Electronic Supplementary Material (ESI) for Metallomics.

Supplementary Material

Targeting metals rescues the phenotype in an animal model of tauopathy

Authors:-

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Supplementary Figure 1. Nuclear Magnetic Resonance (NMR) data for PBT2 and Met-PBT2.

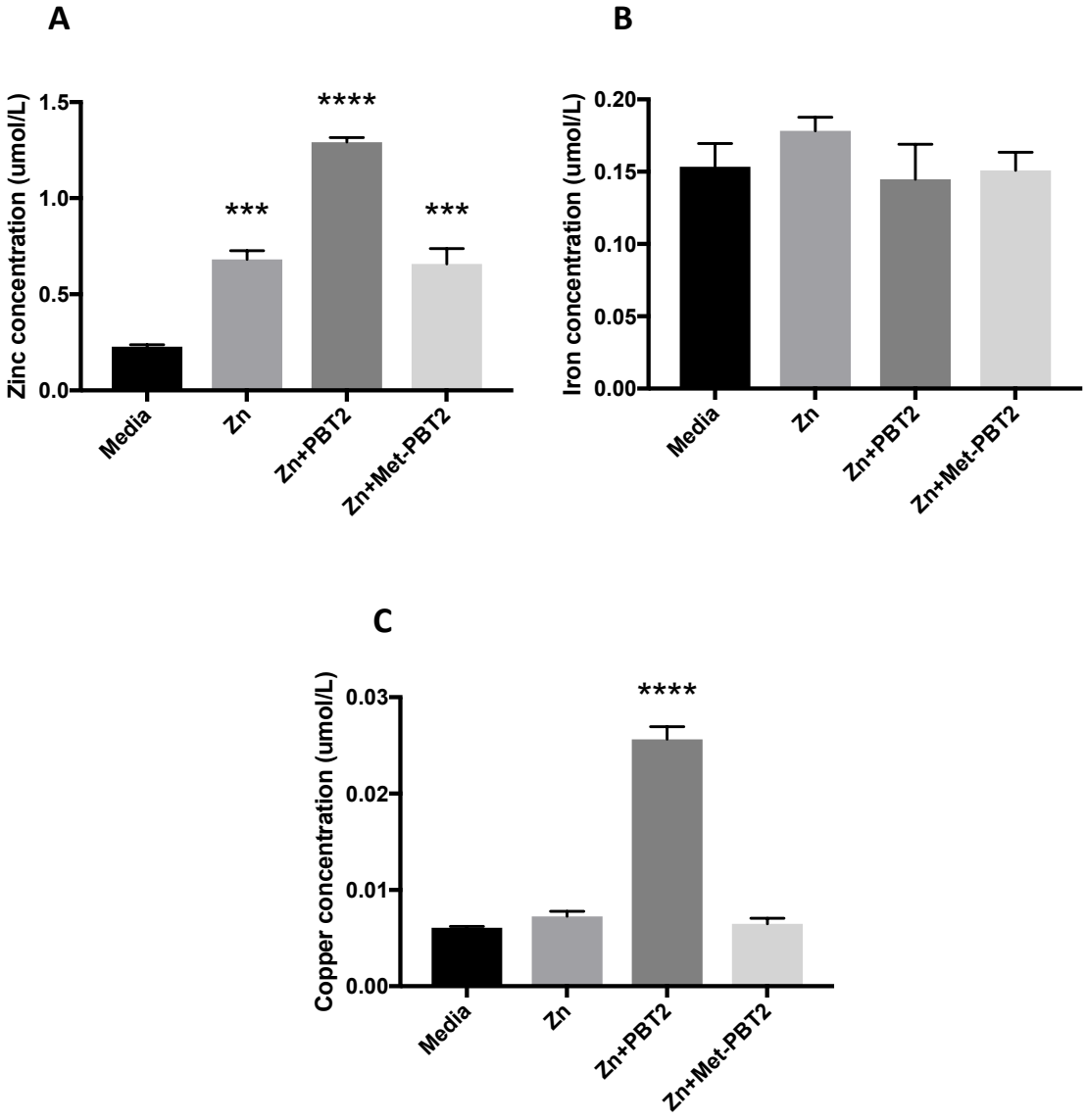
PBT2

5,7-Dichloro-2-((dimethylamino)methyl)quinolin-8-ol-hydrochloride (PBT2):1H NMR (400 MHz, d6-DMSO) δ 2.91 (s, 6H, NMe_2), 4.81 (s, 2H, CH_2N), 7.75 (d, *J*=8.4Hz, 1H, ArH), 7.89 (s 1H, ArH), 8.59 (d, *J*=8.4Hz, 1H, ArH), 10.49 (s, 1H), 10.78 (s, 1H).

Met-PBT2

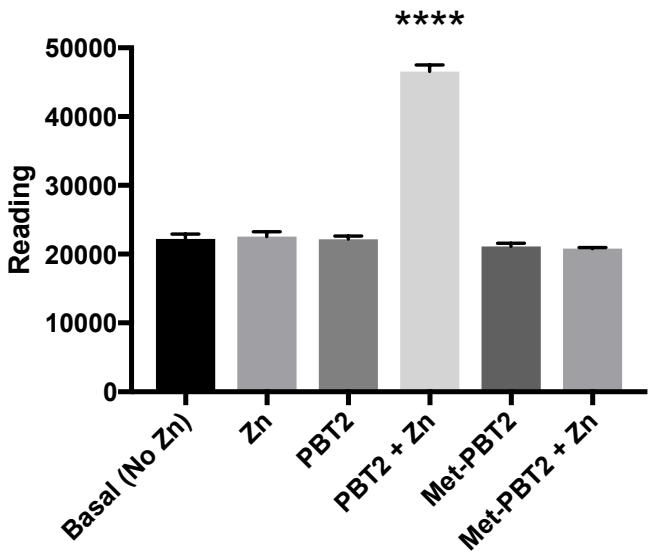
