Supplementary Materials

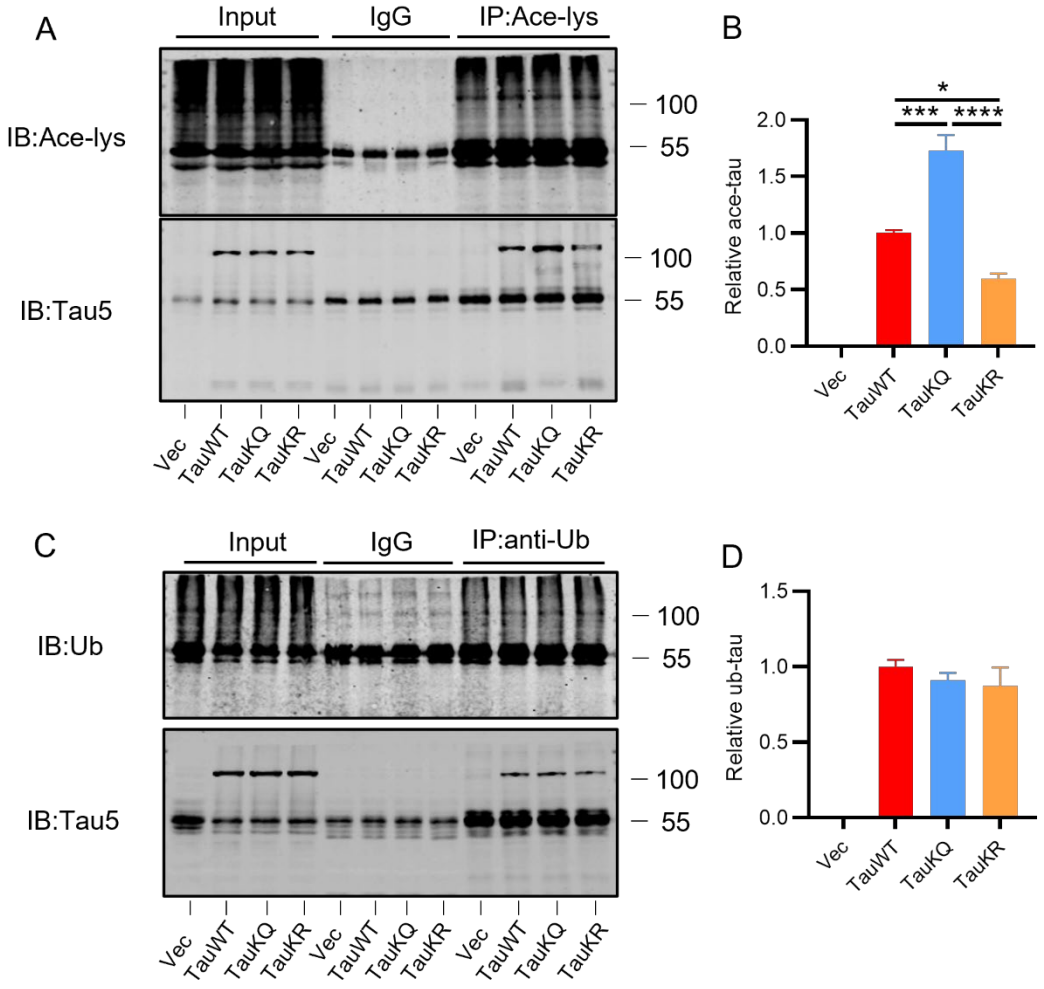


Fig. S1. The K274/281Q and K274/281R mutations altered only the level of tau acetylation, not the level of tau ubiquitination.

(A, B) The hippocampal CA1 subset of 2-month-old C57 mice were infected with the one of three types of tau or empty vector virus for one month, and then the homogenates of CA1 region (virus transfection) were used to immunoprecipitate by anti-acetylated lysine (Ace-lys) and Western blotting by anti-Ace-lys and Tau5 (detecting total Tau) (A). The exogenous tau (~106kDa, human tau) was used to quantify (B) (n = 4 biological replicates each group).

(C, D) The hippocampal CA1 subset of 2-month-old C57 mice were infected with the one of three types of tau or empty vector virus for one month, and then the homogenates of CA1 region (virus transfection) were used to immunoprecipitate by anti-Ubiquitin (Ub) and Western blotting by anti-Ub and Tau5 (detecting total Tau) (C). The exogenous tau

(~106kDa, human tau) was used to quantify (D) (n = 4 biological replicates each group). All data were presented as mean ± SEM. One-way ANOVA test followed by Tukey's post hoc test. *, $p < 0.05$, ***, $p < 0.001$, ****, $p < 0.0001$.

