

Targeting Lipid Remodeling, Circular RNAs And Autophagy To Modulate Amyloid- β Dynamics In Alzheimer's Disease

Skarleth Cardenas-Romero¹, Mei-Yu Lai², Matthew J Rosene¹, Natalia López Herrera², Gabriel Pagan Gonzalez¹, Christina Muratore³, Bruno A. Benitez¹

¹ Department of Neurology, Movement Disorder Division, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts,

² Department of Neurology, Movement Disorder Division, Beth Israel Deaconess Medical Center, Boston, Massachusetts,

³ Department of Neurology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

Background

Protein palmitoylation is an emerging regulator of Alzheimer's disease (AD) pathology, modulating key enzymes involved in A β production, including amyloid precursor protein (APP), BACE1, and γ -secretase components¹. Palmitoylation promotes their localization to lipid rafts and enhances A β generation, whereas loss of palmitoylation retains these proteins in the endoplasmic reticulum and limits A β production. Palmitoyl protein thioesterase-1 (PPT1) is the primary lysosomal depalmitoylation enzyme, and we recently showed that PPT1 hemizyosity increases A β plaque burden in 5xFAD mice². However, the role of lysosomal depalmitoylation in A β dynamics remains poorly understood. Here, we examine how PPT1 integrates lipid remodeling, autophagy, and the regulation of circular RNA (circRNA) to modulate A β metabolism.

Methods

Cells: iPSC-derived neurons (iNs) from patients carrying the London familial AD (fAD) APP mutation (V717I), as described previously³, and N2a-APP695 cells overexpressing human APP.

Drug treatments: N2a695 cells and iNs were treated with the PPT1 mimetic *N*-(tert-Butyl)hydroxylamine (NtBuHA; 250 μ M, 500 μ M, 1 mM, 2 mM) for 24 or 48 h. In separate experiments, N2a695 cells were treated for 24 h with Palmostatin B (APT inhibitor; 10–20 μ M), 2-bromohexadecanoic acid (2-BP; 1–50 μ M), or DC661 (PPT1 inhibitor; 1–3 μ M).

ELISA: Cell lysates and conditioned media were collected after treatment, and total protein was quantified using a BCA assay. Human A β 40 and A β 42 levels in conditioned media were measured by ELISA (Thermo Scientific). Hippocampal tissue from 7–8-month-old mice was fractionated into soluble (PBS) and insoluble (5 M guanidine) fractions. A β species were quantified by sandwich ELISA using HJ2 and HJ7.4 antibodies. Absorbance was measured at 650 nm using Super Slow TMB substrate on a BioTek plate reader.

Mouse models: Female and male 5xFAD mice (JAX 034848) were crossed with hemizygous PPT1 mice (B6;129-Ppt1tm1Hof/J; JAX 004313) to generate P5X (5xFAD::PPT1^{+/-}) mice. At 4 months of age, left hemispheres were fixed in 4% paraformaldehyde for histological analyses, and right hemispheres were dissected into regions and stored at –80 °C for immunoblotting.

Immunofluorescence: Coronal brain sections (40 μ m) were stained with X-34 (10 μ M) and co-immunostained with anti-A β (82E1, 1:1000), LAMP1 (1:200) to assess plaque burden and dystrophic neurites. Neuroinflammation was evaluated by co-staining with X-34, CD68 (1:1000), and GFAP (1:1000). Two sections per animal were analyzed.

RNA-seq: For RNA library preparation, TruSeq Stranded Total RNA was extracted with Ribo-Zero to deplete rRNA and generate stranded RNA-seq. RNA depletion using Ribo-Zero removes 28S, 18S, 5.8S, and 5S rRNA.

CircRNA analysis: CircRNA expression in brain cortex samples was analyzed as described by Dube et al. Raw RNA-seq reads were aligned to GRCh38 using STAR (v2.5.3a) in chimeric mode with GENCODE v26 annotation (--sjdbOverhang 150). Each sample underwent three alignment strategies (paired and individual mates). CircRNAs were identified from chimeric junctions using DCC (v0.4.4), followed by filtering and collapsing back-splice junctions to define high-confidence circRNAs.

