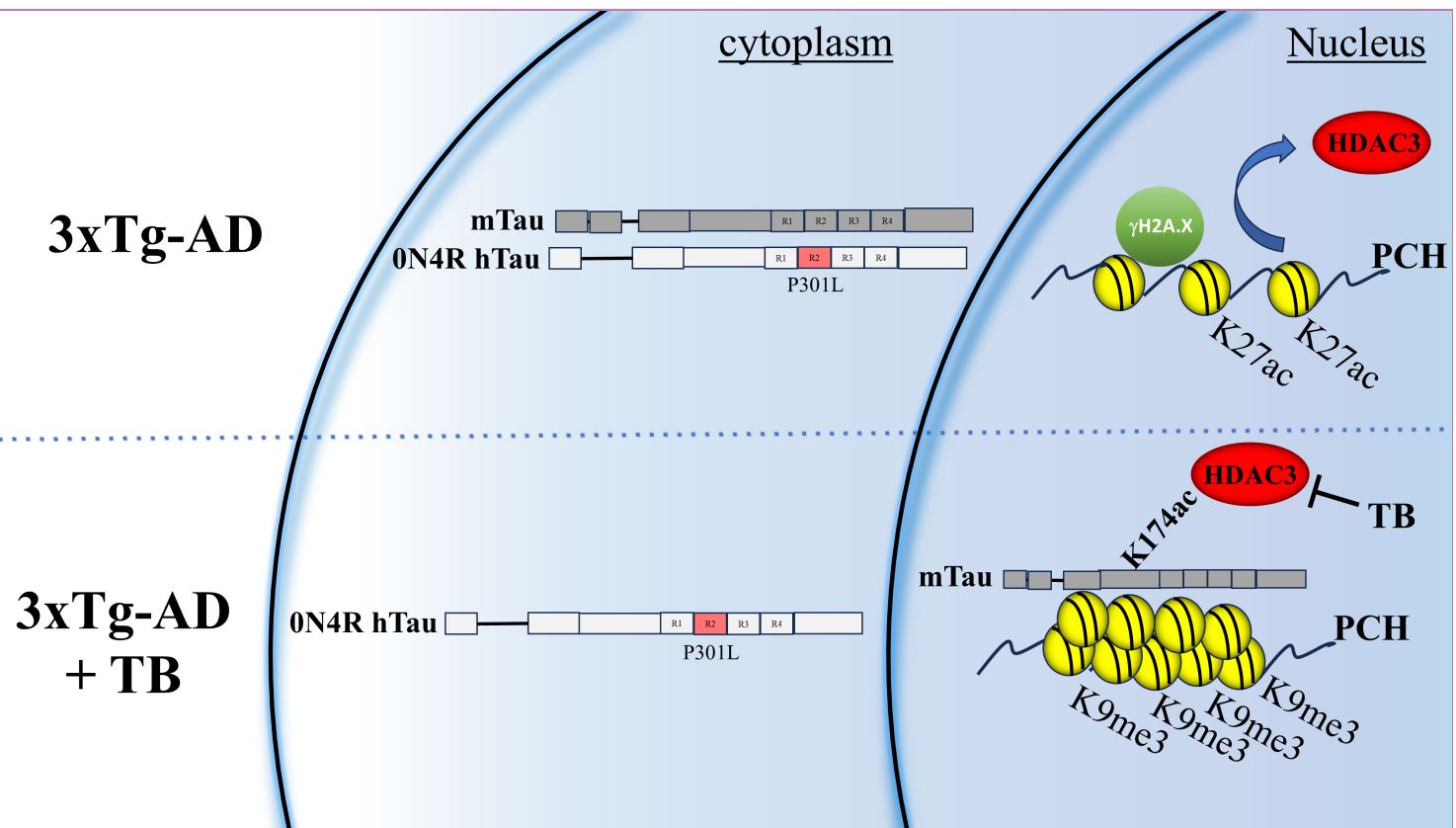


Memory Decline In 3xTg-AD Mice Involves the Loss of Hippocampal Interneuron Function & is Effectively Attenuated by Tributyrin