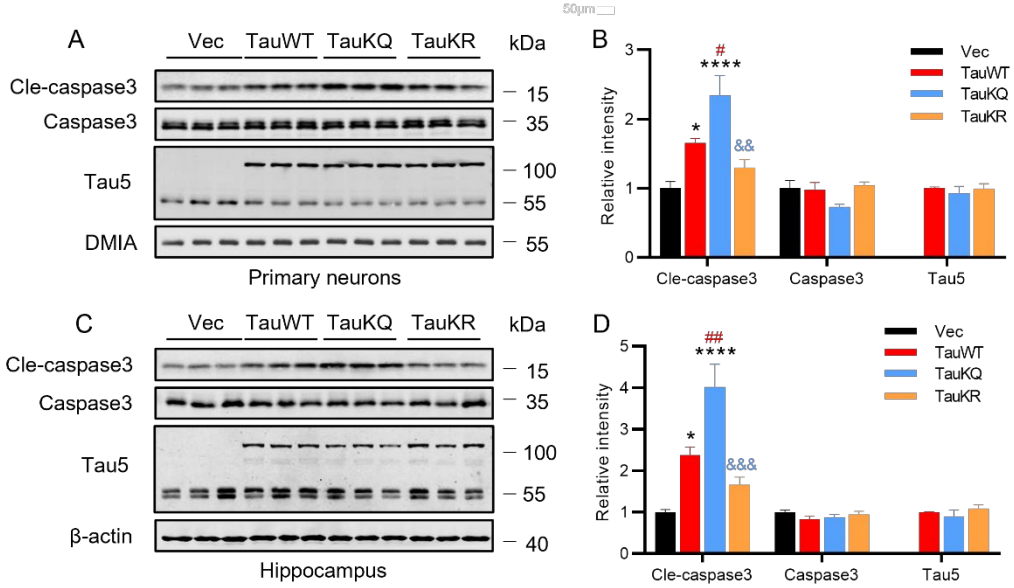


Fig. S2. Overexpressing TauKQ promoted neuron apoptosis.

(A, B) The expression level of Cleaved caspase-3 (Cle-caspase3), Caspase-3 and total Tau (Tau5) were detected by Western blotting (A) and quantitative analysis (B) in primary cultured hippocampal neurons after transfection with all three types of tau lentivirus (n = 6 biological replicates each group). All quantification of Tau5 in this manuscript is done with exogenous tau (~106kDa, human tau).

(C, D) The expression level of Cleaved caspase-3 (Cle-caspase3) and Caspase-3 were detected by Western blotting (C) and quantitative analysis (D) in the extracts of overexpressing the three types of tau or empty vector in hippocampal CA1 subset (n = 6 biological replicates each group).

All data were presented as mean ± SEM. One-way ANOVA test followed by Tukey's post hoc test. *, $p < 0.05$, ****, $p < 0.0001$, vs Vec; #, $p < 0.05$, ##, $p < 0.01$, vs TauWT; &&, $p < 0.01$, &&&, $p < 0.001$, vs TauKQ.

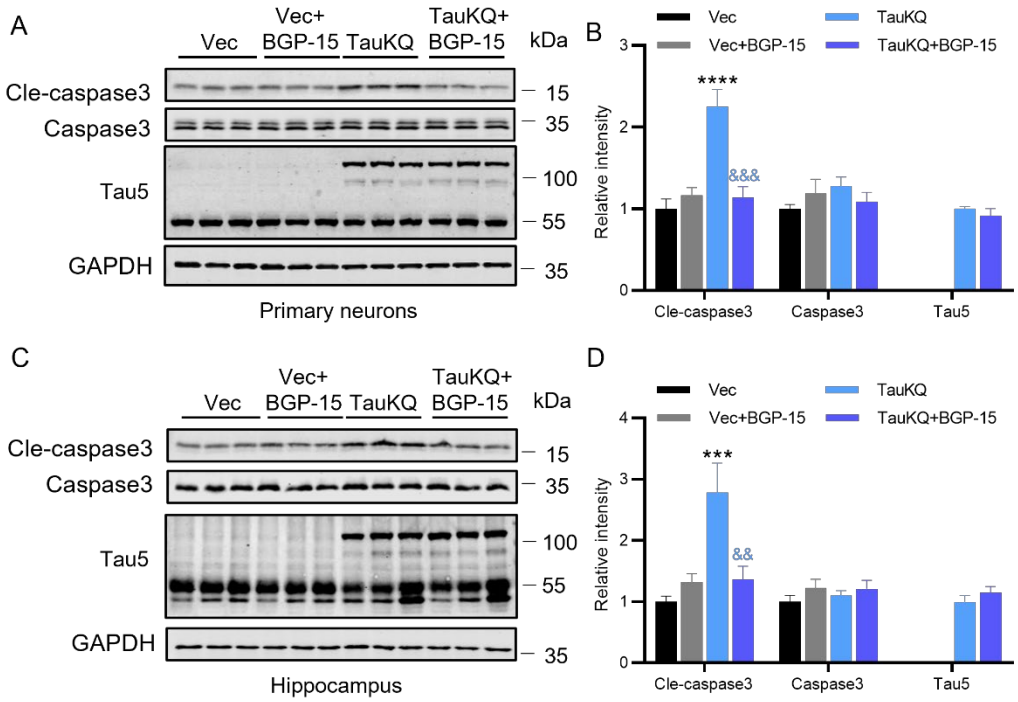


Fig. S3. BGP-15 ameliorated tauKQ-induced apoptosis.

