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# Supplementary Materials for

## Nitrosylation of GAPDH augments pathological tau acetylation upon exposure to amyloid-β

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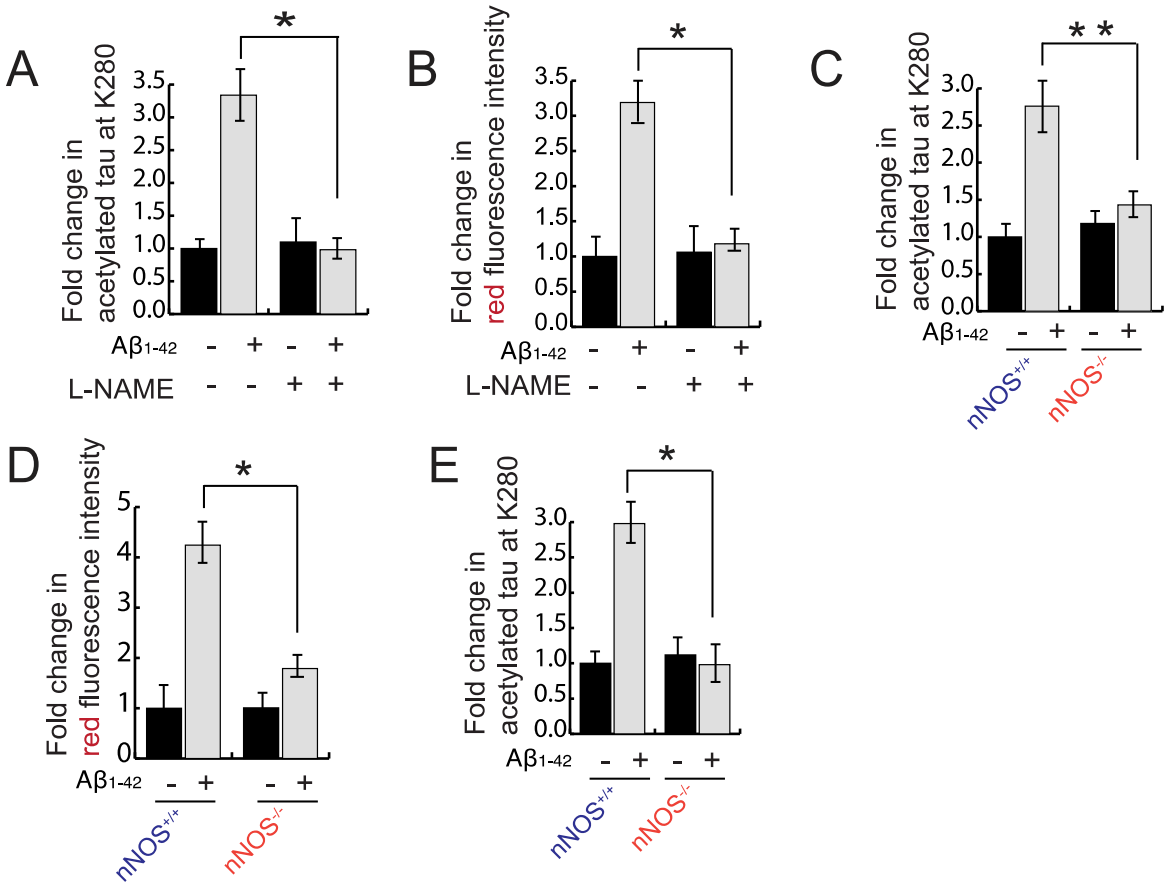


**Fig. S1**. **Quantitative analysis of tau acetylation in vitro and in vivo. (A)** Quantitative analysis of immunoblotting for acetylated tau abundance in primary cortical neurons treated with $A\beta_{1\text{-}42}$ for 24 hours with or without L-NAME. **(B)** Confocal microscopy analysis of the fold change in the red fluorescent intensity of acetylated tau. **(C)** Immunoblotting analysis of the fold change in acetylation of tau at $Lys^{280}$ in cortical neurons from $nNOS^{+/+}$ and $nNOS^{-/-}$ mice after administration of $A\beta_{1\text{-}42}$ with or without L-NAME. D **(D)** Confocal microscopy analysis of the fold change in the red fluorescent intensity indicating acetylation of tau in cortical neurons from $nNOS^{+/+}$ and $nNOS^{-/-}$ mice. **(E)** Immunoblotting analysis of the fold change in tau acetylation at $Lys^{280}$ in cortical neurons from $nNOS^{+/+}$ and $nNOS^{-/-}$ mice after administration of $A\beta_{1\text{-}42}$. All data are mean ± S.E.M, n=8-10, except for (C) (n=3-5); *p<0.05, **p<0.001 by student's *t*-test.