5,7-Dichloro-8-methoxyquinolin-2yl)-*N*,*N*-dimethylmethanamine hydrochloride (Met-PBT2) : 1H NMR (600 MHz, d6-DMSO) δ 2.91 (s, 6H, NMe_2), 4.12 (s, 3H, OMe), 4.73 (s, 2H, CH2N), 7.95 (d, *J*=8.4Hz, 1H, ArH), 8.02 (s, 1H, ArH), 8.85 (t, d= 8.4Hz, 1H, ArH), 10.99 (s, 1H, HCl).

Supplementary Figure 2

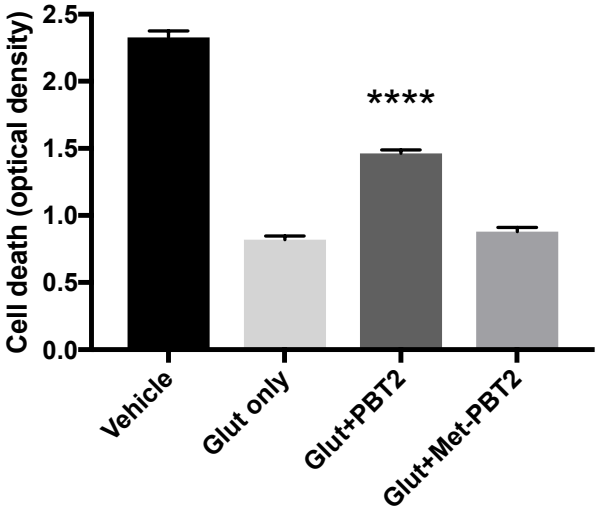


Supplementary Figure 2. Comparison of the effects of PBT2 and Met-PBT2 in an *in vitro* metal ionophore assay. Both the zinc and the compounds are used at 10µM. The change in the three elements, zinc (A; overall ANOVA, $p<0.0001$), iron (B; overall ANOVA, $p>0.5$) and copper (C; overall ANOVA, $p<0.0001$), was assessed by ICPMS. (A) In the presence of excess zinc there is a significant increase in zinc levels in the cells (as compared to "Media", which does not have