(A, B) The primary cultured hippocampal neurons were infected with the lenti-TauKQ at 2 div, and 10 μM BGP-15 was treated at 3 div, half-change the culture medium every other day until sample collection at 8 div. The expression level of Cleaved caspase-3 (Cle-caspase3), Caspase-3 and total tau were detected by Western blotting (A) and quantitative analysis (B) (n = 6 biological replicates each group).

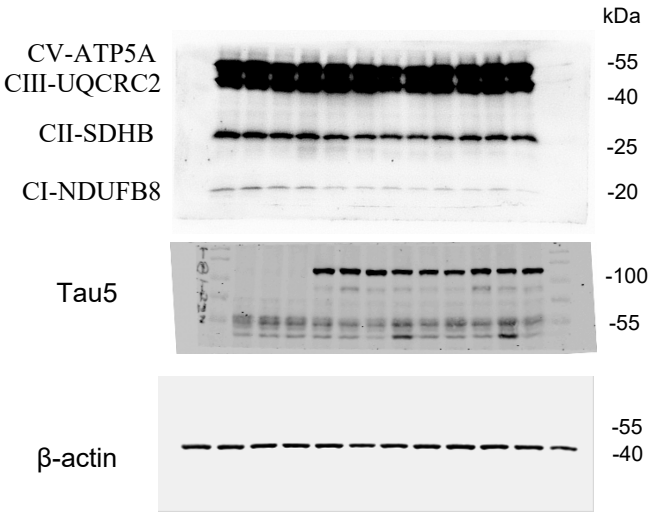
(C, D) The expression level of Cleaved caspase-3 (Cle-caspase3), Caspase-3 and total tau in the extracts of hippocampal CA1 region were detected by Western blotting (C) and quantitative analysis (D) (n = 6 biological replicates each group).

All data were presented as mean ± SEM. One-way ANOVA test followed by Tukey's post hoc test. ***, $p < 0.001$, ****, $p < 0.0001$, vs Vec; &&, $p < 0.01$, &&&, $p < 0.001$, vs TauKQ.

Table S1. Antibodies used in the present study

Antibody	Source / Identifier	Dilution
Anti-Tau5	Abcam, ab80579	WB: 1:1000
Anti-Mitofusin 1	Abcam, ab126575	WB: 1:1000
Anti-Mitofusin 2	CST, 9482S	WB: 1:1000
Anti-OPA1	Abcam, ab157457	WB: 1:300
Anti-DRP1	Abcam, ab184247	WB: 1:1000
Anti-Fis1	Proteintech, 66635-1-Ig	WB: 1:300
Anti-PGC1 alpha	Abcam, ab54481	WB: 1:500
Anti-PGC1 alpha	Proteintech, 66369-1-Ig	WB: 1:500
Anti-NRF1	ABclonal, A3252	WB: 1:2000
Anti-TFAM	Proteintech, 22586-1-AP	WB: 1:300
Anti-TFAM	Abcam, ab131607	WB: 1:500
Anti-TOMM40	Santa Cruz, sc-365467	WB: 1:500
Anti-Caspase3	CST, 9662S	WB: 1:500
Anti-Cleaved caspase3	CST, 9661S	WB: 1:300
Anti-LC3B	Abcam, ab51520	WB: 1:500
Anti-P62/SQSTM1	Abcam, ab56416	WB: 1:1000
Anti-mitochondrial OXPHOS	Abcam, ab110413	WB: 1:1000
Anti-MAP2	Abcam, ab32454	IF: 1:500
Anti-NeuN	Millipore, ABN78	IF: 1:200
Anti-GAPDH	Proteintech, 60004-1-Ig	WB: 1:5000
Anti-β-actin	Abclonal, AC026	WB: 1:3000
Anti-α-tubulin/DMIA	Abclonal, AC012	WB: 1:3000
Anti-DAPI	Beyotime, C1002	IF: 1:1000
Anti-Acetylated-Lysine	CST, 9441S	IP: 1:50
Anti-Ubiquitin	Santa Cruz, sc-8017	IP: 1:50