In vitro results

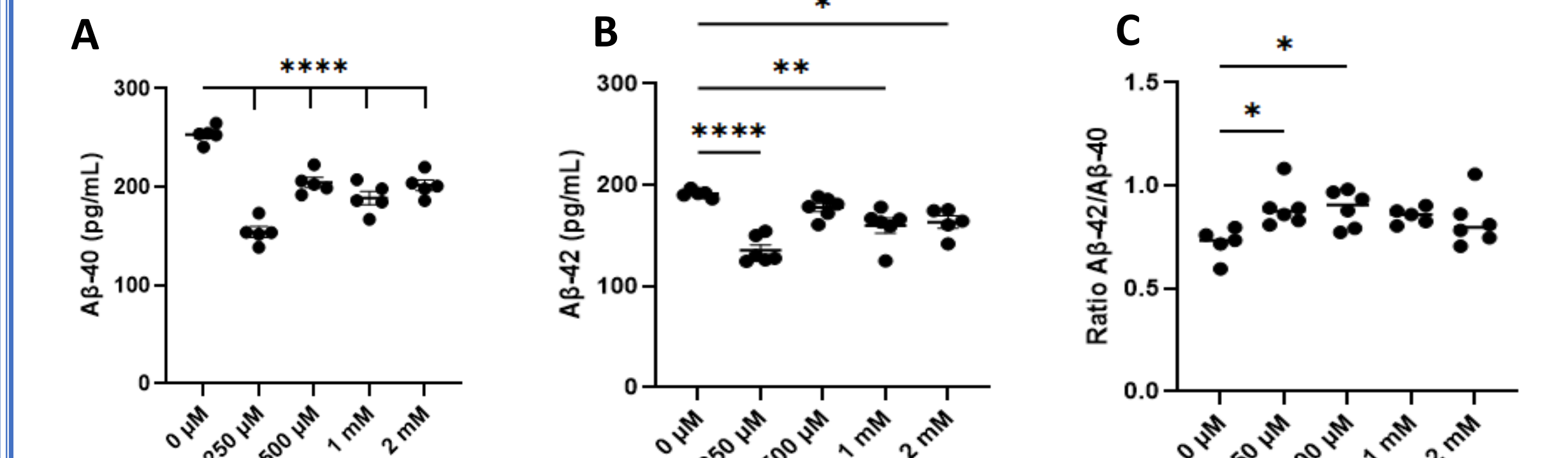


Figure 1. NtBuHA reduces amyloid-related peptides in iNs. (A) Soluble A β 40 (n = 9), (B) soluble A β 42 (n = 6), and (C) A β 42/A β 40 ratio across treatment groups. Data are shown as mean $\pm$ SEM. ns, not significant; *p $\leq$ 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

