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Supplementary Materials for

Tau reduction prevents neuronal loss and reverses pathological tau deposition and seeding in mice with tauopathy

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Supplemental Materials

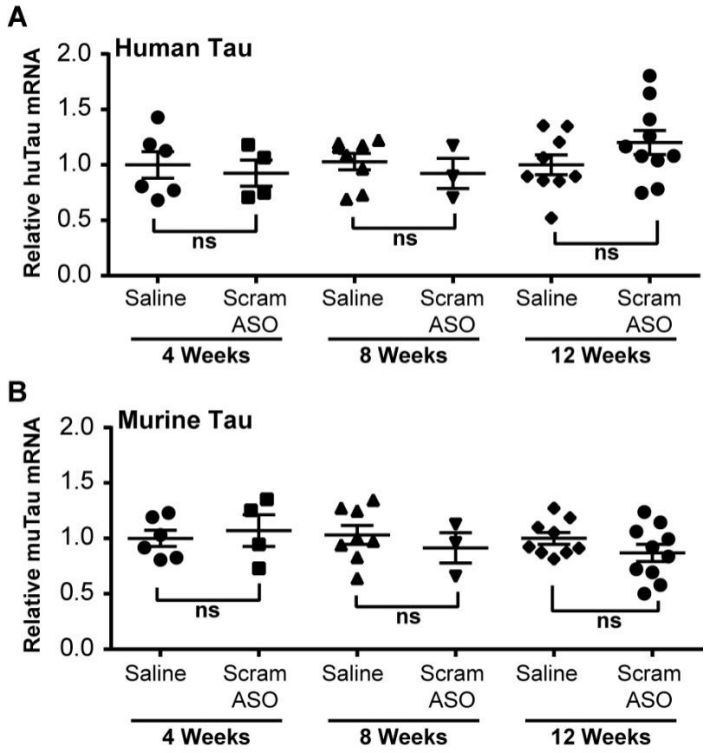

Fig. S1. Human and murine tau mRNA in control-treated PS19 mice. PS19 mice were treated with either Saline or Scrambled control ASO (30µg/day for 28 days) and mice sacrificed at 4 (saline n=6, scrambled n=4), 8 (saline n=8, scrambled n=3), and 12 (saline n=9, scrambled n=10) weeks post treatment initiation. Human and murine total tau mRNA levels were measured for each of the time points. The scrambled ASO did not alter human or mouse tau mRNA levels at any of the time points analyzed. One-way ANOVA, Sidak post-hoc analysis. Data are shown as individual data points overlaid with mean ± SEM. ns, non-significant.

