Supplementary Material

Loss of tau and Fyn reduces compensatory effects of MAP2 for tau and reveals a Fyn-independent effect of tau on signaling

Guanghao Liu, Ramasamy Thangavel, Jacob Rysted, Yohan Kim, Meghan B Francis, Eric Adams, Zhihong Lin, Rebecca J Taugher, John A Wemmie, Yuriy M Usachev, Gloria Lee

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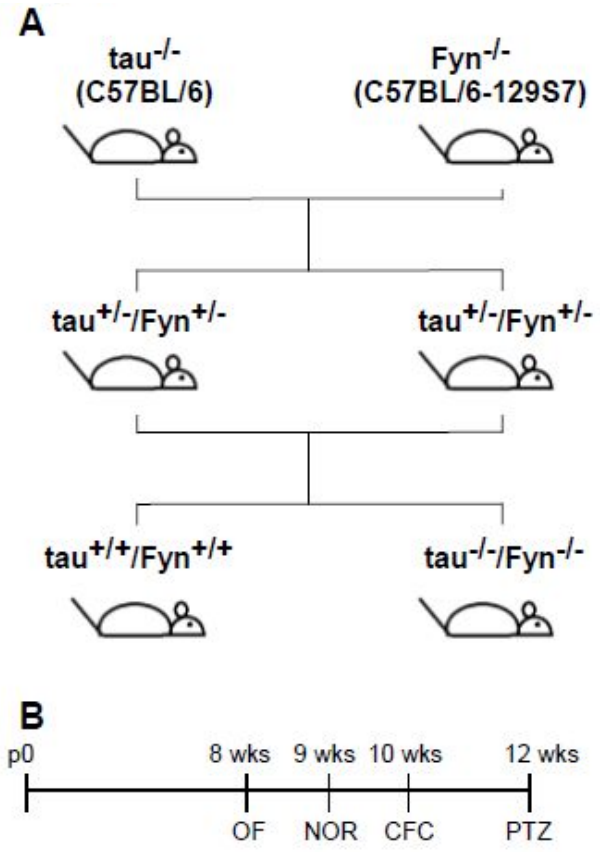

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MHV	**Sendai**	**PVM**
Reo3	**TMEV**	**Ectromelia**
Mouse adenovirus 1 & 2	**M. pulmonis**	**LCMV**
EDIM	**Parvo virus (MVM)**	**Parvo virus (MPV)**
Pin worms	**Furmites**	

Fig. S1: Breeding scheme to generate DKO and WT mice, behavior testing time line, and colony pathogen status

A) Tau$^{-/-}$ and Fyn$^{-/-}$ mice were mated to create tau$^{+/-}$/Fyn$^{+/-}$ heterozygote mice. The heterozygotes were then mated to generate tau$^{+/+}$/Fyn $^{+/+}$ (WT) and tau$^{-/-}$/Fyn$^{-/-}$ (DKO) mice.

B) Behavioral experiment set up: Mice underwent the following tasks, ordered from the least traumatizing, to the most traumatizing: Open field (OF, 8 wks), novel object recognition (NOR, 9 wks), contextual fear conditioning (CFC, 10 wks), and lastly, pentylenetetrazole induced seizures (PTZ, 12 wks).