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- acetylated tau within nuclear pericentric heterochromatin (PCH) declines with age; this loss is exacerbated by 0N4R P301L tau
- loss of PCH acetylated tau correlates with lower H3K9me3 & higher PCH localization of the double-strand break marker γH2A.X
- administration of the HDAC antagonist tributyrin reversed the loss of PCH-acetylated tau, increased H3K9me3, & lowered γH2A.X
- tributyrin restored loss of BDNF, cFos, & parvalbumin⁺ neurites of inhibitory interneurons in 0N4R P301L tau overexpressing mice
- tributyrin reduced expression of activity dependent, immediate early genes within CA1 pyramidal excitatory neurons



INTRODUCTION

METHODS

RESULTS

CONCLUSIONS

The microtubule-associated protein tau (MAPT) is intricately associated with the pathophysiology of several tauopathies, including Alzheimer’s disease (AD). Alternative splicing of the *Mapt* transcript produces three brain-specific isoforms (0N4R, 1N4R, & 2N4R) in the adult mouse. The 0N4R & 2N4R isoforms exhibit minimal nuclear localization. In contrast, 1N4R is predominantly localized within neuronal nuclei.^{1,2} Importantly, acetylation of tau at lysine residue 174 (K174ac) promotes its nuclear translocation in response to DNA damage.³

Higher 0N-tau & lower 1N-tau isoforms were identified in the brains of Huntington’s disease patients. Additionally, exon 2 inclusion is differentially disrupted in distinct tauopathies, with an accumulation of 0N isoforms in AD brains.⁴

Growing attention has been given to the role of tau in the regulation of heterochromatin stability. Tau interacts with the minor groove of DNA & forms protein-DNA complexes.⁵ Additionally, tau binds to core histone H3 & can prevent histone posttranslational modification (PTM) by maintaining a more compact chromatin structure.⁶ Nuclear tau preserves nucleic acid integrity under stress conditions⁷ & stabilizes pericentromeric heterochromatin (PCH) organization by regulating the expression of PCH non-coding RNAs.⁸

In AD, the extent of memory deficits & cognitive decline strongly correlates with progression of the anatomical spread of misfolded tau.⁹ Misfolded, aggregated & hyperphosphorylated forms of tau first appear in AD within the middle entorhinal cortex (mEC), spreading through the perforant pathway to the hippocampus in a prion-like fashion.^{10,11} Extracellular vesicle (EV)-associated phosphorylated tau (P-tau) derived from AD patients accumulates predominantly within GABAergic interneurons, exhibiting preferential EV-mediated interneuronal tau propagation.¹² This phenotype is accompanied by reduced amplitude of spontaneous inhibitory postsynaptic currents & reduced GABAergic transmission to the CA1, leading to dysfunction.

Chronic administration of the butyrate prodrug tributyrin (TB), a histone deacetylase (HDAC) antagonist, was previously shown to reverse an age-associated decline in butyrate-producing bacteria & enteric dysbiosis in the 3xTg-AD mouse model.¹³ These changes correlated with a reduction in both memory deficits & hyperphosphorylated tau accumulation within the hippocampus. In the current study, we investigated the effects of TB-mediated HDAC inhibition on tau localization & AD pathology.

Animals
Homozygous triple transgenic mice modeling early-onset AD, B6;129-Tg(APP^{Swe}, tau^{P301L})1Lfa Psen1tm1Mpm/Mmjax (3xTg; RRID:MG1:3720782), developed at the University of California by Frank LaFerla, were purchased from MMRC. Control B6129SF2/J mice (strain #: 101045; RRID: IMSR_JAX:101045) were purchased from The Jackson Laboratory. All mice were bred at UofL & housed in a specific pathogen-free facility. Experiments were conducted according to federal & institutional guidelines with approval of the UofL Committee for Animal Welfare.

Experimental Design
Female 3xTg-AD mice were left untreated or were given 2 mg/kg TB by oral gavage twice weekly for 10 months, beginning at 12 months of age. At 18 months, mice were anesthetized & transcardially perfused with PBS followed by 4°C 4% PFA. Perfused brains were collected, dissected into hemispheres, fixed overnight in 4% PFA & incubated in 30% sucrose for 72h at 4°C.