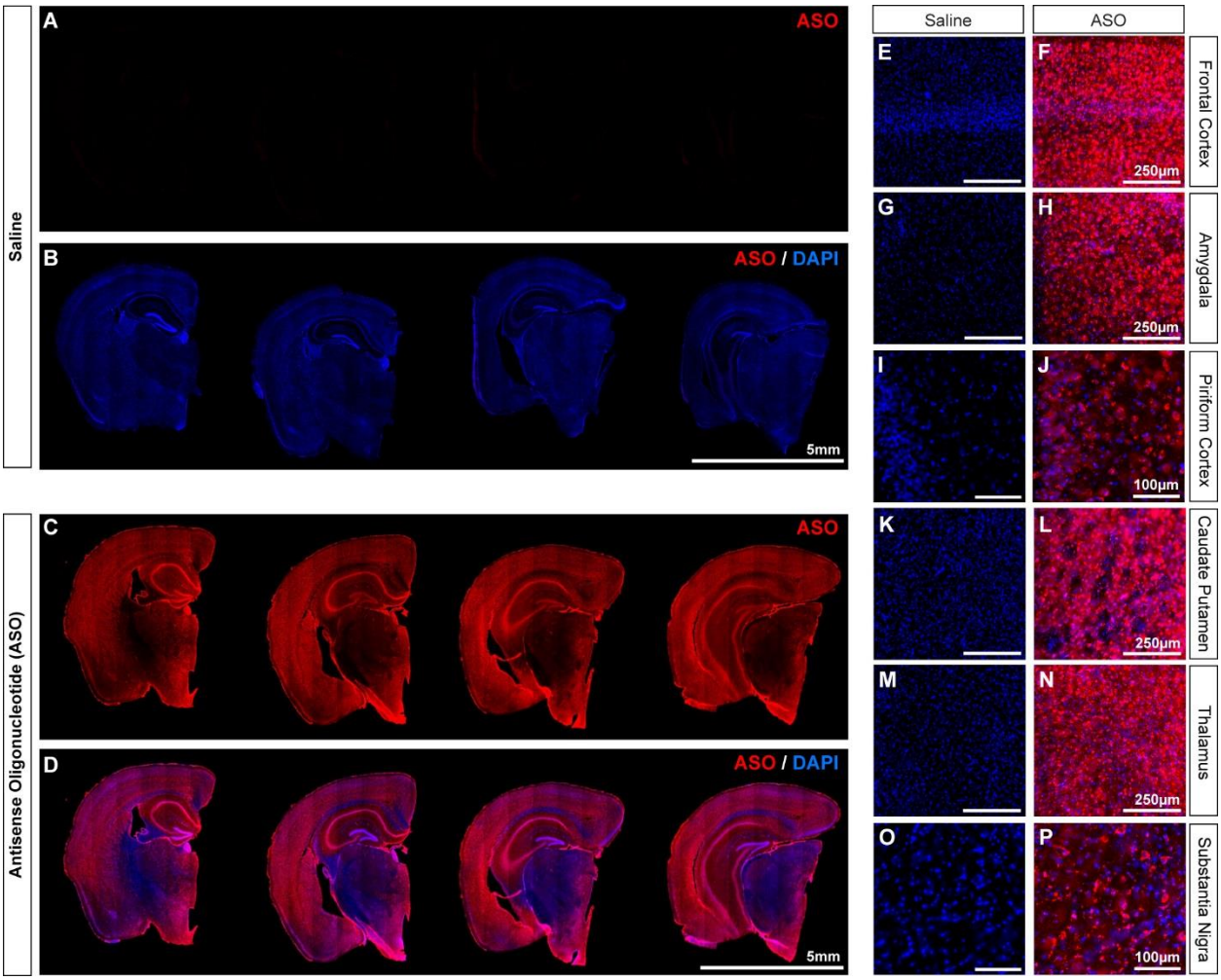

Fig. S2. ASOs are distributed throughout adult mouse brain. (**A-D**) Saline or Scrambled control ASO was delivered by ICV infusion at 30μg/day into adult PS19 mice for 1 month. In mice collected 12 weeks post-pump implantation, brain sections on the contralateral side of the catheter (left hemisphere) were stained with an ASO-antibody (red) and counterstained with nuclear stain DAPI (blue). ASO is found throughout the adult mouse brain (A-D), including Frontal Cortex (**E-F**), Amygdala (**G-H**), Piriform Cortex (**I-J**), Caudate Putamen (**K-L**), Thalamus (**M-N**), and Substantia Nigra (**O-P**).