Source Data for Figure 2A



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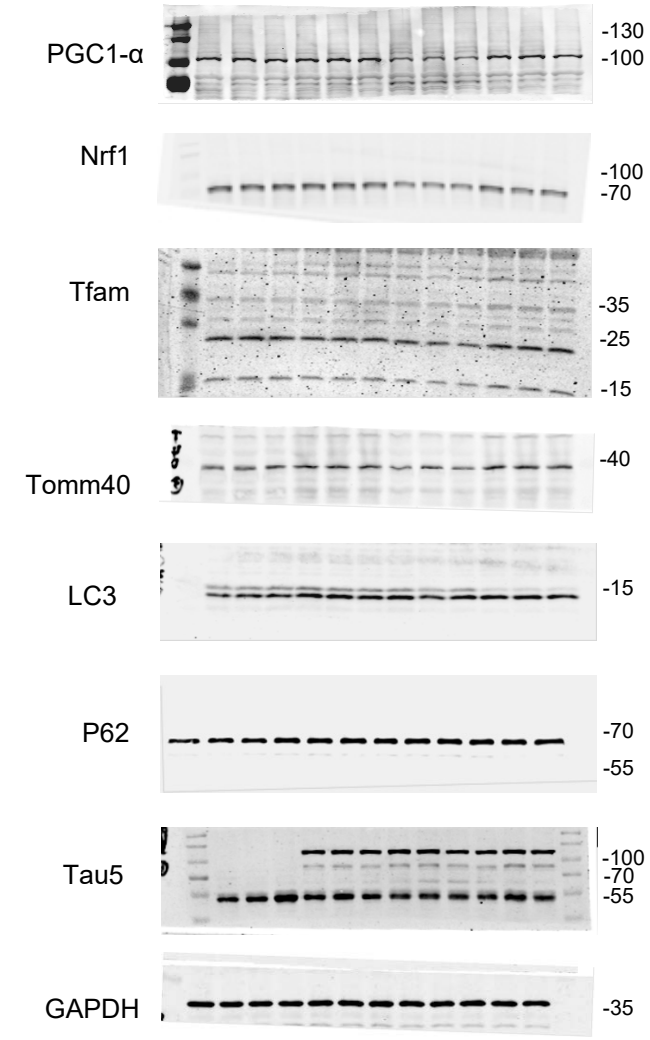

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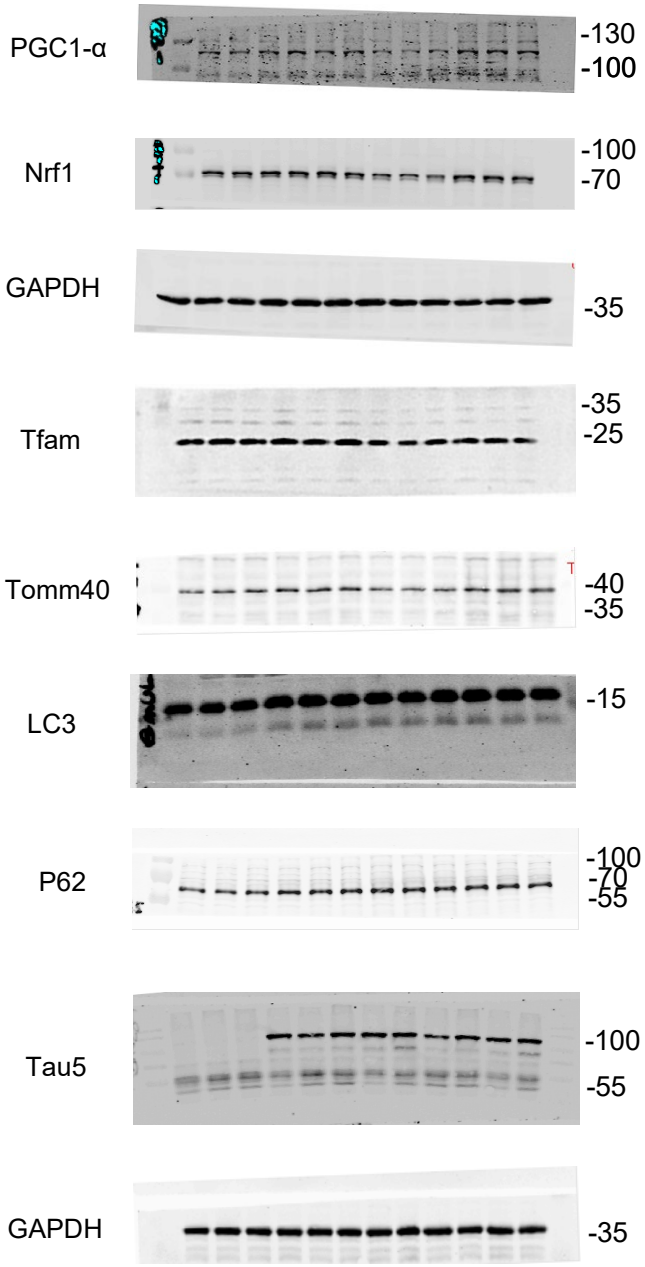

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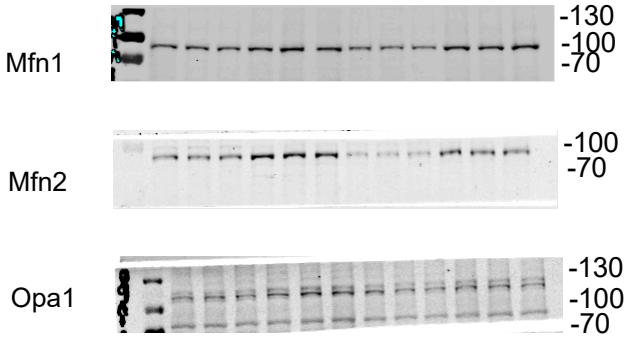

