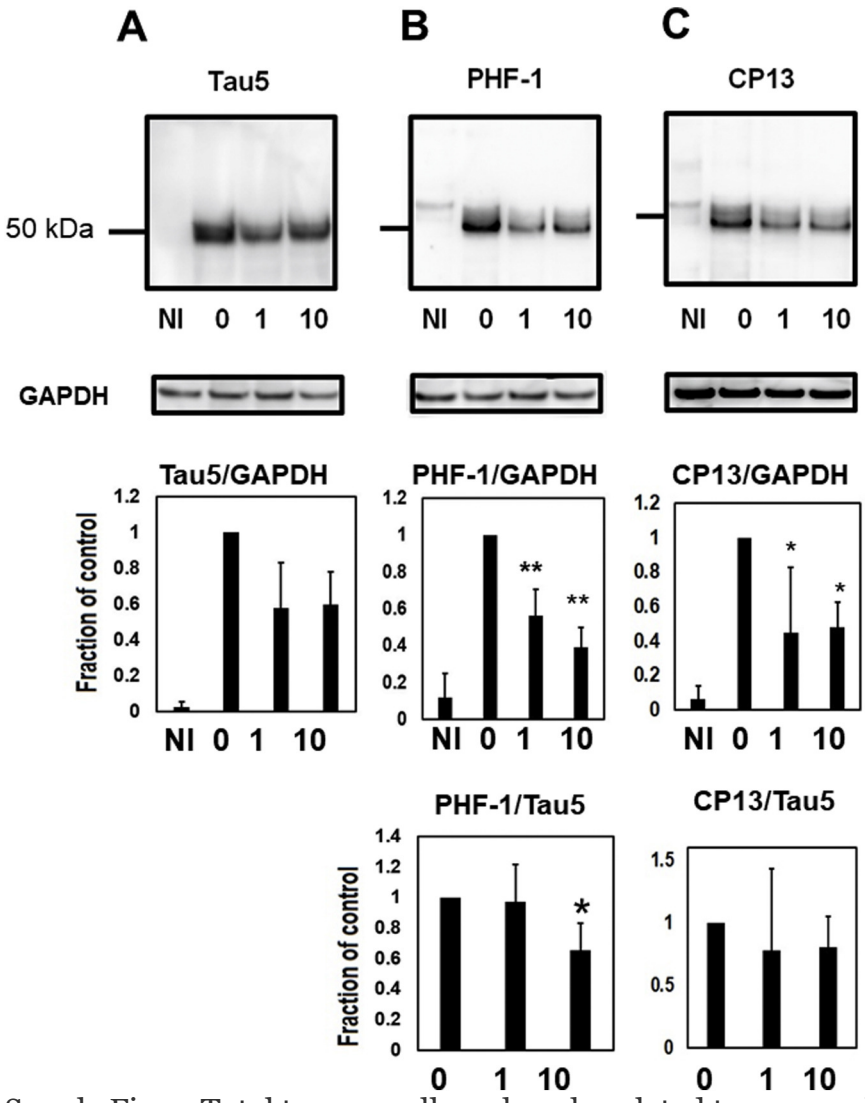
Supple Method

Thioflavin S staining of P301L mouse Hemisphere sections were mounted onto a glass slide and allowed to air dry prior to staining. After washing with 70, and 80% EtOH, the slides were incubated in filtered thioflavin S (Sigma) solution (1% in 80% Et-OH) for 15 min. Slides were incubated with 80% EtOH for 1 min, and then washed with DDW twice (Ly et al., 2011).

Reference

Ly, P.T., Cai, F., Song, W., 2011. Detection of neuritic plaques in Alzheimer's diseasemouse model. J. Vis. Exp. 26, pii: 2831.