Immunohistochemistry & immunofluorescence
Brain hemispheres were paraffin embedded & 5 μm sagittal sections were cut, mounted onto slides & incubated at 60°C for 48h. Sections were deparaffinized, dehydrated in 100% EtOH in two 10 min incubations & rehydrated. All sections were antigen unmasked using 10 mM sodium citrate, pH 6.0. After washing in TBS, sections were blocked in buffer (10% Normal Donkey Serum, 0.5% BSA, 0.1% Triton X-100, 1xTBS, pH 7.4) for 1 hr at RT. All antibody reactions were incubated in blocking buffer at 4°C shaking overnight. Images were taken with a Nikon C2+ confocal microscope with intelligent acquisition & an ORCA-Fusion Gen-III sCMOS monochrome camera using a Plan Apo λ 20x objective. Post-acquisition, volumes were visualized in NIS-Elements AR (Nikon, ver. 5.30.05). Immunofluorescent positive areas were quantified by a scorer blinded to genotype, age, or treatment.

Statistical analyses
Quantification data are presented as means ± standard error obtained from 4-9 animals per stain using 2-3 tissue sections per animal. Comparisons between two groups were performed by tailed paired Student’s t-tests. Multiple comparisons were performed by either 1- or 2-way ANOVA followed by Tukey’s post hoc test. Statistical analyses were performed using GraphPad Prism [Ver 10.3.1 (509)]. Data points three standard deviations beyond the mean were excluded. A significant difference was assumed at P < 0.05 with a 95% confidence interval.