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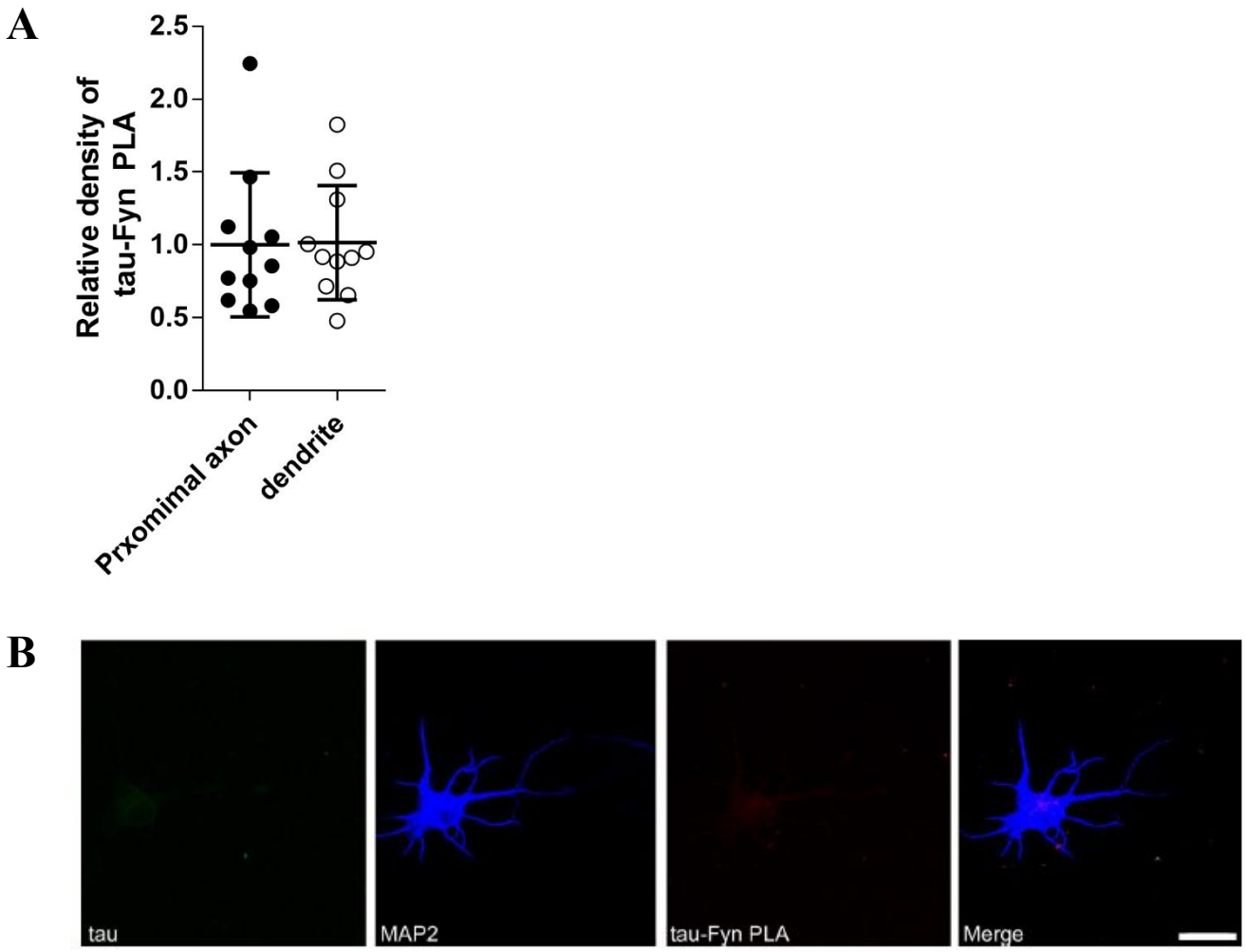

Fig. S2: Density of tau-Fyn PLA complexes in WT proximal axons and their absence in tau KO hippocampal neurons

A) The density of tau-Fyn complexes in WT dendrites were plotted relative to proximal axons with proximal axon as 1. There was no significant difference between the two (p=0.9346; statistical results are shown in Table 3.) n=11 images.

B) Using PLA, tau KO neurons did not show tau-Fyn complexes. Scale bar: 25 um.

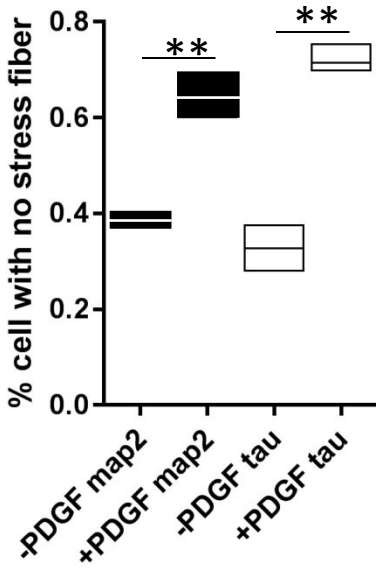

Fig. S3: MAP2c resembled tau in potentiating SFK activity in cells

The ability of MAP2c to potentiate SFK activity in cells was assayed using 3T3 cells responding to PDGF, as described by Sharma et al (2007). It is well established that upon PDGF treatment, after 10 min, 3T3 cells lose their stress fibers; then, after 7 hrs, stress fibers are re-gained. It has also been shown that the change in stress fiber pattern relies on Src activity. That is, at 10 min, Src is activated and after 7 hrs, Src has returned to its basal activity level. In Sharma et al (2007), we had demonstrated that in PDGF-stimulated 3T3 cells expressing tau, after 7 hrs, the percent of cells with no stress fibers was significantly higher than that in the non-transfected cells or the –PDGF culture (open bars). In examining 3T3 cells transfected with MAP2c, 7 hrs after PDGF stimulation, the percent of cells with no stress fibers resembled that of tau transfected cells (compare +PDGF black bar with +PDGF open bar), with no significant difference between MAP2c and tau transfected cells at 7 hours ($p=0.0771$). The absence of stress fibers at 7 hrs was indicative of persistent activated Src in MAP2c expressing cells. N=3 experiments. Tau –PDGF vs +PDGF: **$p=0.0027$; MAP2c –PDGF vs +PDGF: **$p=0.0061$. Statistical results are shown in Table 3. 3T3 cells have not been reported as contaminated or misidentified (International Cell Line Authentication Committee, October 14, 2018).