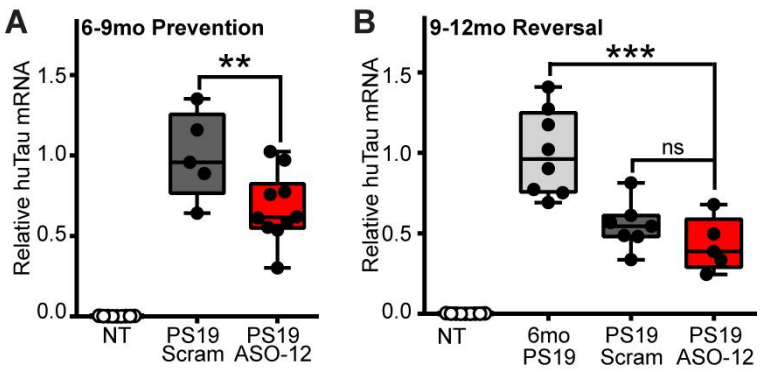

Fig. S3. Human tau mRNA is decreased after Tau^{ASO-12} treatment in the 6- to 9-month-old prevention cohort and 9- to 12-month-old reversal cohort. (**A**) In the 6-9mo prevention cohort, huTau mRNA (one-way ANOVA, Sidak post-hoc p=0.004) levels were significantly reduced in the Tau^{ASO-12} group as compared to the scrambled control. (**B**) In the older 9-12mo reversal cohort, huTau mRNA levels in the Tau^{ASO-12} treated group were significantly decreased when compared to 6mo PS19 mice (p=0.0003, one-way ANOVA, Sidak post-hoc). Interestingly, the level of huTau mRNA in the 9-12mo PS19 Scrambled treated cohort was greatly reduced compared to the 6mo PS19 mice, likely due to neuronal loss in the aged PS19 mice. This made it increasingly difficult to get an accurate huTau mRNA percent reduction as the Tau^{ASO-12} treated PS19 mice did not experience the same level of neuronal loss. Thus the mRNA comparison was made between the neuronally intact 6 month PS19 mice and 9-12mo Tau^{ASO-12} PS19 cohort. **p<0.01, ***p<0.001 Graphical data are represented as box and whisker plots with individual points overlaid, where error bars represent maximum and minimum values and the boxed line represents the median.

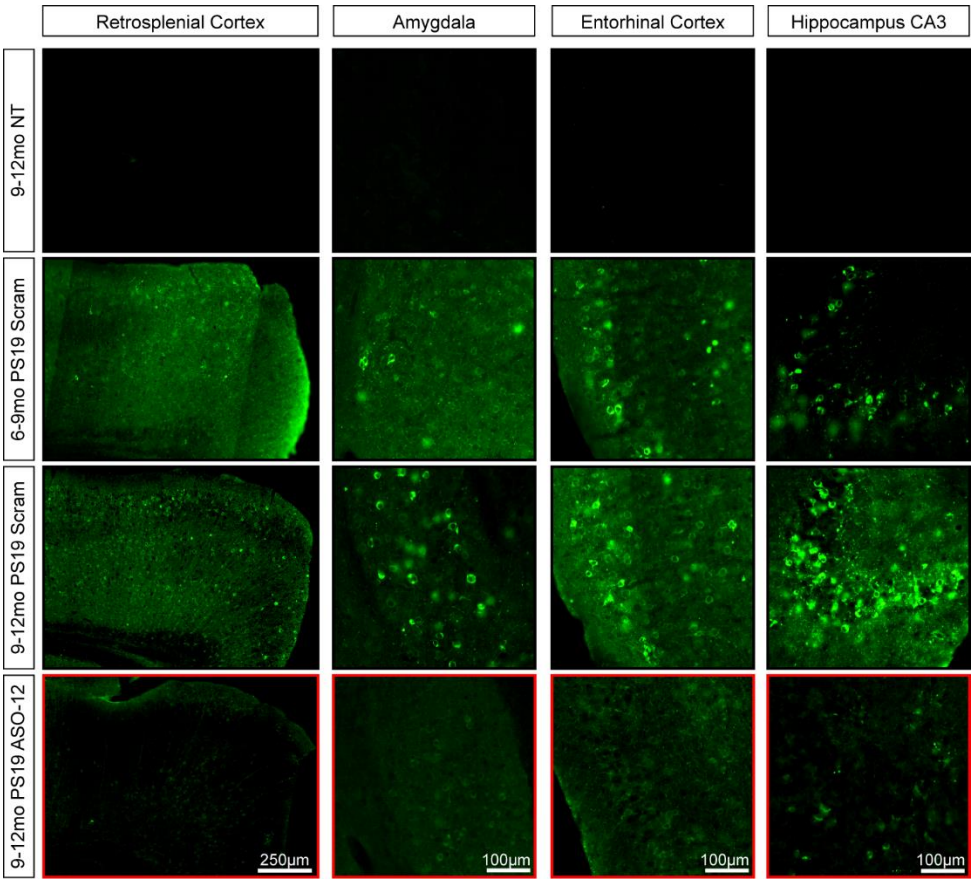

Fig. S4. Phosphorylated tau is reversed across multiple brain regions after reducing human tau. Non-transgenic and PS19 mice brain sections were stained for phosphorylated tau using the tau AT8 antibody. 9 month PS19 mice treated with $Tau^{ASO\text{-}12}$ had less AT8 positive pathology as compared to both the age-matched 9-12mo PS19 Scrambled mice as well as the starting 6-9mo PS19 Scrambled treated mice across multiple brain regions, including the Retrosplenial Cortex, Amygdala, Entorhinal Cortex, and Hippocampus.