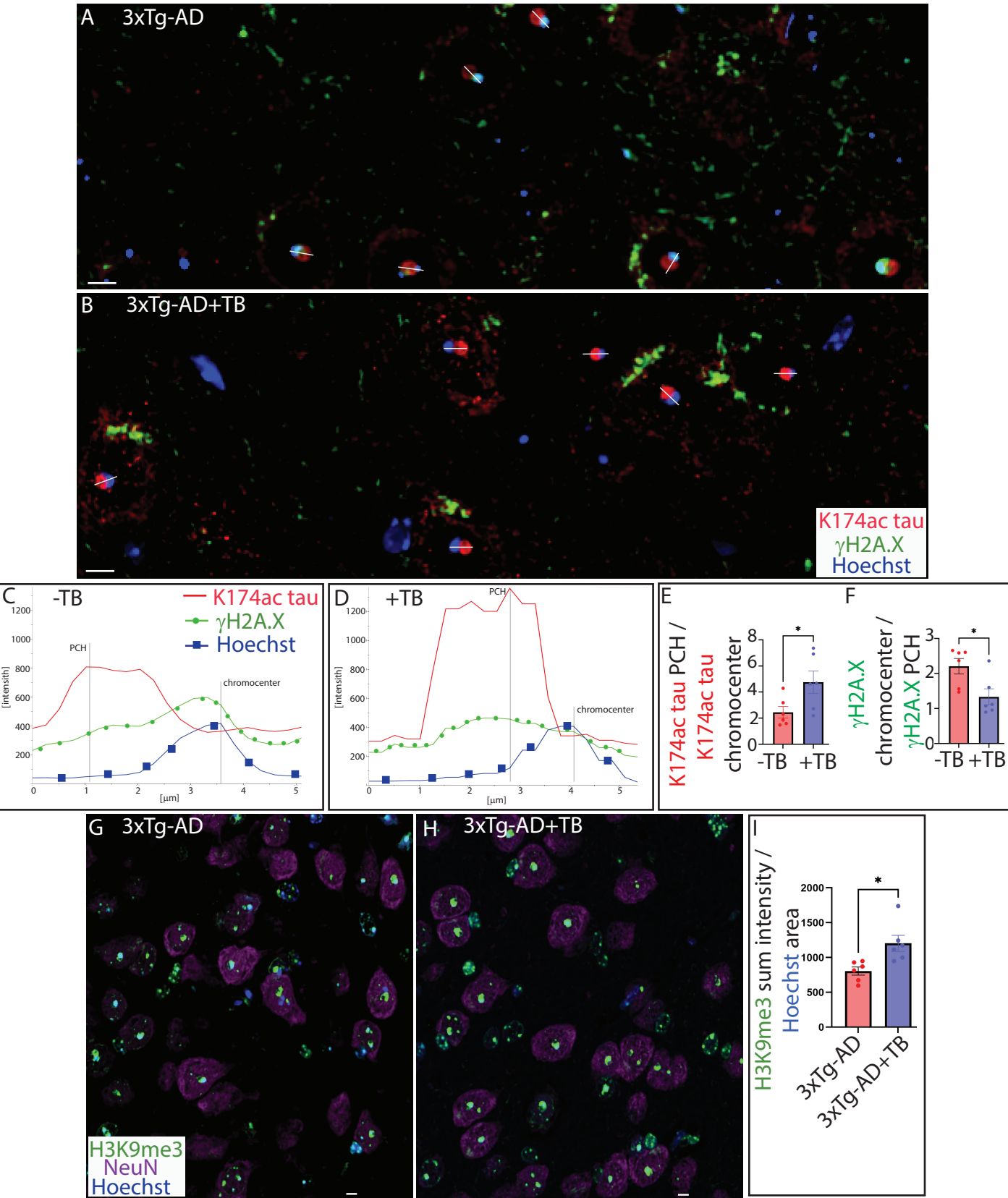


Figure 1. Tributyrin increases PCH localization of acetylated tau, reduces chromocenter-localized γH2A.X, & increases nuclear H3K9me3. Neurons within layer II of the mEC of 18-month-old 3xTg-AD mice treated with (B) or without (A) TB for 10 months & stained with K174ac tau, γH2A.X, & Hoechst. Line histograms span the PCH & chromocenters of control (A, C) or TB-treated (B, D) mice. Mean ratios of PCH/chromocenter of K174ac tau localization (E) or chromocenter/PCH of γH2A.X localization (F). mEC layer II neurons of control (G) or TB-treated (H) mice stained with H3K9me3, NeuN, & Hoechst. (I) H3K9me3 intensity (arbitrary units) / nuclear area were measured in 18-month-old mice. Each data point represents a mean ratio of ~100 cells per mouse ± SEM. n=6; +TB, n=6). Scale bars = 5 μm. *P < 0.05.