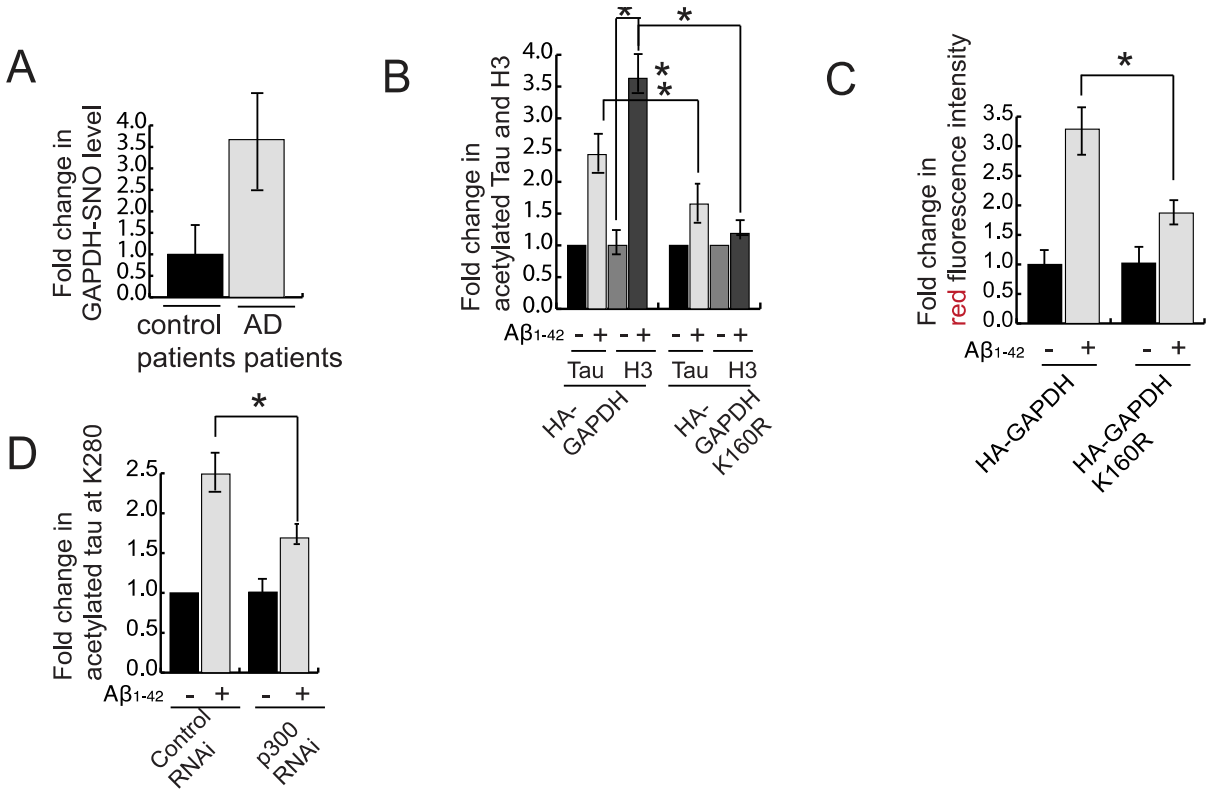


**Fig. S2**. **Quantitative analysis of GAPDH nitrosylation after Aβ$_{1–42}$ administration. (A)** Quantitative analysis of immunoblotting for the fold change in the abundance of nitrosylated GAPDH in post-mortem cortical brain tissue samples from control and AD patients. **(B)** Quantitative analysis of immunoblotting for the fold change in the acetylation of tau and H3 in cultured cortical neurons after overexpression of either HA-GAPDH or HA-GAPDH-K160R. **(C)** Quantitative analysis of immunoblotting for the Fold change in tau acetylation, assessed by red fluorescence intensity in confocal microscopy, in the cortex of mice overexpressing HA-GAPDH or HA-GAPDH-K160R and injected with Aβ$_{1-42}$. **(D)** Fold change in tau acetylation at Lys$^{280}$ after administration of Aβ$_{1-42}$ to isolated cortical neurons transfected with control or p300-targeted siRNA (RNAi). All data are mean ± S.E.M., n= 5-7; *p<0.05, **p<0.001 by student's *t*-test.