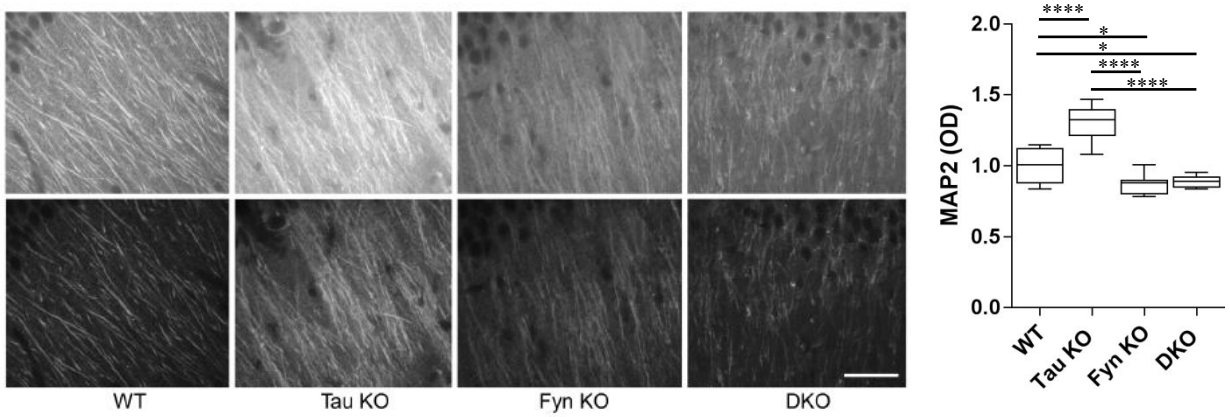

Fig. S4: MAP2 expression in the hippocampus was altered in the Fyn KO and DKO mice.

MAP2 was labeled in mouse hippocampal sections from the 4 genotypes. Top row shows the raw images captured from each genotype using identical photographic conditions. Bottom row shows MAP2 staining after thresholding the images using identical thresholding conditions in ImageJ. Scale bar: 50 μm. MAP2 values obtained from thresholded images are shown on the right. 9 images from each genotype were examined using Image J. WT-Fyn: *p=0.0271; WT-tau: ****p<0.0001; WT-DKO: *p=0.0165; tau KO-Fyn KO: ****<0.0001; tau KO-DKO: ****p<0.0001. Statistical results are shown in Table 3.

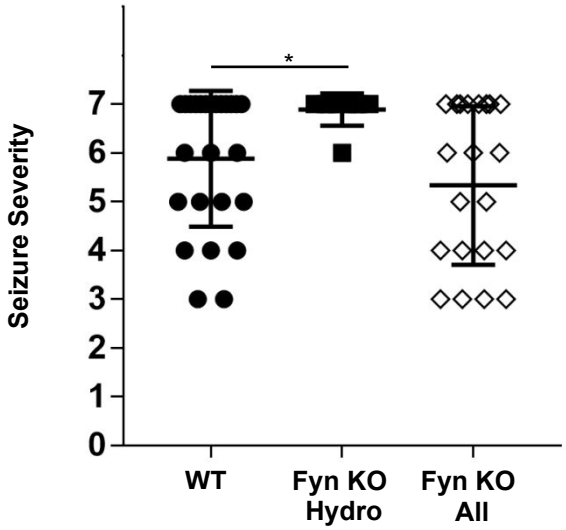

Fig. S5: Hydrocephalus increased Fyn KO mice's susceptibility to PTZ induced seizures.

In one cohort, Fyn KO mice with moderate hydrocephalus reached higher seizure stages after PTZ injection compared to WT (WT vs Fyn KO Hydro: *p=0.0410) while non-hydrocephalic Fyn KO mice were protected and reached lower seizure stages (Fig. 3F). Since the numbers of Fyn KO Hydro and Fyn KO normal mice in this cohort were very similar (9 vs 12), when the two groups were combined ("all" = hydro + normal), the mean maximum seizure stage reached resembled that of WT (WT vs Fyn KO all: p=0.2255). n=25 WT, 9 Fyn KO hydro, 12 Fyn KO normal. Statistical results are shown in Table 3.