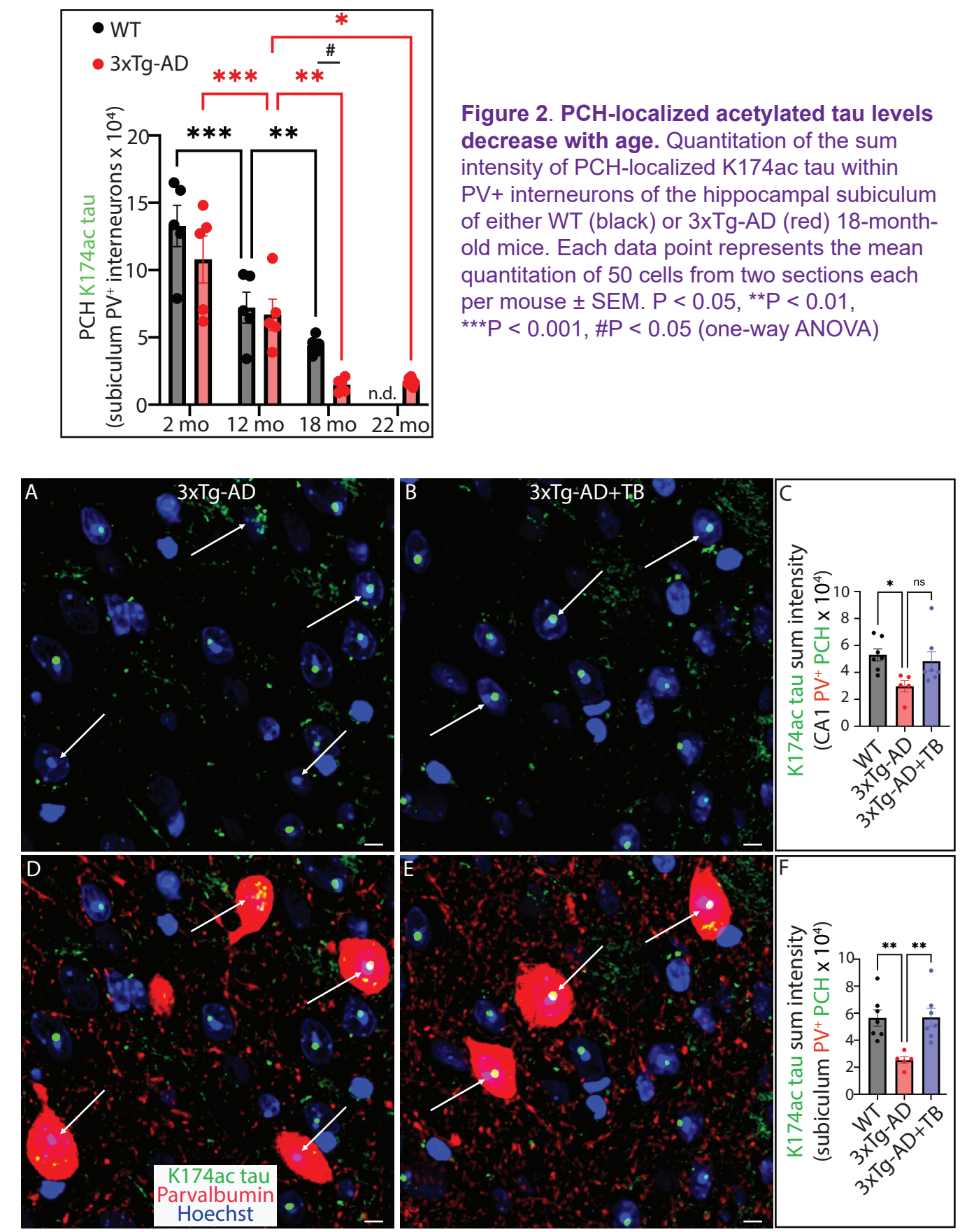


Figure 2. PCH-localized acetylated tau levels decrease with age. Quantitation of the sum intensity of PCH-localized K174ac tau within PV+ interneurons of the hippocampal subiculum of either WT (black) or 3xTg-AD (red) 18-month-old mice. Each data point represents the mean quantitation of 50 cells from two sections each per mouse ± SEM. P < 0.05, **P < 0.01, ***P < 0.001, #P < 0.05 (one-way ANOVA)