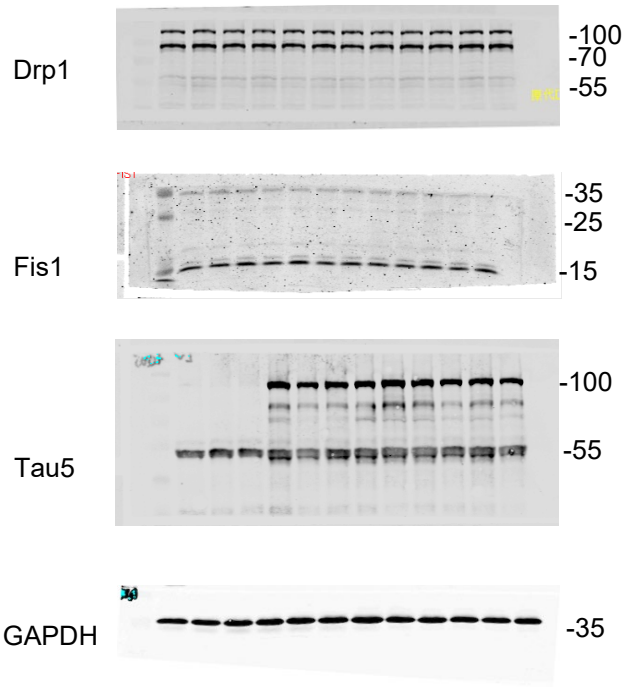

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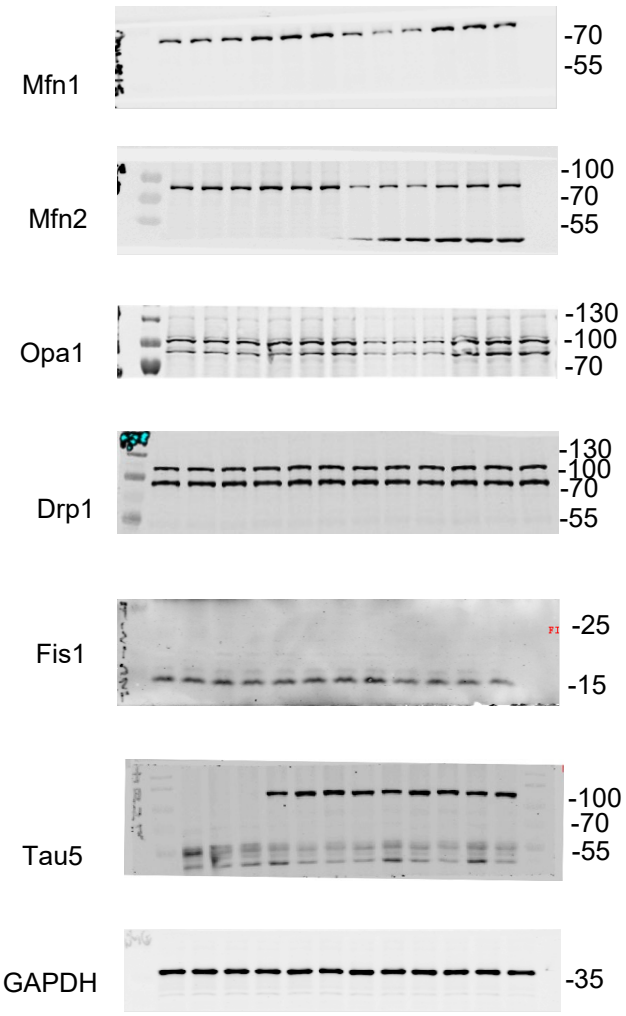

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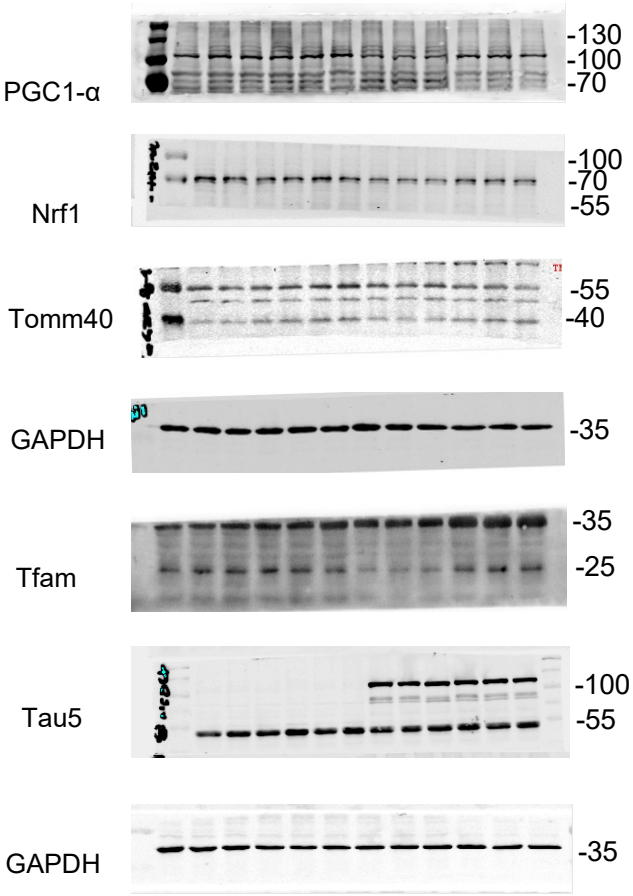