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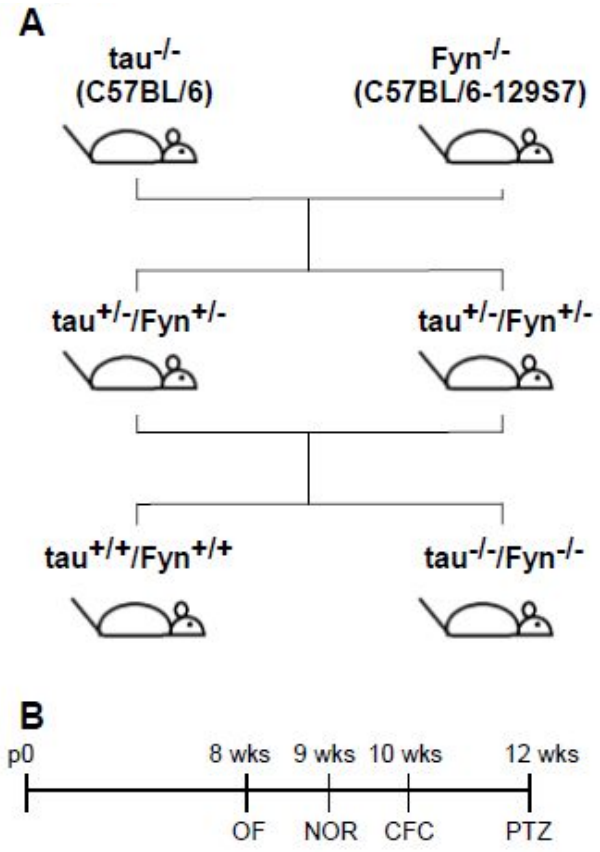

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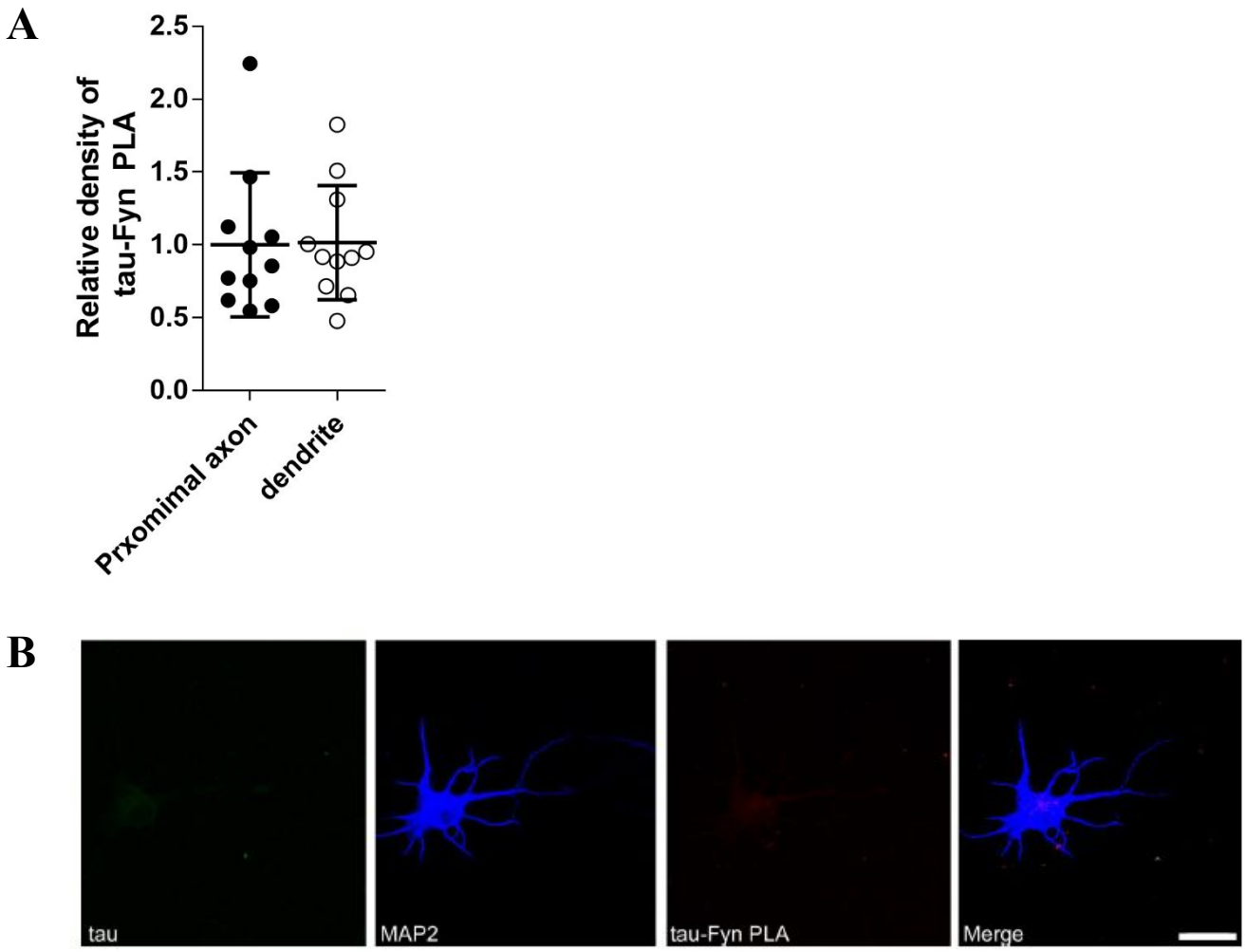