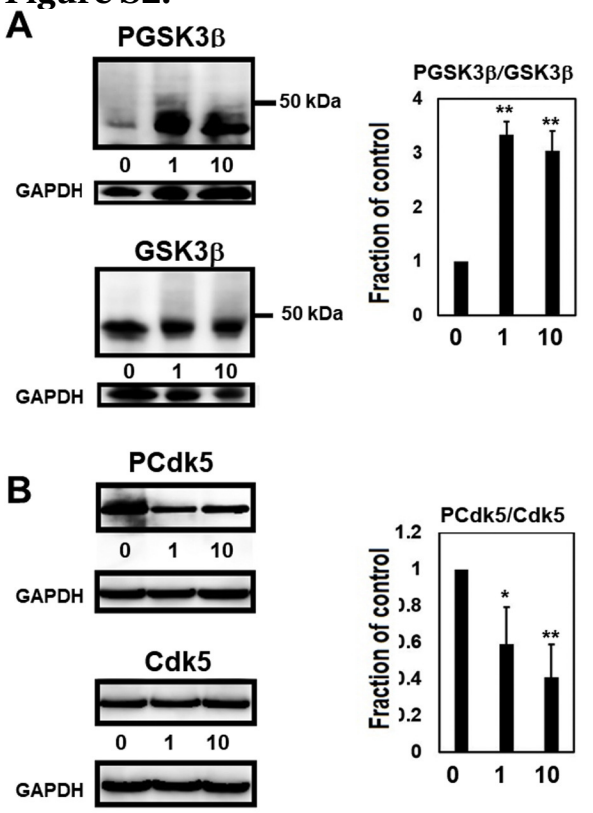
Supplemental Figures
Figure S1.



Supple Fig. 1. Total tau, as well as phosphorylated tau was reduced by the ROCK inhibitor (Y-27632) in a dose-dependent manner. To examine the effects of ROCK inhibitors on tau, M1C cells were induced to express tau for 5 days and exposed to the ROCK inhibitor (1 or 10 μM) during the final day of the induction period. Control cultures were treated with dimethyl sulfoxide (DMSO), the vehicle used to dilute the ROCK inhibitor. Lysates from cultures were analyzed by Western blotting using the antibody Tau5, PHF-1, and CP13. These samples were probed with anti-GAPDH to confirm that loading among lanes were equal. Total tau levels were reduced in a dose dependent manner following ROCK inhibitor treatment. In cultures treated with 1 μM ROCK inhibitor, Tau 5 detected 45-60-kDa bands at 57.9% of the levels exhibited by the vehicle control. Phosphorylated tau detected by PHF-1, and CP13 were also reduced by ROCK inhibitor. NI, non-induced cells, 0: 0 μM ROCK inhibitor, 1: 1 μM ROCK inhibitor, 10: 10 μM ROCK Inhibitor. N = 5, $**p < 0.01$, $*<0.05$, Bar: ± SD. Data from Tau5/GAPDH, PHF-

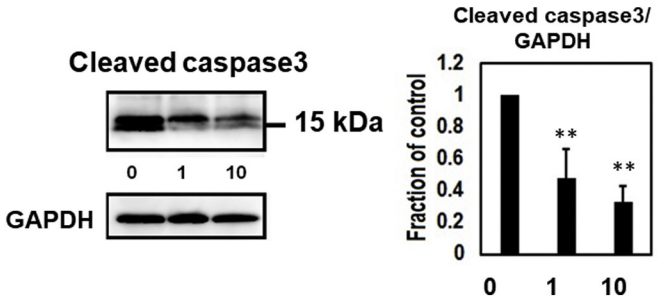
1/GAPDH, and PHF-1/Tau5 followed a normal distribution and were analyzed with one-way ANOVA followed by Bonferroni's post-hoc test, while Kruskal Wallis test followed by Dunn's post hoc test was used for CP13/GAPDH and CP13/Tau5 since the data deviated from a normal distribution.

Figure S2.