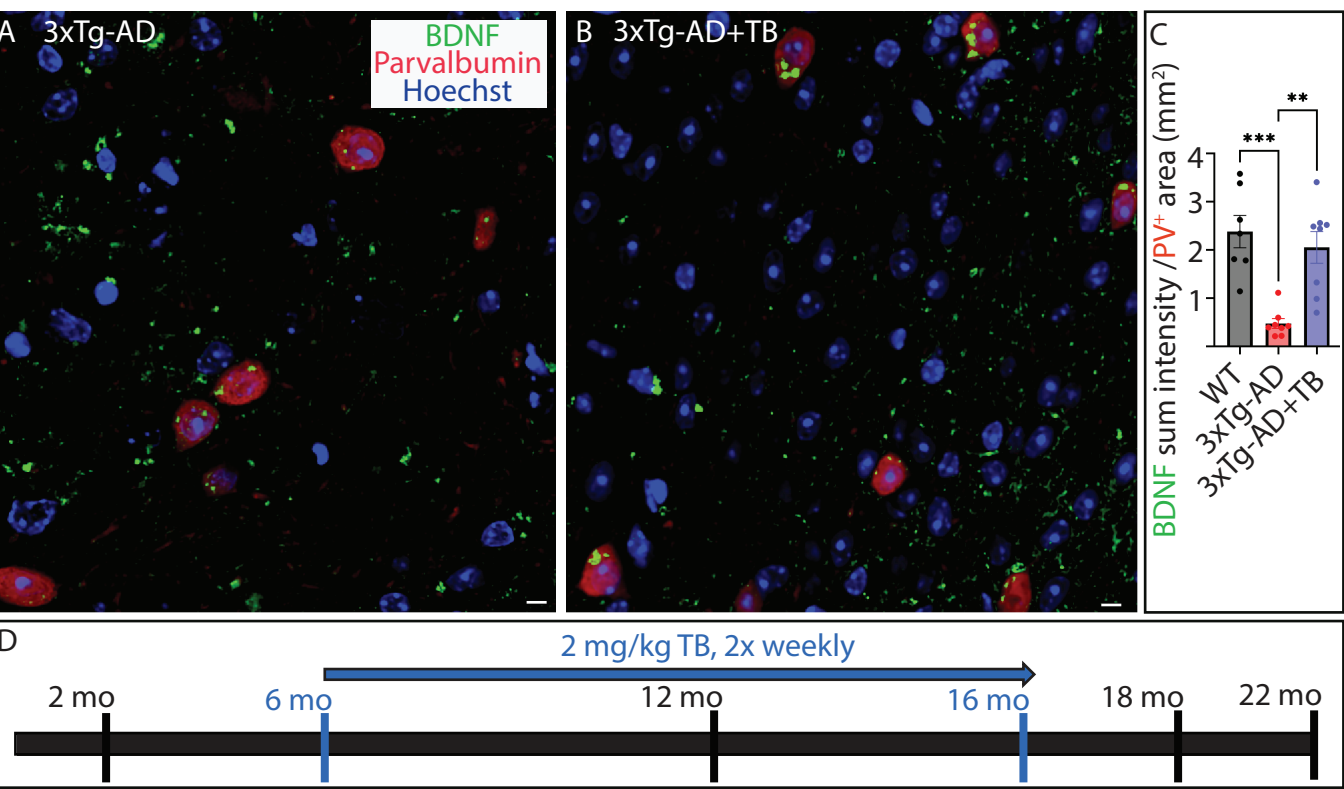


Figure 4. Tributyrin increases BDNF expression within interneurons. Subiculum interneurons of control (A) or TB-treated (B) mice stained with BDNF, parvalbumin, & Hoechst. Co-localization of BDNF & PV was quantified & divided by PV+ area. (C) Each data point represents quantitation of 40–50 subiculum interneurons per mouse ± SEM. (D) Schematic of TB oral administration. Scale bars = 5 μm. **P < 0.01, ***P < 0.001.