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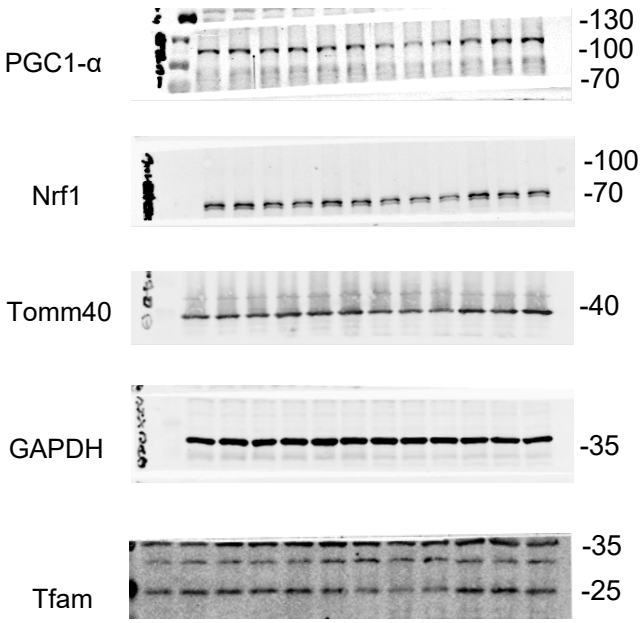

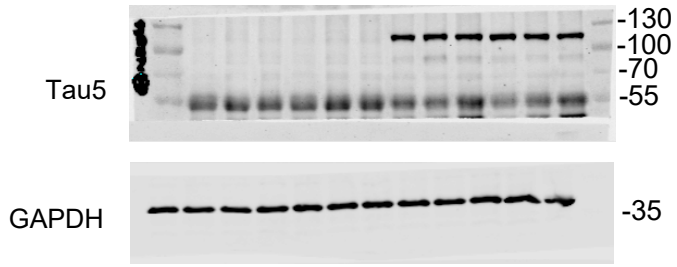

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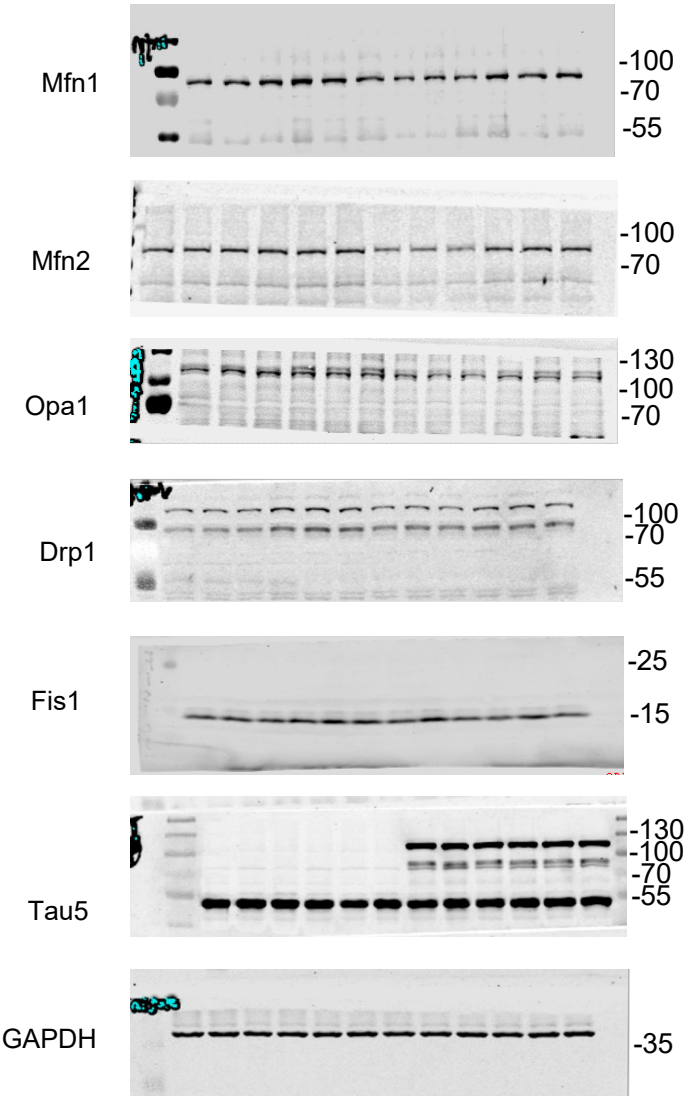