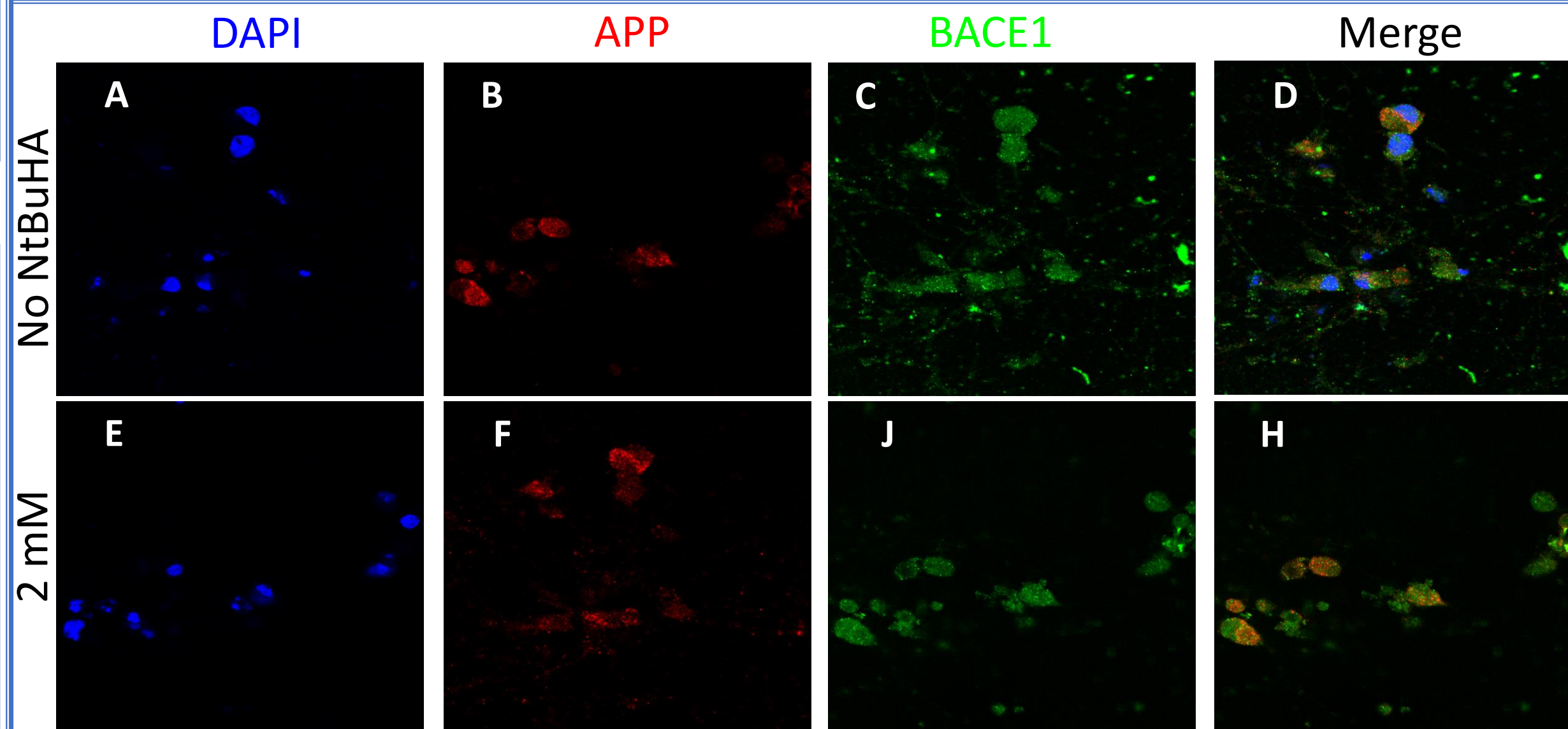


Figure 2. NtBuHA increases APP–BACE1 colocalization in iNs. Representative immunofluorescence images showing DAPI (A, E), APP (B, F), BACE1 (C, G), and merged channels (D, H).