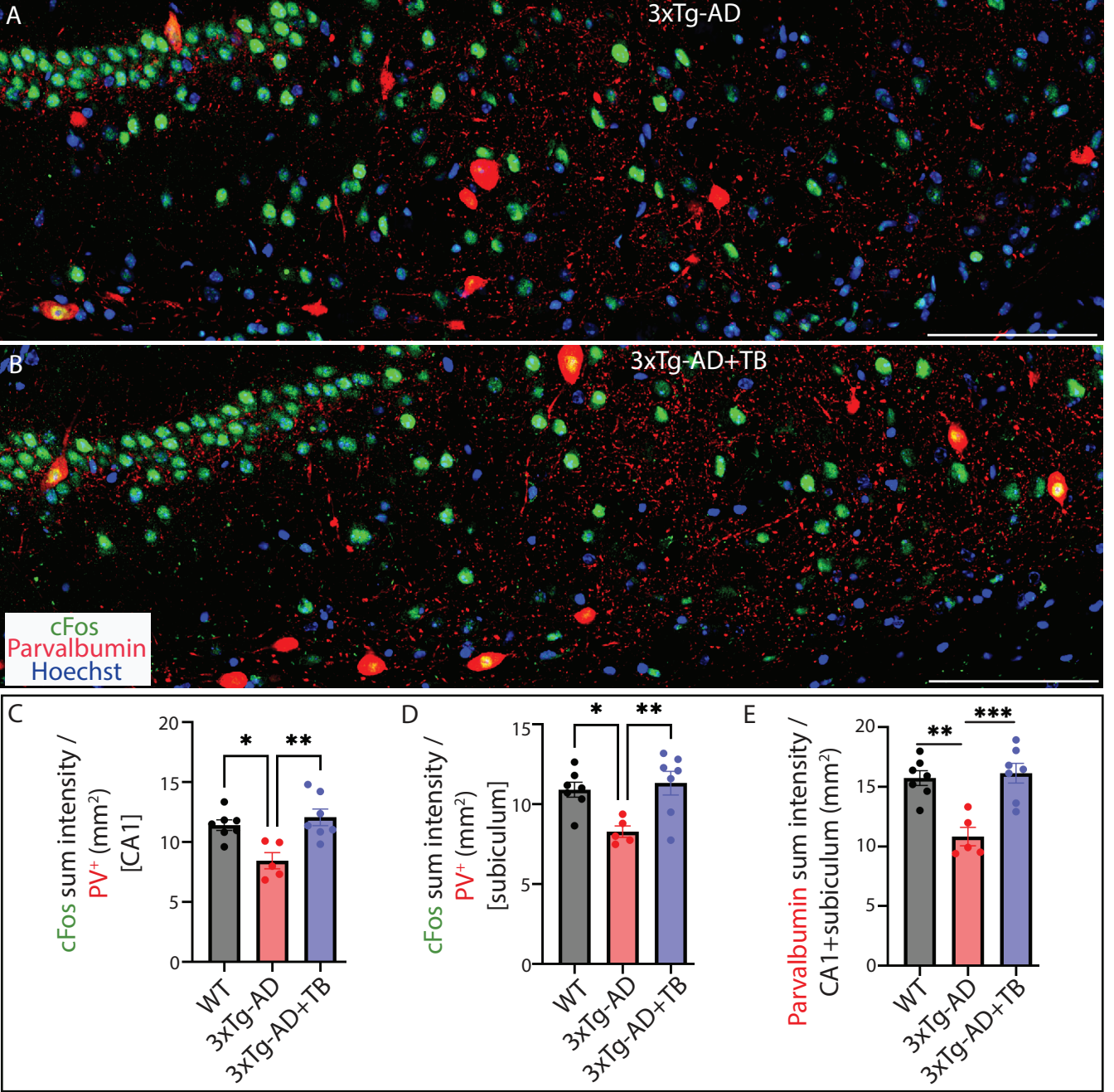


Figure 5. Tributyrin mitigates loss of cFos expression & neurites of PV+ inhibitory interneurons. 18-month-old 3xTg-AD control (A) & TB-treated (B) mice stained with cFos, parvalbumin, & Hoechst. (C) Quantitation of cFos expression within CA1-localized PV+ interneurons. (D) Quantitation of cFos within subiculum-localized PV+ interneurons. (E) Quantitation of parvalbumin sum intensity within the entire CA1 field & subiculum. Each data point represents the mean quantitation of two sections per mouse ± SEM. Scale bars = 100 μm. *P < 0.05, **P < 0.01, ***P < 0.001.