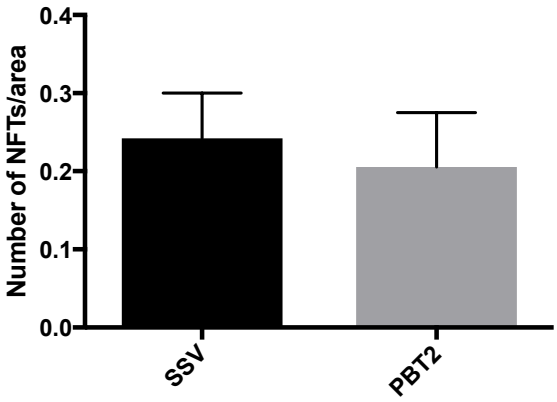
any added zinc), but this is unaffected by the presence of Met-PBT2 , which does not increase zinc levels above the zinc alone condition (but it is still elevated above Media alone). In contrast, PBT2 significantly increases zinc levels above both the zinc alone and the Met-PBT2 conditions, and also the Media alone control. (B) There is no change in the levels of iron as a result of the presence of excess zinc, and neither PBT2 or Met-PBT2 have any significant effect on cellular iron content. (C) PBT2 treatment results in a significant increase in cellular copper content, but the zinc alone and Met-PBT2 groups have no effect on copper levels in the cell. ***$p<0.001$. ****$p<0.0001$. All data are mean ± SEM.



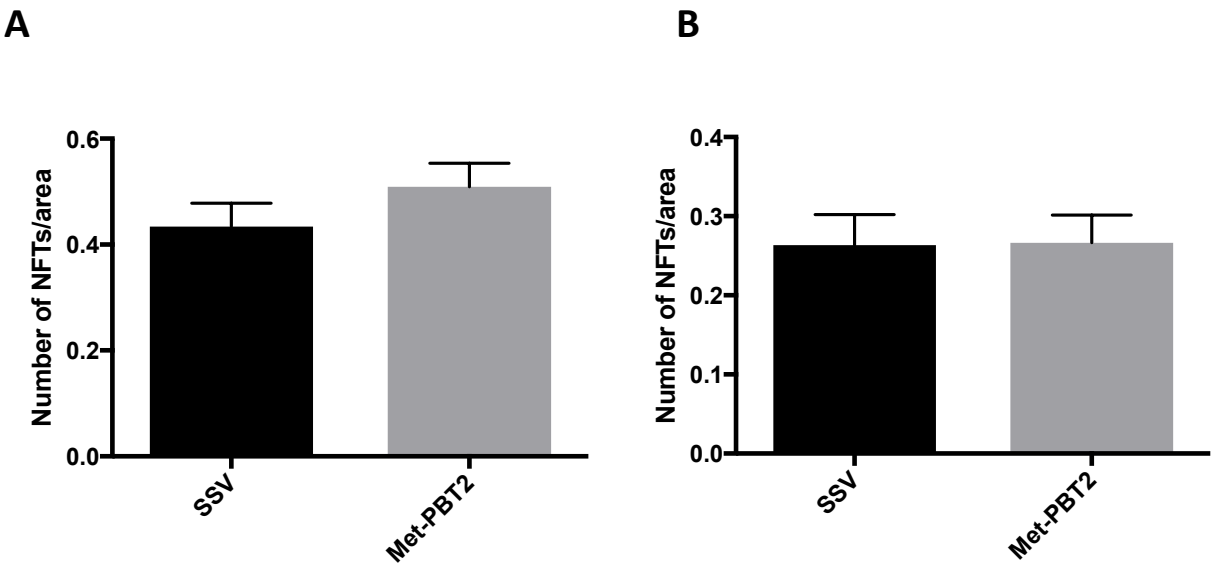
Supplementary Figure 3. *In vitro* assessment of potential biological efficacy of PBT2 and Met-PBT2. In this figure, we compare PBT2 and Met-PBT2 using the AlphaScreen *SureFire* GSK3B (p-Ser9) assay (Perkin Elmer). We have previously demonstrated (Crouch et al., 2011; reference in main article) that PBT2 promotes a metal-dependent phosphorylation of GSK3ß at ser9 (an inhibitory phosphorylation site) – and hence, decreases GSK3ß activity. We utilize this assay as a surrogate for a biological readout of the ionophore-like activity of compounds – as metal movement into the cell is required to activate this cell signaling pathway. We utilize this high throughput assay according to manufacturer's recommendations, with all assays conducted in SH-SY5Y cells (between passage 12-19; plated at a density of 600,000 cells/well in a 96 well plate format; maintained in Locke's media). The data demonstrate that PBT2+Zn significantly ($p<0.0001$) increases the phosphorylation of GSK (ser9), but Met-PBT2 does not. There was no effect of zinc alone, or compounds alone in the assay – demonstrating the requirement of zinc and zinc binding/movement into the cell to cause GSK3ß phosphorylation. ****$p<0.0001$. All data are mean ± SEM.