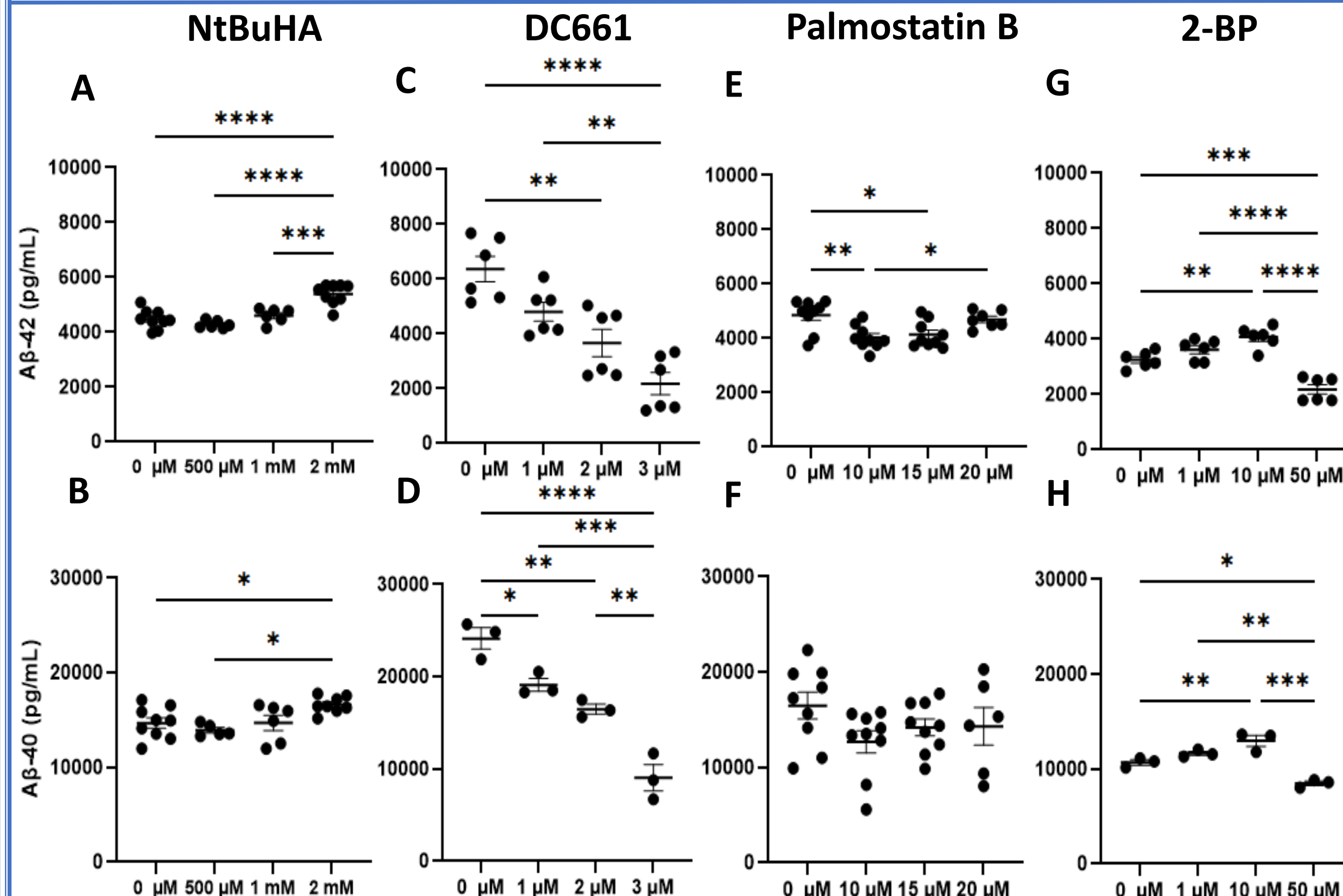


Figure 3. Pharmacological modulation of A β dynamics. N2A695 cells treated with NtBuHA (A–B), DC661 (C–D), Palmostatin B (E–F), or 2-BP (G–H). Soluble A β 40 and A β 42 levels (n = 3–9) are shown. Data are presented as mean $\pm$ SEM. *p $\leq$ 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