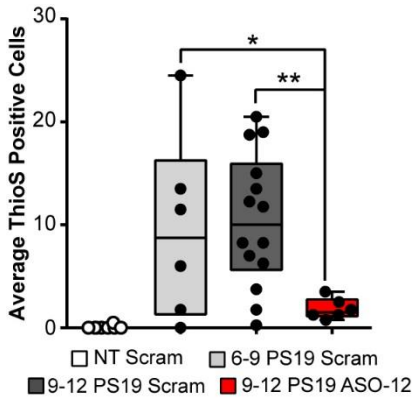

Fig. S5. Human tau reduction reverses Thioflavin S–positive NFTs in the piriform cortex of aged PS19 mice. The number of ThioflavinS (ThioS) positive neurofibrillary tangle (NFT) bearing cells in the piriform cortex was counted in 12 month Non-Transgenic (NT), 9 month PS19, 9-12 month PS19 (both untreated and treated with Scrambled ASO), and 9-12 month $Tau^{ASO\text{-}12}$ PS19 mice. The $Tau^{ASO\text{-}12}$ treatment group demonstrated not only a significant prevention of ThioS pathology, but also a significant reversal of ThioS accumulation in aged PS19 mice. One-way ANOVA, Sidak post hoc analysis. *$p<0.05$, **$p<0.01$. Graphical data are represented as box and whisker plots with individual points overlaid, where error bars represent maximum and minimum values and the boxed line represents the median.

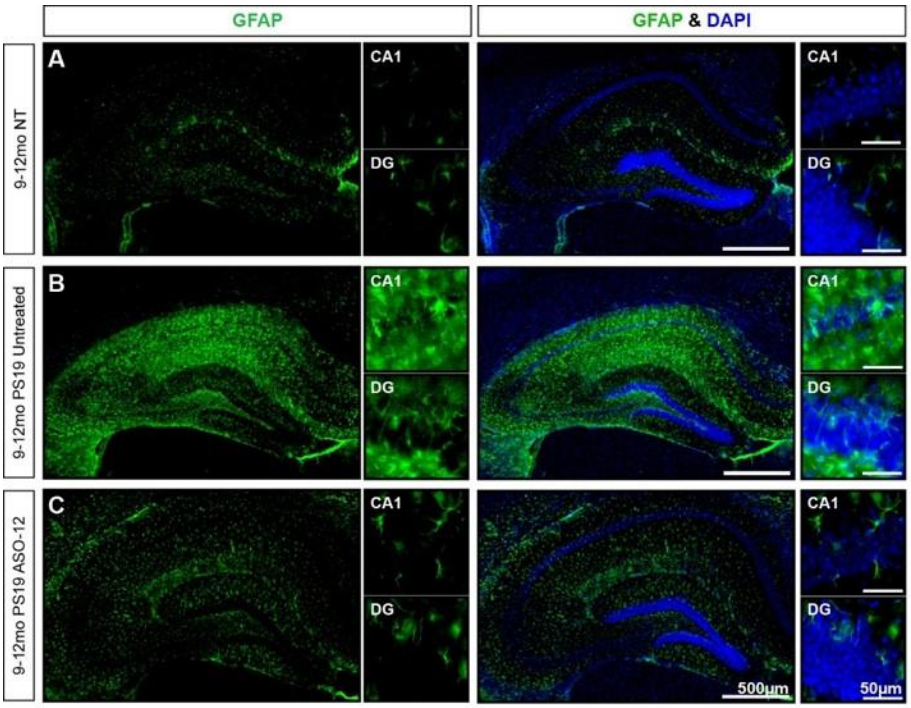

Fig. S6. Astrogliosis is reduced after Tau$^{ASO\text{-}12}$ treatment in aged PS19 mice. (**A-C**) Hippocampal sections stained for the astrogliosis marker, glial fibrillary acidic protein (GFAP). Representative images shown of the full Hippocampus, CA1, and Dentate Gyrus (DG) from 12mo Non-Transgenic (NT) mouse (A), 12 month PS19 untreated mouse (B), and 9-12mo Tau$^{ASO\text{-}12}$ treated PS19 mouse (C).