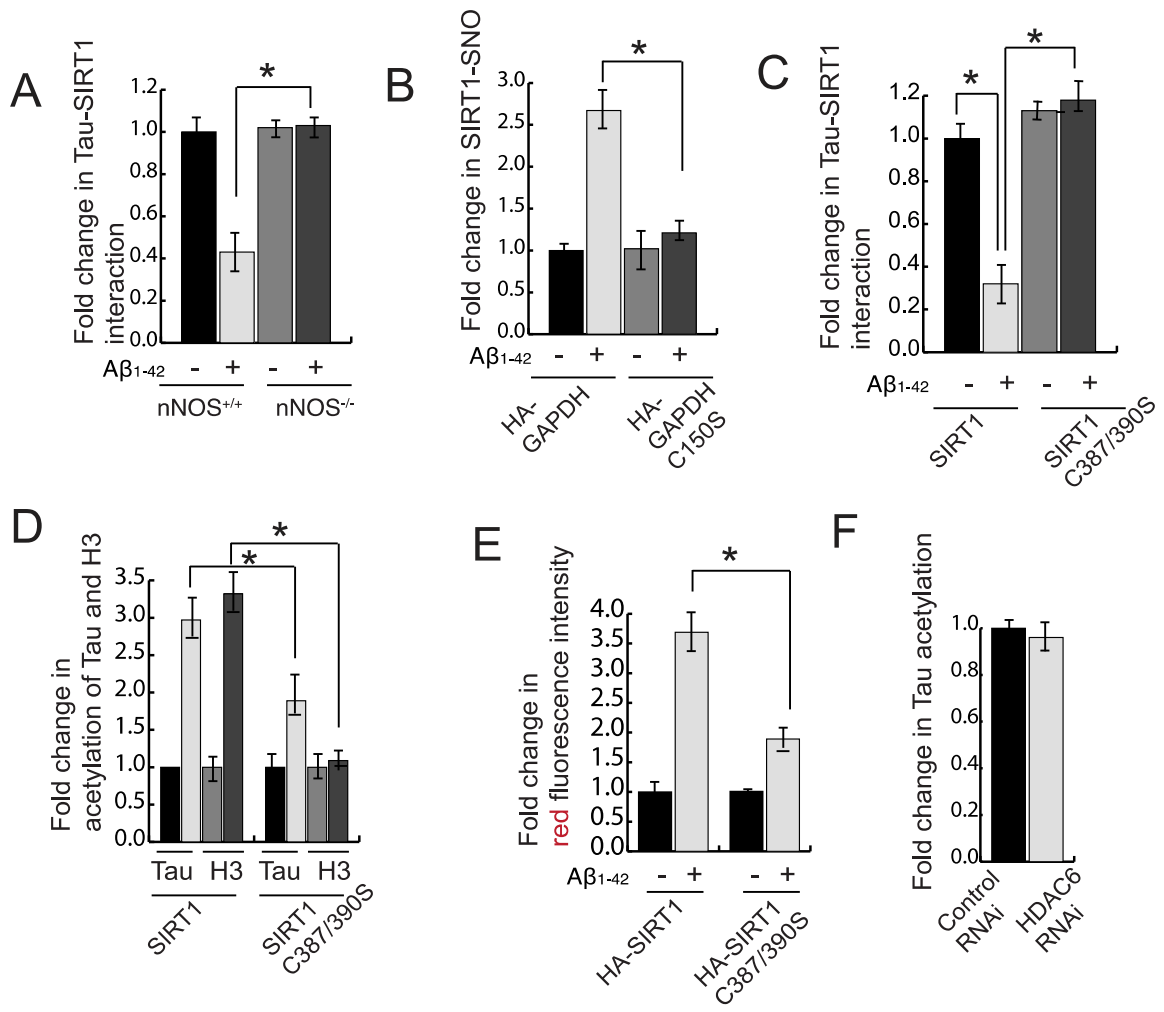


**Fig. S3. Quantitative analysis of transnitrosylation of SIRT1 and its effect on tau acetylation. (A)** Quantification of co-immunoprecipitation assays to measure the interaction between tau and SIRT1 in the cortex of nNOS$^{+/+}$ and nNOS$^{-/-}$ mice, presented as fold change relative to controls. **(B)** Quantification of immunoblotting for the fold change in SIRT1 nitrosylation in the cortex of nNOS$^{+/+}$ and nNOS$^{-/-}$ mice injected with HA-GAPDH or HA-GAPDH-K160R then A$\beta_{1\text{-}42}$. **(C)** Quantification of co-immunoprecipitations assessing the interaction between tau and SIRT1 in cultured primary cortical neurons transfected with SIRT1 or SIRT1-C387/390S. **(D)** Quantification of immunoblotting for the fold change in acetylation of tau and H3 in cells described in (C). **(E)** Fold change in red flurorescence intensity, indicating tau acetylation, after overexpression of either SIRT1 or SIRT1-C387/390S in the cortex. **(F)** Quantification of immunoblotting for the fold change in tau acetylation in cultured primary cortical neurons after administration of either control or HDAC6 siRNA. All data are mean ± S.E.M., n=8-10 (A) or 5-7 (B-F); *p<0.05, **p<0.001 by student's *t*-test.