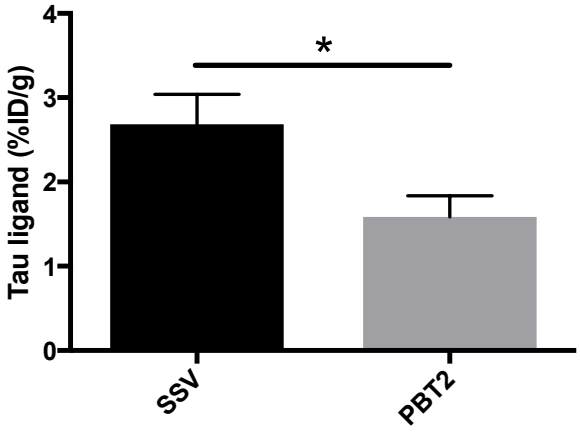
Supplementary Figure 4. *In vitro* assessment of potential biological efficacy of PBT2 and Met-PBT2. In order to characterize our compounds we utilize several different assays that may have some relevance in an *in vivo* setting. In this figure, we compare PBT2 and Met-PBT2 in a model of glutamate excitotoxicity. We have previously published data showing that PBT2 inhibits glutamate-mediated excitotoxicity (Johanssen et al., 2015). Utilizing this published protocol (which uses a CCK8 colorimetric assay as a cell death readout), we demonstrate that Met-PBT2 (2.5µM) has no effect on glutamate (10µM)-mediated cell death, whereas PBT2 (2.5µM) provides a significant ($p<0.0001$) rescue (overall ANOVA, $p<0.0001$). ****$p<0.0001$. All data are mean ± SEM.