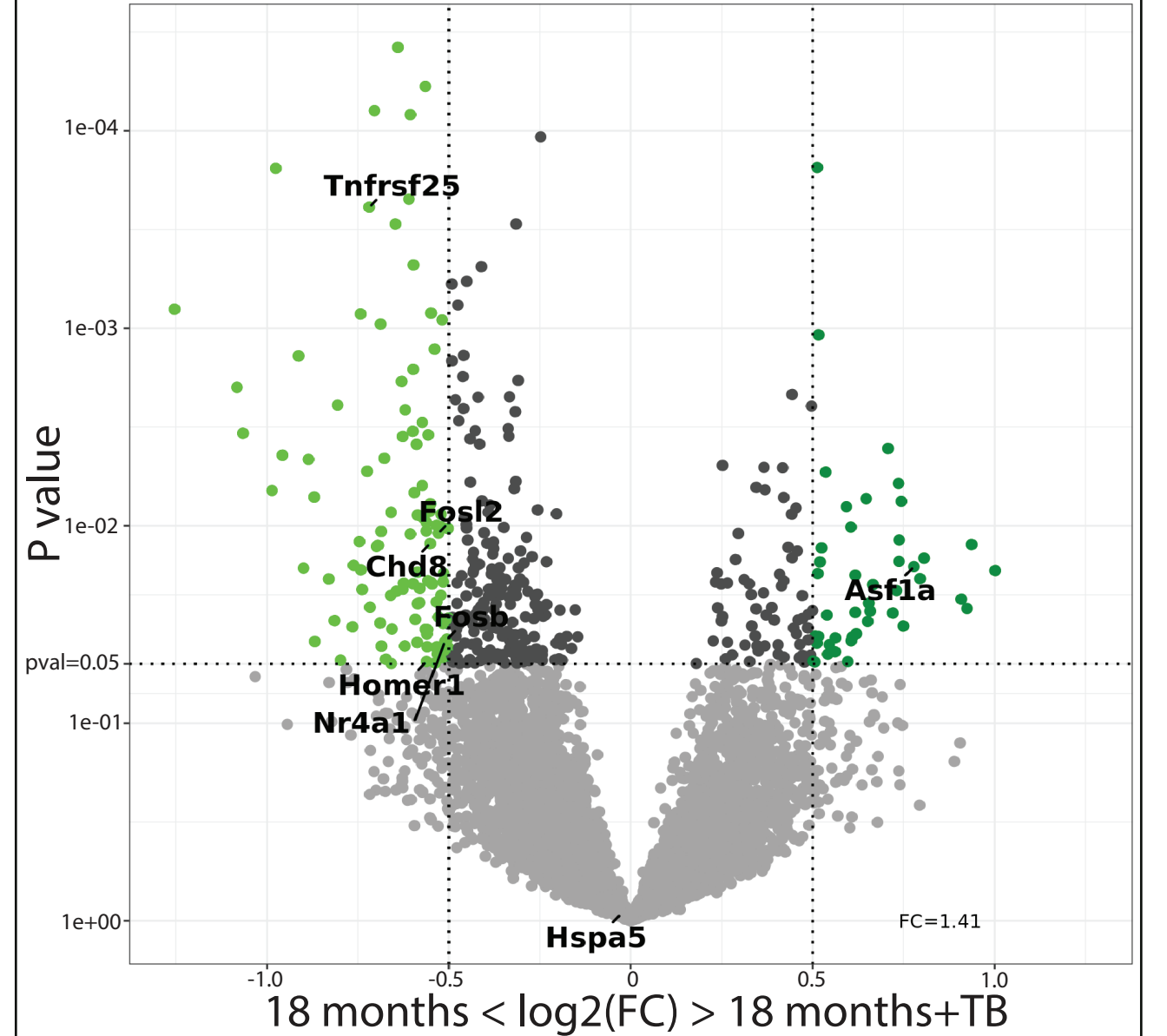


Figure 6. Tributyrin downregulates activity-dependent, immediate early gene (IEG) expression within CA1 neurons. Volcano plots of a spatial profiling analysis of NeuN+ CA1 pyramidal neurons between 18-month-old 3xTg-AD control & 18-month-old 3xTg-AD + TB mice using Nanostring’s™ Whole Transcriptome Atlas. Significantly up- or down-regulated transcripts (*P < 0.05) are shown in green.

- Age- & AD-related dysfunction in hippocampal inhibitory interneurons involves the loss of PCH-localized acetylated tau & increased DNA breaks.
- Overexpression of 0N4R tau appears to act as a 'prion sink' within the cytosol & leads to increased paired helical filament formation with endogenous tau, depleting the nucleus of heterochromatin tau
- Loss of PCH acetylated tau reduces heterochromatin stability & reduces inhibitory interneuron input onto excitatory CA1 pyramidal neurons.
- Our observed reduction in memory deficits¹³ by tributyrin administration is at least partially due to a restoration of heterochromatin stability & improvement in inhibitory interneuronal function

REFERENCES

1. Younas N, Saleem T, Younas A, Zerr I. Acta Neuropathol Commun. Dec 12 2023;11(1):196.
2. Bowles KR, Pugh DA, Oja LM, et al. Feb 2022;143(2):225-243.
3. Portillo M, Eremenko E, Kalusi S, et al. Cell Rep. Apr 27 2021;35(4):109035.
4. Petry S, Nateghi B, Keraudren R, et al. Neuroscience. May 10 2023;518:54-63.
5. Wei Y, Qu MH, Wang XS, et al. PLoS One. Jul 2 2008;3(7):e2600.
6. Rico T, Gilles M, Chauderlier A, et al. Front Cell Dev Biol. 2021;9:740550.
7. Violet M, Delattre L, Tardivel M, et al. Front Cell Neurosci. 2014;8:84.
8. Mansuroglu Z, Benhelli-Mokrani H, Marcato V, et al. Sci Rep. Sep 8 2016;6:33047.
9. Arriagada PV, Growdon JH, Hedley-Whyte ET, Hyman BT. Neurology. Mar 1992;42(3 Pt 1):631-9.
10. Asai H, Ikezu S, Tsunoda S, et al. Nat Neurosci. Nov 2015;18(11):1584-93.
11. Winston CN, Goetzl EJ, Akers JC, et al. Alzheimers Dement (Amst). 2016;3:63-72.
12. Ruan Z, Pathak D, Venkatesan Kalavai S, et al. Feb 12 2021;144(1):288-309.
13. Chilton PM, Ghare SS, Charpentier BT, et al. Gut Microbes. Jan-Dec 2024;16(1):2389319.