In vivo results

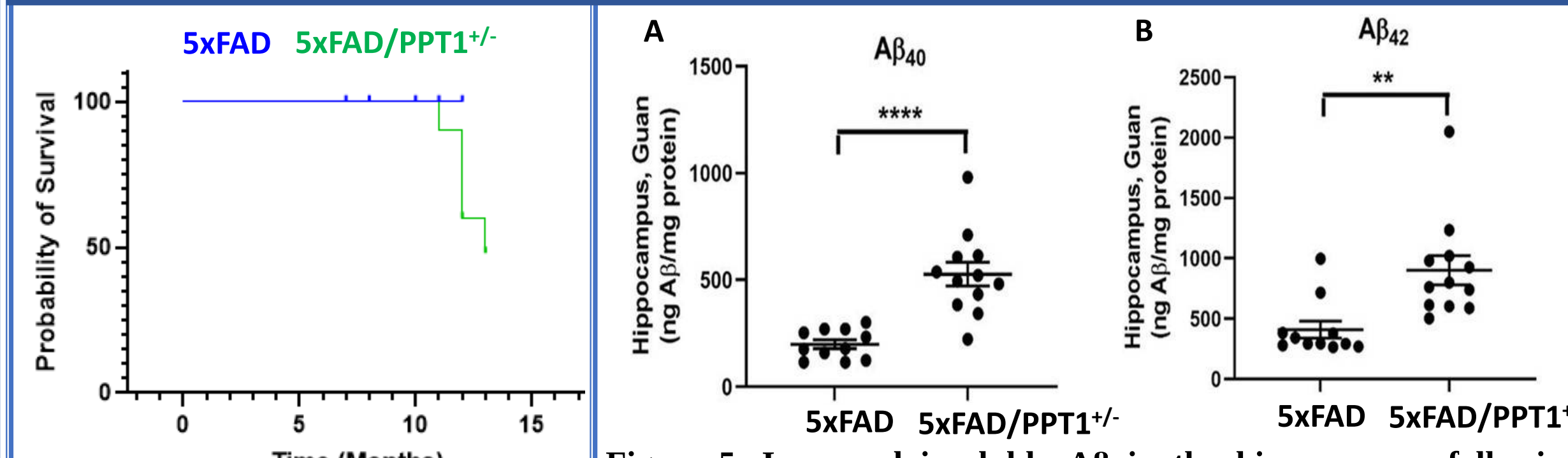


Figure 4. Survival Curve. Survival analysis comparing 5xFAD/PPT1^{+/-} (green line, n = 20), and 5xFAD (blue line, n = 19) mice. ****p < 0.0001.