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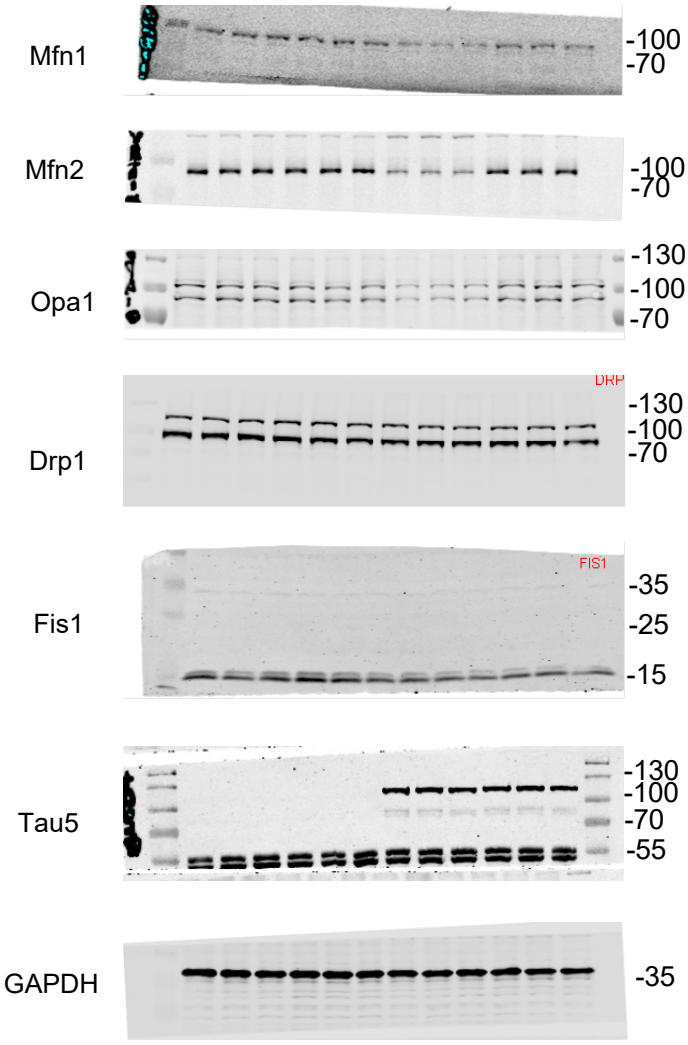

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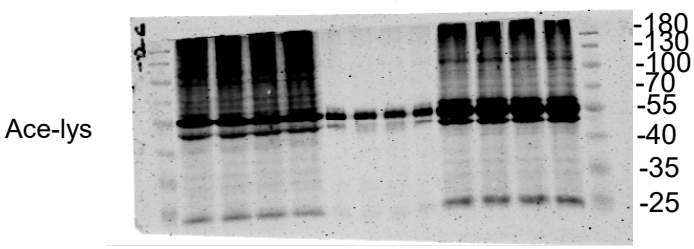
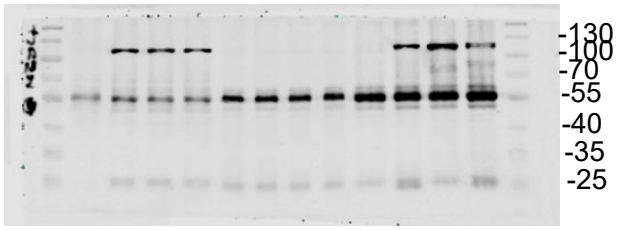


Tau5



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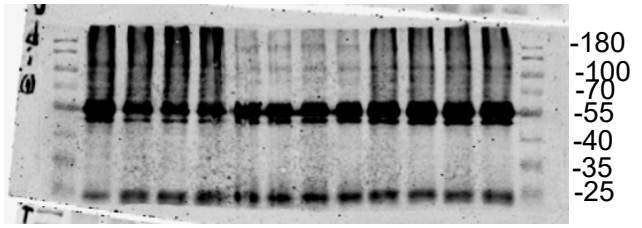

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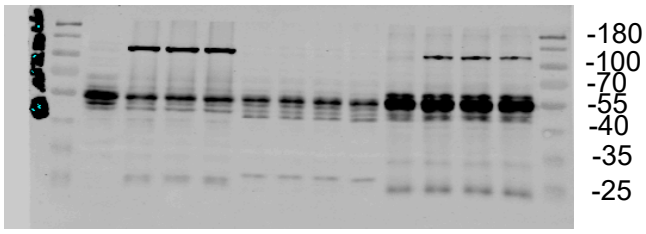

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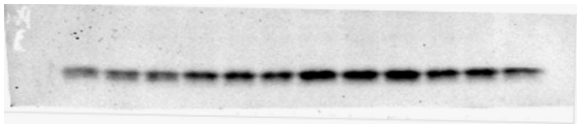
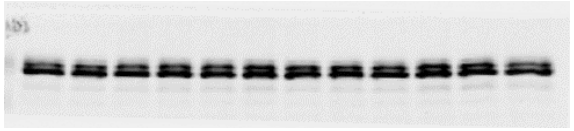


Caspase3



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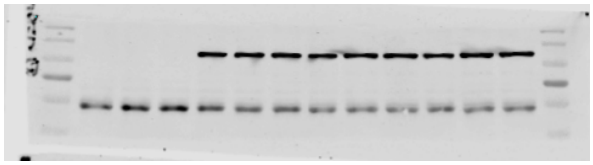

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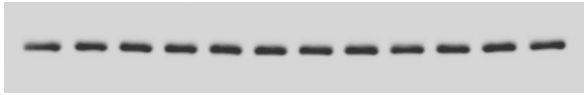