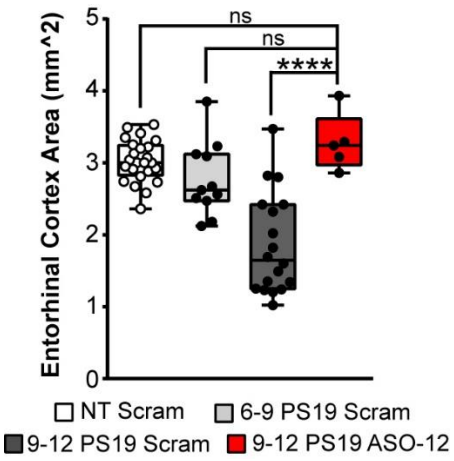

Fig. S7. Reducing human tau prevents entorhinal cortex atrophy in aged PS19 mice. The entorhinal cortex area was measured in 9 and 12 month Non-Transgenic (NT), 9 month PS19, 9-12 month PS19 (both untreated and treated with Scrambled ASO), and 9-12 month $Tau^{ASO\text{-}12}$ PS19 mice. The entorhinal cortex area in each of the sections was measured blindly (n=5-24). The $Tau^{ASO\text{-}12}$ treatment group demonstrated significant entorhinal cortex loss prevention. One-way ANOVA, Sidak *post hoc* analysis. ****$p<0.0001$. Graphical data are represented as box and whisker plots with individual points overlaid, where error bars represent maximum and minimum values and the boxed line represents the median.

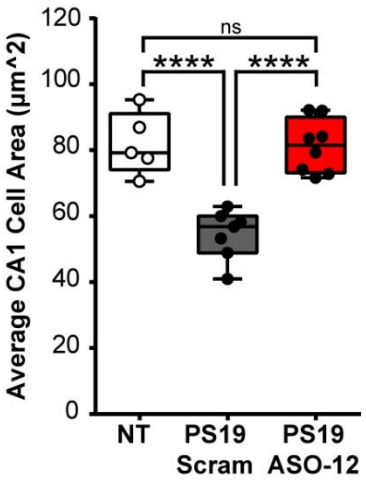

Fig. S8. Reducing human tau prevents a reduction in cell size in the CA1 region of the hippocampus in aged PS19 mice. The area of cells in the CA1 region of hippocampus of 9 and 12 month Non-Transgenic (NT), 9-12 month PS19 (both treated with Saline and Scrambled ASO), and 9-12 month $Tau^{ASO\text{-}12}$ PS19 mice. The cell size area in each of the sections was measured blindly (n=5-8). The $Tau^{ASO\text{-}12}$ treatment group demonstrated significant cell size loss prevention. One-way ANOVA, Sidak *post hoc* analysis. ****$p<0.0001$. Graphical data are represented as box and whisker plots with individual points overlaid, where error bars represent maximum and minimum values and the boxed line represents the median.

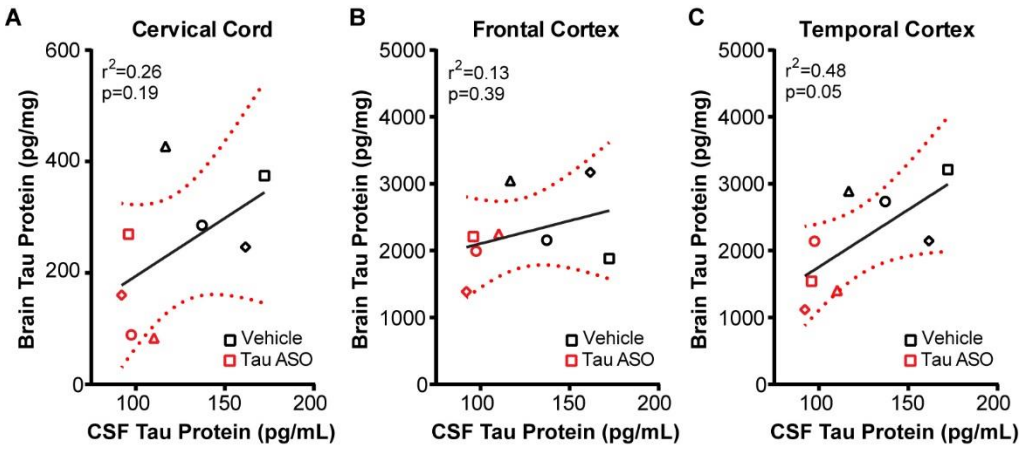

Fig. S9. Correlation of total tau protein between brain and CSF in nonhuman primates. (**A-C**) CSF tau protein levels were measured and directly compared to the total tau protein levels in the spinal cord (A) as well as frontal cortex (B) and temporal cortex (C) of vehicle and Tau ASO treated Non-Human Primates. Linear Regression.