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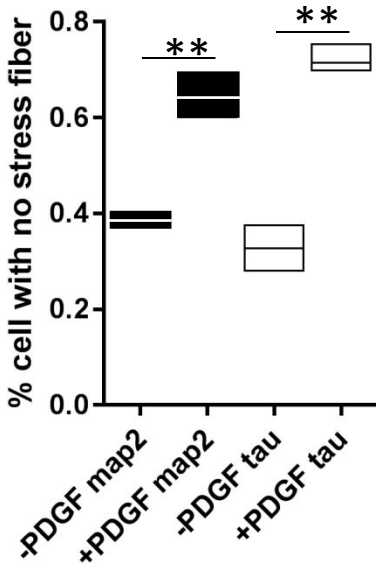

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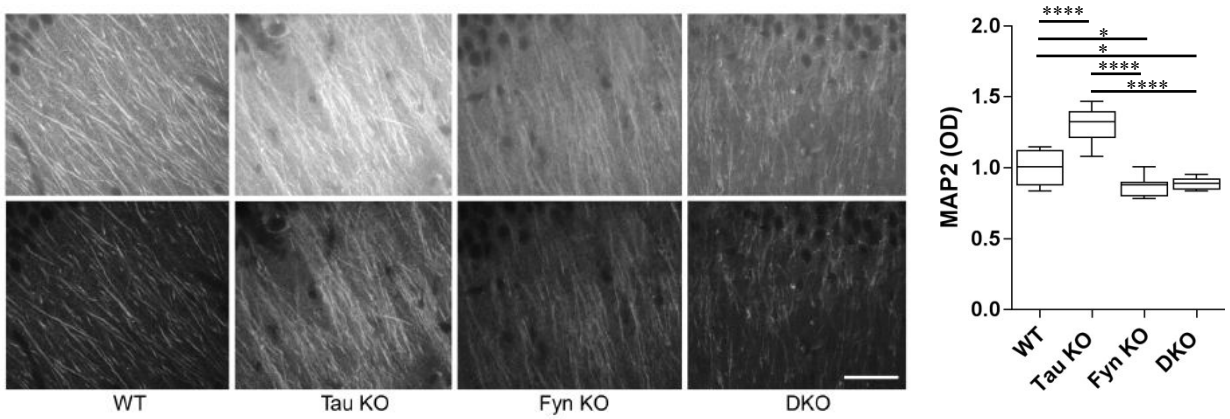

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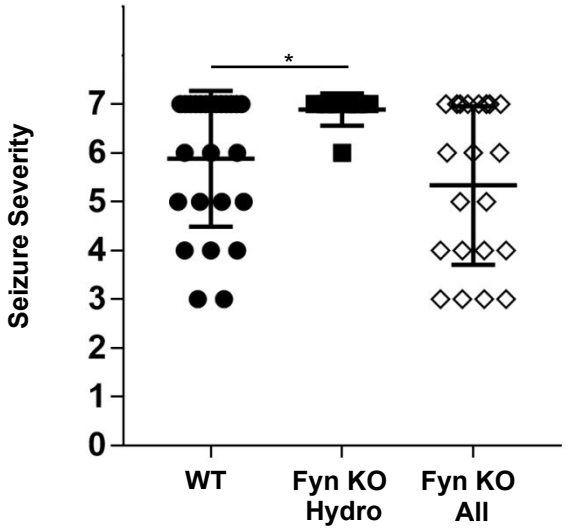

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