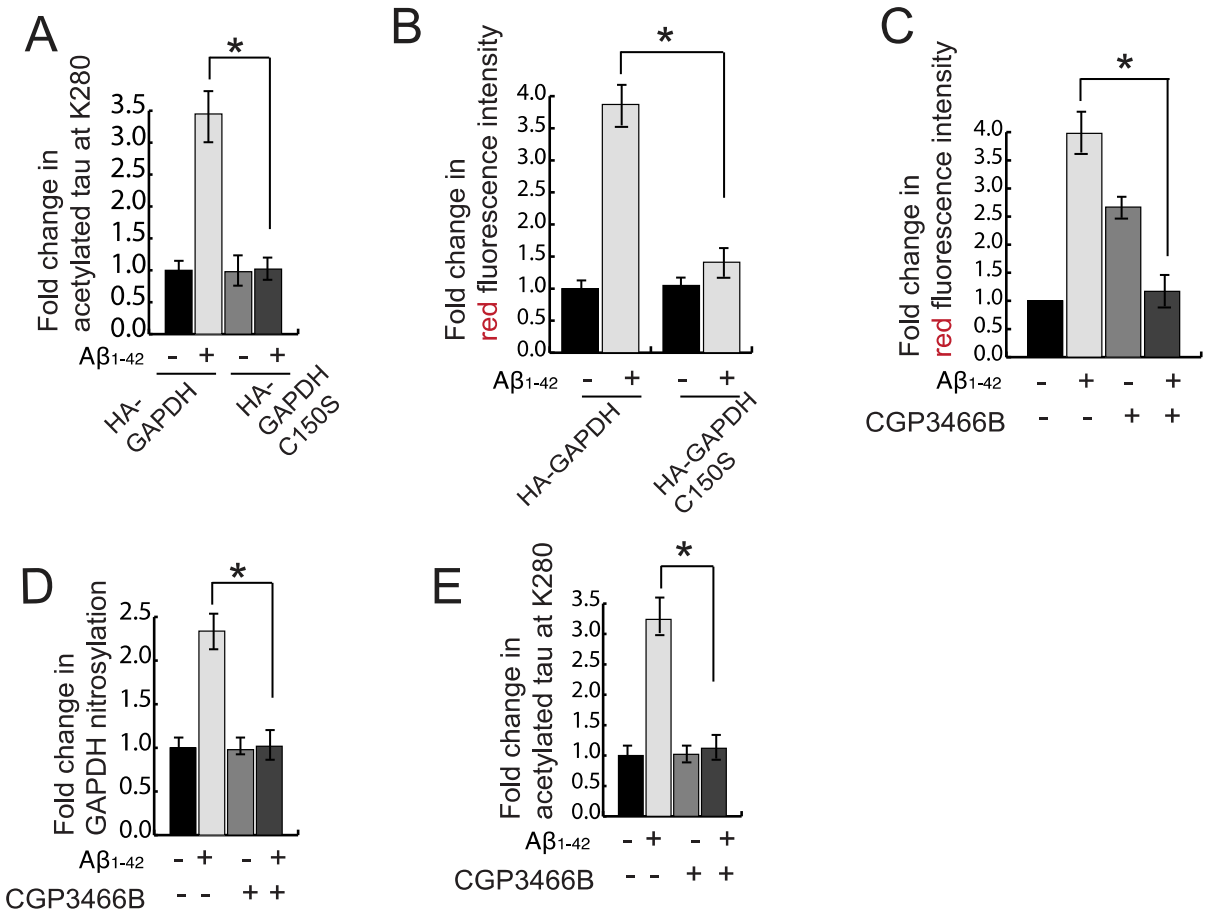


**Fig. S4. Quantitative analysis of tau acetylation after inhibition of GAPDH nitrosylation.** **(A)** Quantification of co-immunoprecipitation assays, presented as fold change in tau acetylation at $Lys^{280}$ in cortical lysates from mice that were intracortically injected with HA-GAPDH or HA-GAPDH-K160R then $A\beta_{1\text{-}42}$. **(B)** Quantification of confocal microscopy analysis as fold change in red fluorescence intensity, indicating acetylated tau, in the cortex after overexpression of either HA-GAPDH or HA-GAPDH-C150S then $A\beta_{1\text{-}42}$. **(C)** Quantification of confocal microscopy analysis as fold change in red fluorescence intensity, indicating acetylated tau, in the cortex after treatment with CGP3466B then $A\beta_{1\text{-}42}$. **(D and E)** Quantification of blotting analysis GAPDH nitrosylation (D) and tau acetylation (E) in cortical lysates from mice treated with CGP3466B (2.5 mg/kg) then $A\beta_{1\text{-}42}$. All data are mean ± S.E.M., n=5-7; *$p<0.05$, student's *t*-test.