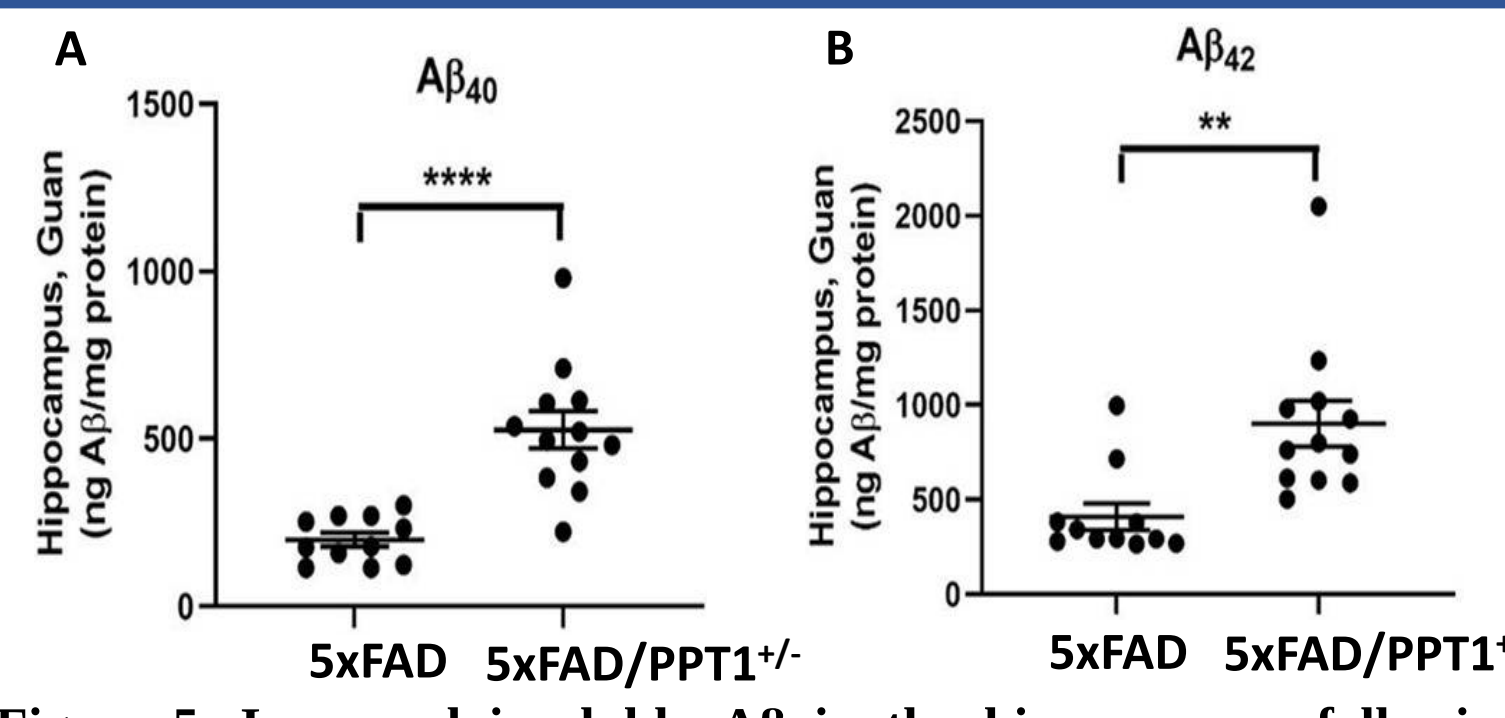


Figure 5. Increased insoluble A β in the hippocampus following PPT1 reduction. Quantification of insoluble A β 40 (A) and A β 42 (B) in fractionated hippocampal tissue from 5xFAD::PPT1^{+/-} (n = 12) and 5xFAD (n = 11) mice. Data are shown as mean $\pm$ SEM. **p < 0.01, ****p < 0.0001.