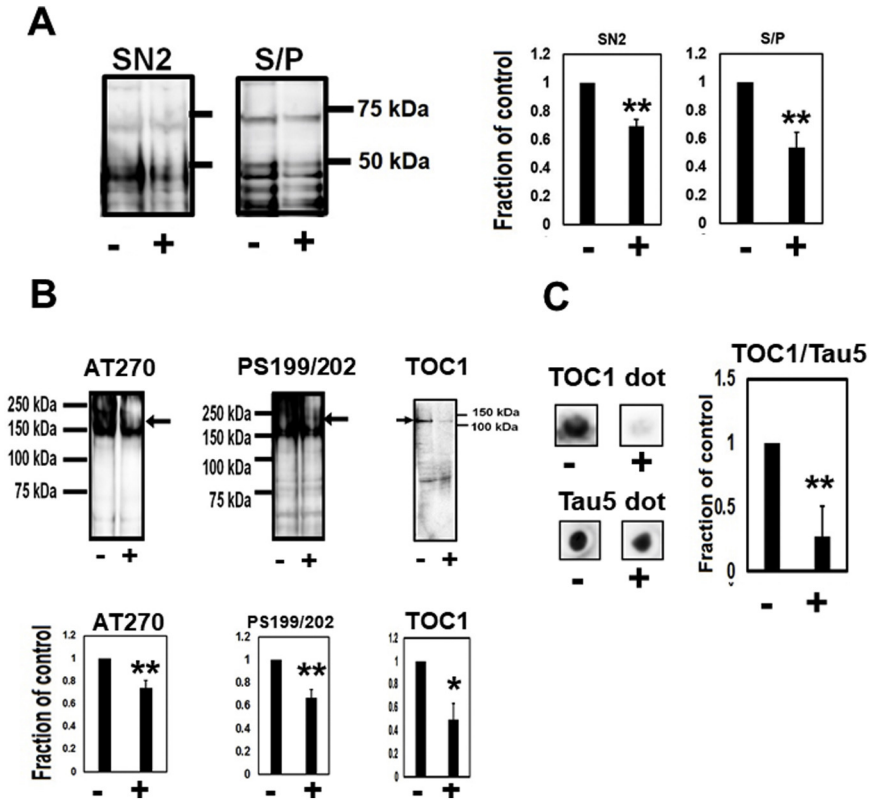
Supple Fig. 2. Tau kinases were inactivated by the ROCK inhibitor (Y27632). GSK3β was inactivated by the ROCK inhibitor (A). 0: 0 μM ROCK inhibitor, 1: 1 μM ROCK inhibitor, 10: 10 μM ROCK inhibitor. Cdk5, another tau kinase, was inactivated by ROCK inhibitor treatment (B). $**p < 0.01$, $*<0.05$, Bar: ± SD. Data from PGSK3β/GSK3β and PCDK5/CDK5 followed a normal Distribution and were analyzed with one-way ANOVA followed by Bonferroni's post-hoc test.

Figure S3.



Supple Fig. 3. ROCK inhibitor inactivated caspase-3 (Y27632). The amount of cleaved caspase-3 was reduced by the ROCK inhibitor. 0: 0 μM ROCK inhibitor, 1: 1 μM ROCK inhibitor, 10: 10 μM ROCK Inhibitor. N = 4, **p < 0.01, Bar: ± SD. Data from cleaved caspase3 followed a normal distribution and were analyzed with one-way ANOVA followed by Bonferroni's post-hoc test.

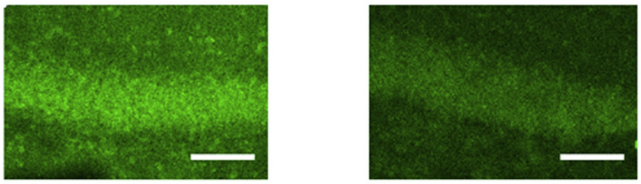
Figure S4.



Supple Fig. 4. Fractionation study revealed that the ROCK inhibitor (Y-27632) reduced tau in the Tris insoluble, sarkosyl-soluble fraction (SN2) and sarkosyl insoluble fraction (S/P). N = 5, **p < 0.01, Bar: ± SD (A). The ROCK inhibitor (Y-

27632) reduced the amount of phosphorylated high- molecular weight tau (AT270, PS199/202) (arrow). The ROCK inhibitor (Y-27632) also reduced the amount of tau oligomer complex 1 (TOC1) positive oligomeric tau in the Tris-insoluble, sarkosyl- soluble fraction under non-reducing conditions. N = 4, **p < 0.01, *<0.05, Bar: ± SD (B). Dot blot analysis using lysate confirmed the reduction of oligomeric tau by ROCK inhibitor treatment. -: DMSO control, +: 1 μM of ROCK inhibitor (Y-27632). N = 3, **p < 0.01, Bar: ± SD (C). Data from Tau5 from SN2, and SP fraction, phosphorylated high-molecular-weight tau (AT270, PS199/202, and TOC1), and dot blotanalysis of TOC1/Tau5 followed a normal distribution and were analyzed with Student's t-test.

Figure S5.



Supple Fig. 5. ROCK inhibitor reduced thioflavin S staining in P301L mice Thioflavin S staining was reduced by ROCK inhibitor (fasudil) treatment in the cerebral cortex.