **F**) A significant decrease in levels of AT270 p-tau were observed in the Triton-X soluble fraction from cortex after treatment with 20 mg/kg NPT088 ($q_{21} = 2.6$, $P=0.03$). N= 8 (rTg4510-PBS), 5 (rTg4510-0.2 mg/kg), 5 (rTg4510-2 mg/kg), 7 (rTg4510-20 mg/kg).