In vivo results

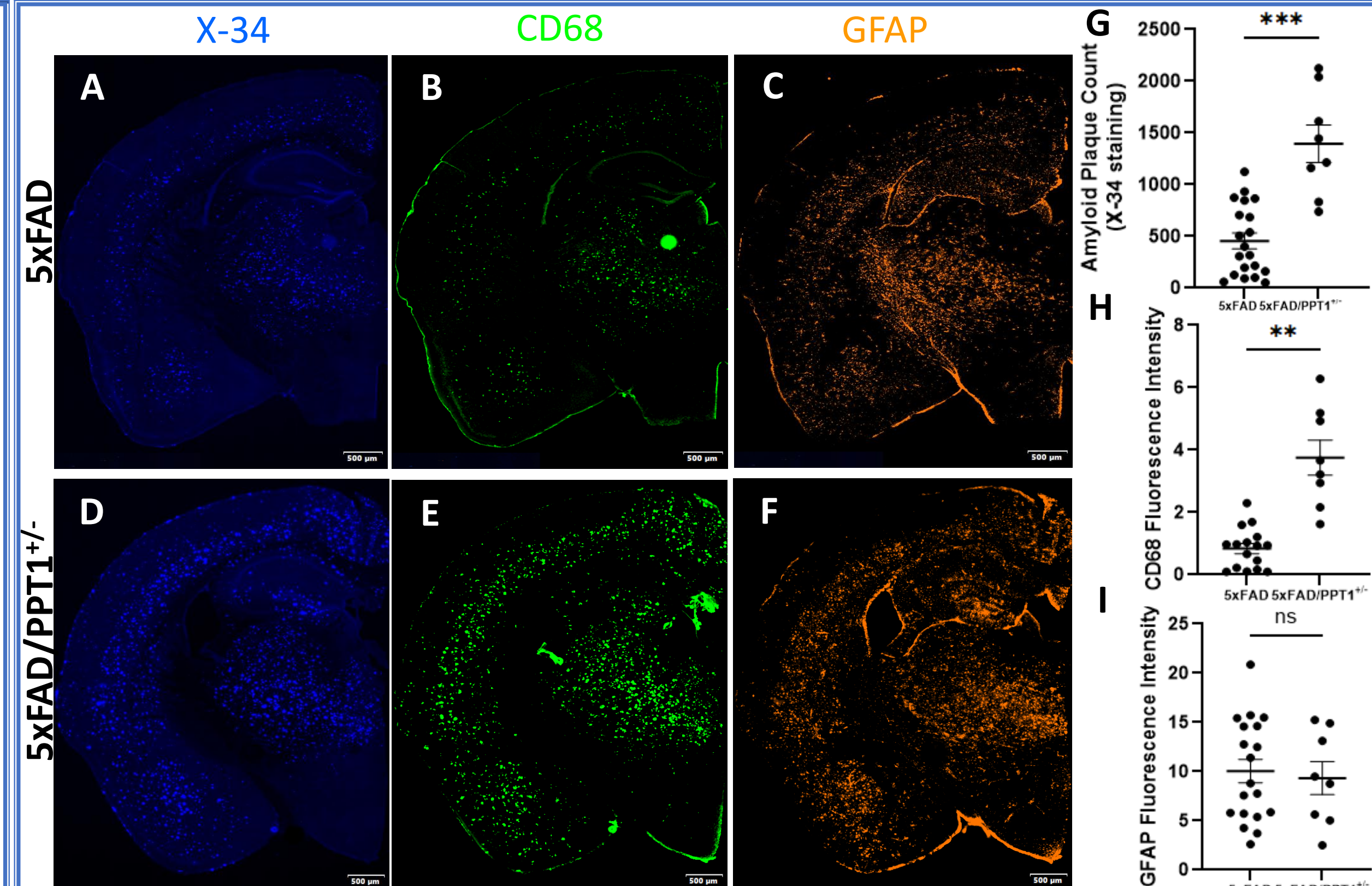


Figure 6. PPT1 loss-of-function increases A β accumulation and neuroinflammation. Representative right hemisphere images from 4-month-old mice (female and male) show X-34 (A, D), CD68 (B, E), and GFAP (C, F) staining in 5xFAD and 5xFAD/PPT1^{+/-} groups. Quantification of X-34 (G), CD68 (H), and GFAP (I) in 5xFAD (n=19) and 5xFAD/PPT1^{+/-} (n=8) mice. Data are mean $\pm$ SEM. **p < 0.01, ****p < 0.0001.