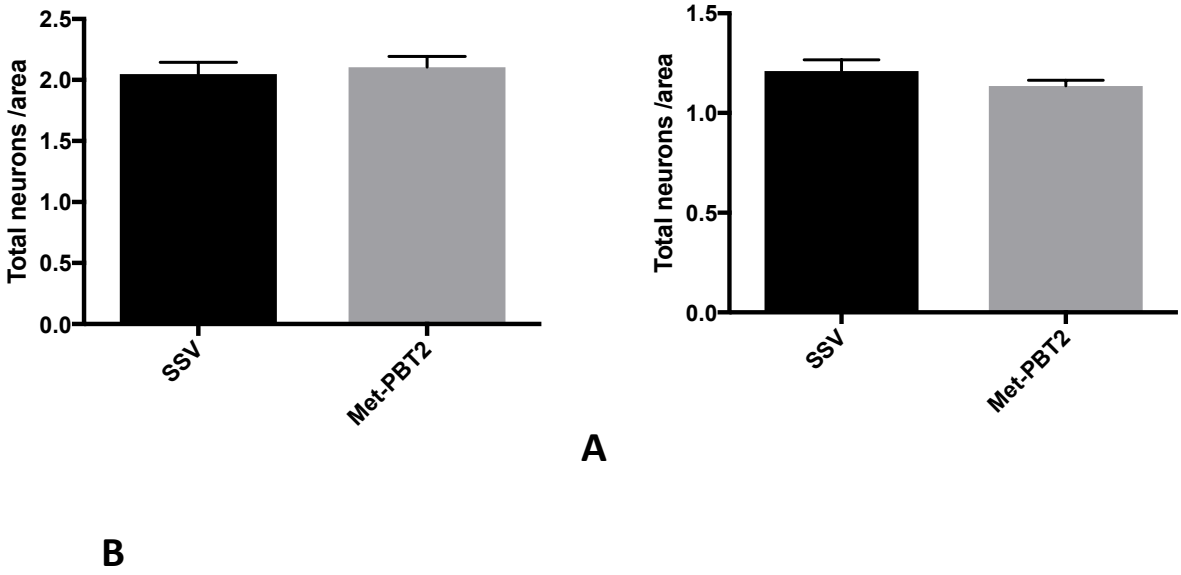
Supplementary Figure 5. Stereological assessment of the number of neurofibrillary tangles in the hippocampus of rTg4510 mice. There was no significant difference between groups. All data are mean ± SEM.



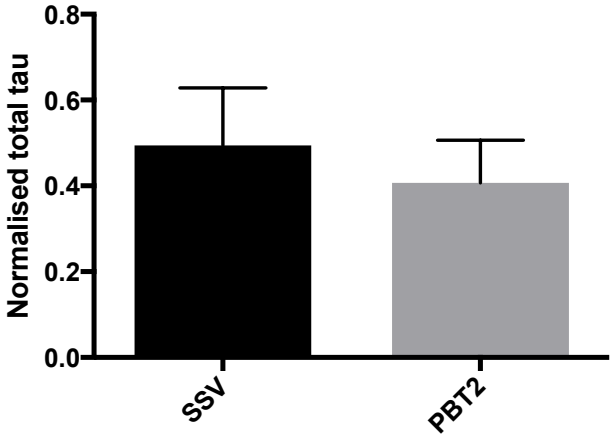
Supplementary Figure 6. Stereological assessment of the number of neurofibrillary tangles in Met-PBT2-treated rTg4510 mice. There was no significant difference in either the cortex (A) or hippocampus (B) following treatment with Met-PBT2. All data are mean ± SEM.



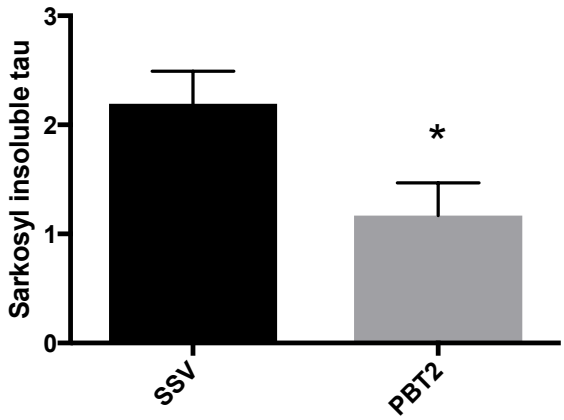
Supplementary Figure 7. Tau microPET imaging in rTg4510 mice. These data are from whole brain, and demonstrate that PBT2-treatment resulted in a significant reduction in tracer retention, consistent with what was shown in the cortex. *$p<0.05$. All data are mean ± SEM.