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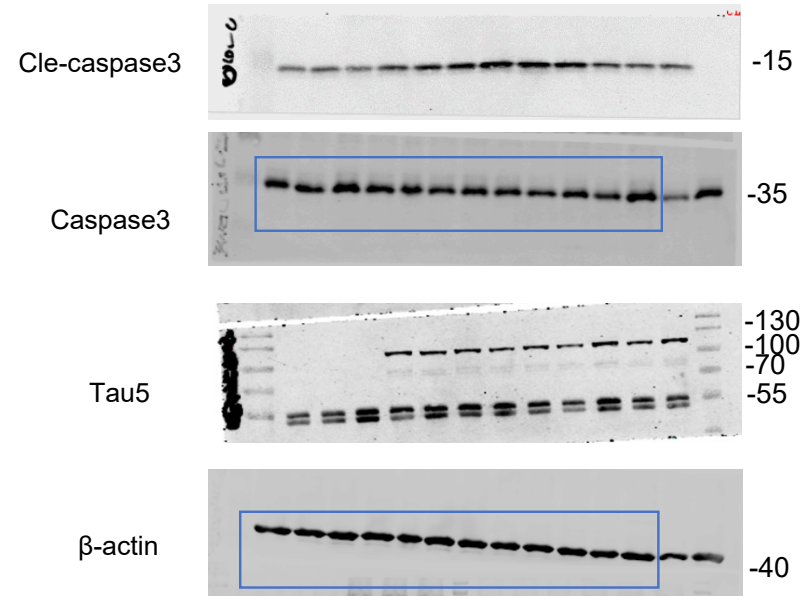

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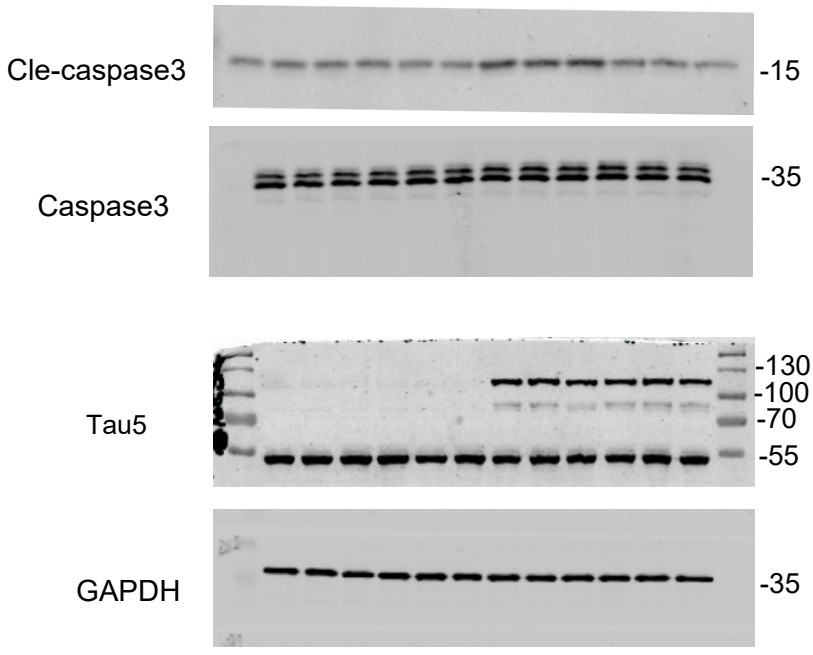
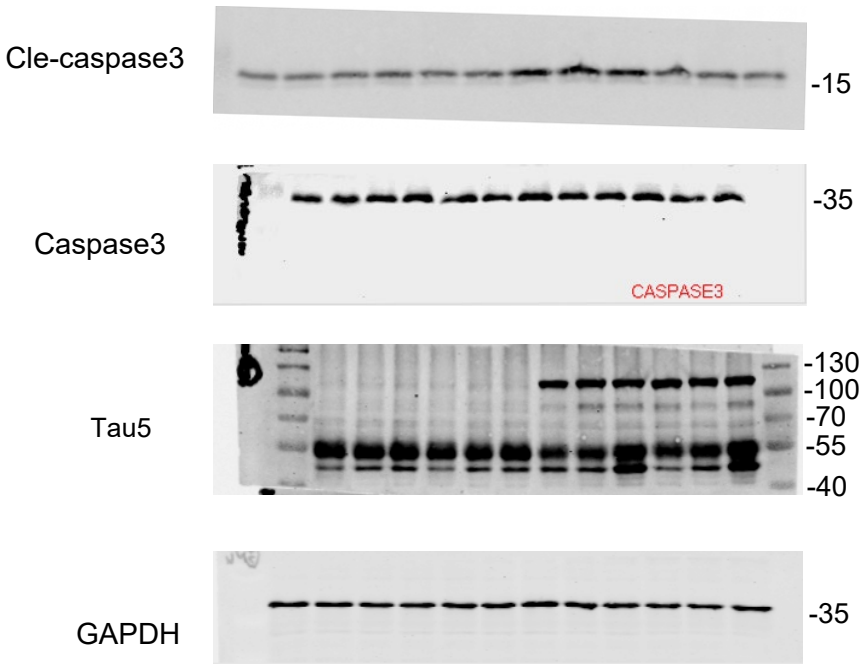
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