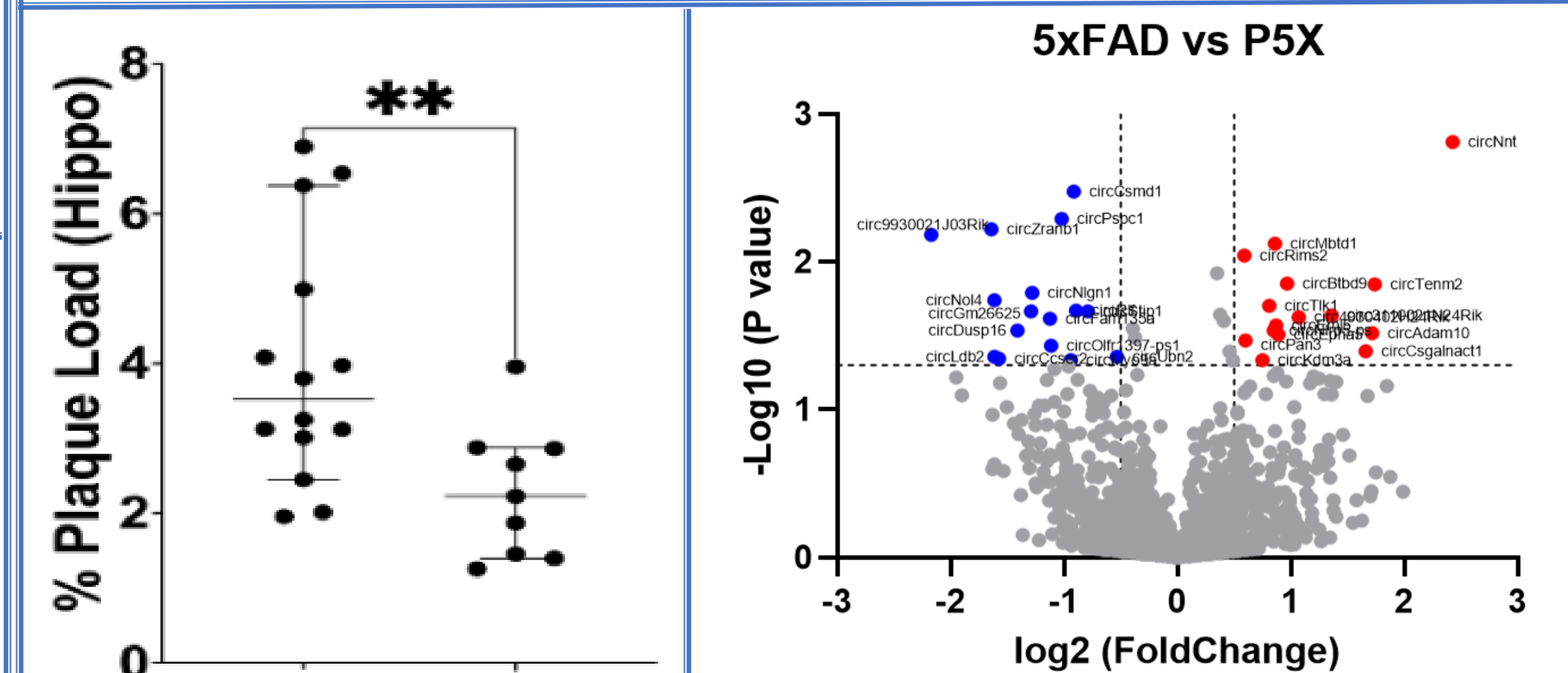


Figure 7. Hippocampal plaque load in untreated vs. AAV9-PPT1-treated 5xFAD/PPT1^{+/-} mice. **p < 0.01.

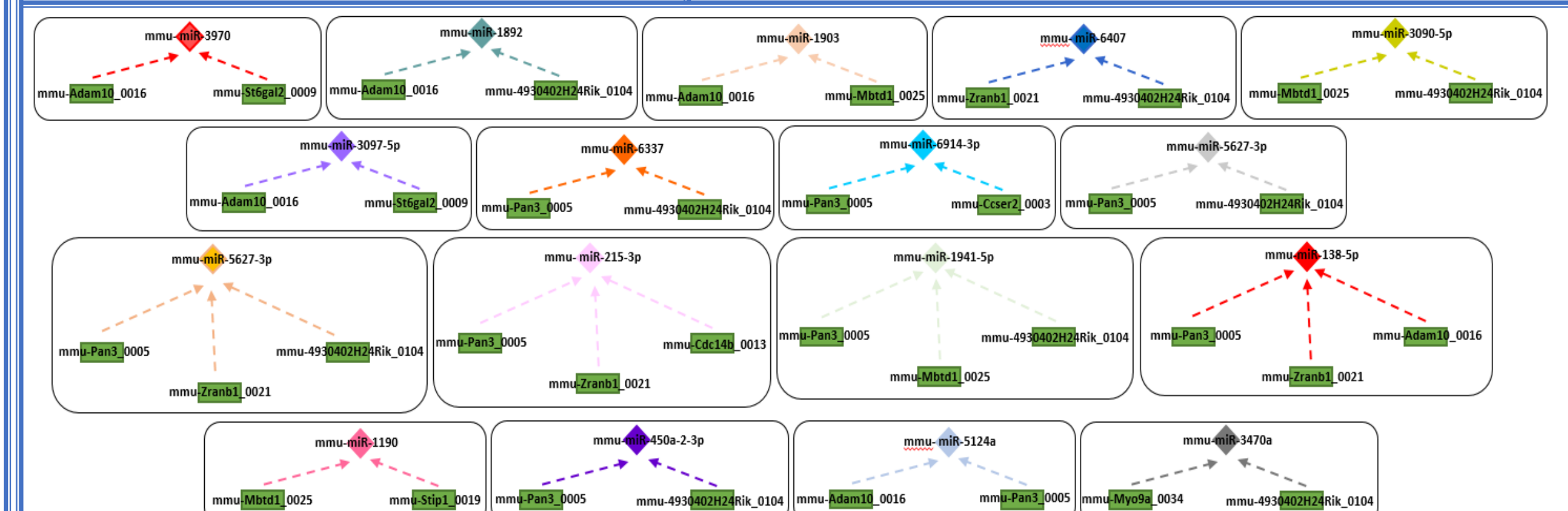


Figure 8. Volcano plots of differential expression for circRNAs. 5xFAD vs P5X. Upregulated circRNAs (Red dots) and downregulated circRNAs (blue dots). Gray dots (n.s.). The dotted vertical lines (1.5-fold up and down), and the horizontal dotted line (nominal p-value of 0.05).