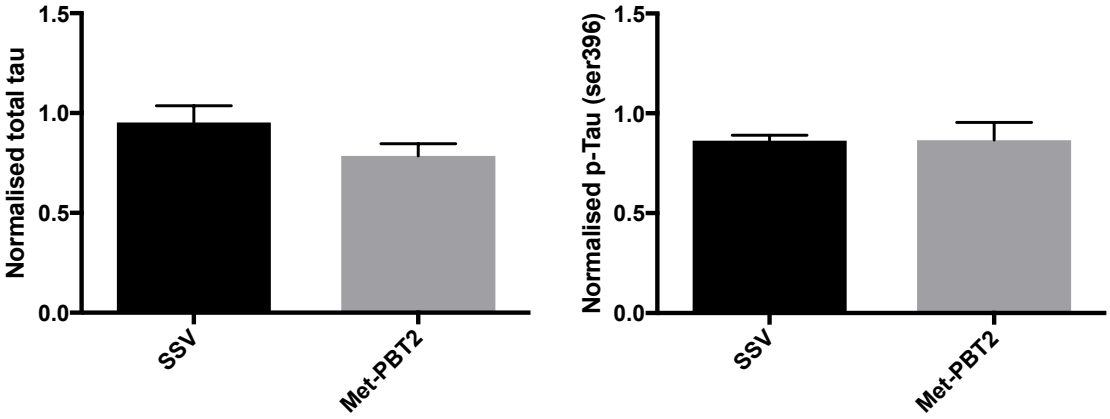
Supplementary Figure 8. Stereological assessment of the number of neurons in both the cortex (A) and hippocampus (B) of rTg4510 mice. There was no significant effect of Met-PBT2 on neuron number. All data are mean ± SEM.



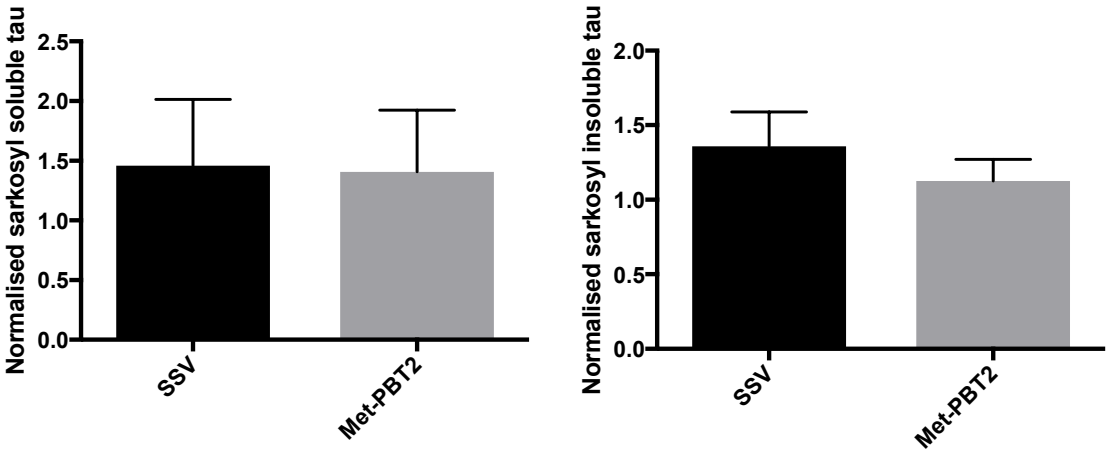
Supplementary Figure 9. Western blot analysis of total tau levels in the cortex, normalized to GAPDH, following PBT2-treatment in the rTg4510 mice. There was no significant effect of PBT2. Due to limited tissue samples, we were unable to assess hippocampal tissues. All data are mean ± SEM.

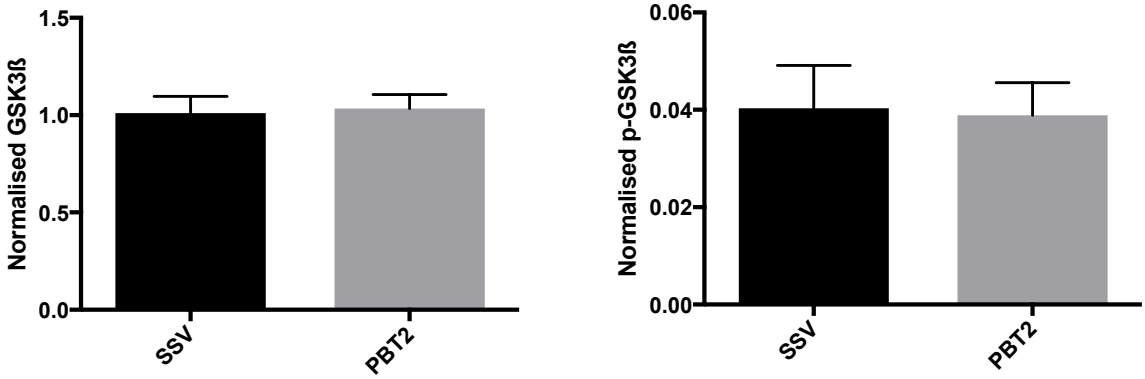


Supplementary Figure 10. Western blot analysis of sarkosyl-insoluble tau levels, showing a significant reduction in the rTg4510 mice following treatment with PBT2. *$p<0.05$. All data are mean ± SEM.

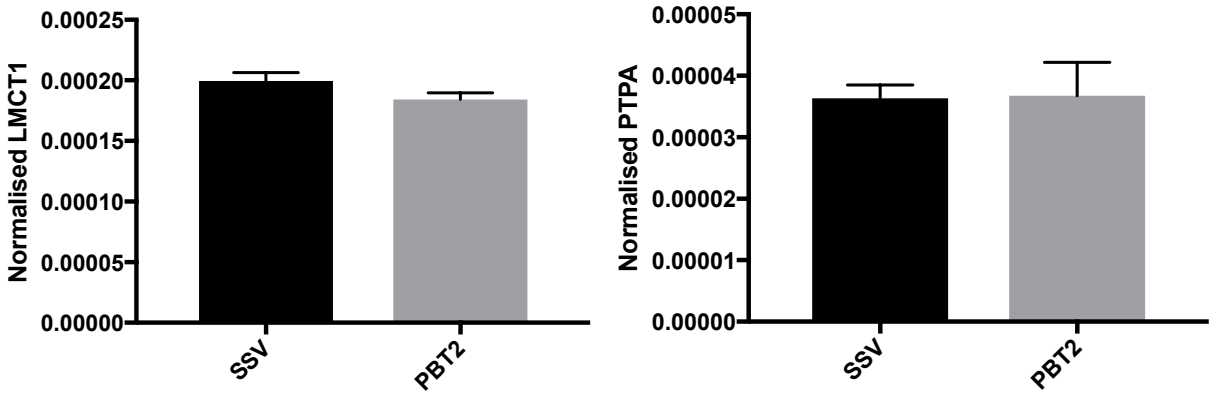


Supplementary Figure 11. Western blot analysis of different tau species in the cortex, normalized to GAPDH, following Met-PBT2-treatment in the rTg4510 mice. There was no significant effect of Met-PBT2. Due to limited tissue samples, we were unable to complete all these assessments in hippocampal tissues. All data are mean ± SEM.





Supplementary Figure 12. Western blot analysis of GSK3ß and p-GSK3ß in the cortex, normalized to GAPDH, following PBT2-treatment in the rTg4510 mice. There was no significant effect of PBT2. Due to limited tissue samples, we were unable to complete all these assessments in hippocampal tissues. All data are mean ± SEM.



Supplementary Figure 13. Western blot analysis of LMCT1 and PTPA in the cortex, normalized to GAPDH, following PBT2-treatment in the rTg4510 mice. There was no significant effect of PBT2. Due to limited tissue samples, we were unable to complete all these assessments in hippocampal tissues. All data are mean ± SEM.

Supplementary Reference

Timothy Johanssen, Nuttawat Suphantarida, Paul S. Donnelly, Xiang M. Liu, Steven Petrou, Andrew F. Hill, Kevin J. Barnham. PBT2 inhibits glutamate-induced excitotoxicity in neurons through metal-mediated preconditioning. Neurobiology of Disease 81 (2015) 176-185.