Supplemental Materials

Molecular Biology of the Cell

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Movie 1. α-Syn-GFP in PFF-treated neurons accumulates in large immobile aggregates. Primary neurons were transfected with α-syn-GFP, treated with PFFs and imaged 7 days later. Images were captured every 5 min for 420 min and the videos are presented at 20 fps.

Movie 2. Synaptic vesicle precursors travel normally in PFF treated neurons. Primary neurons were transfected with Synaptophysin-GFP, treated with PBS or PFF and imaged 7 days later.

Movie 3. Transport of TrkB receptors in the presence of BDNF is reduced in PFF-treated neurons. Primary neurons were transfected with TrkB-GFP, treated with PBS or PFF and imaged 7 days later. Images were captured every 1 sec for 3 min and are presented at 20 fps videos.

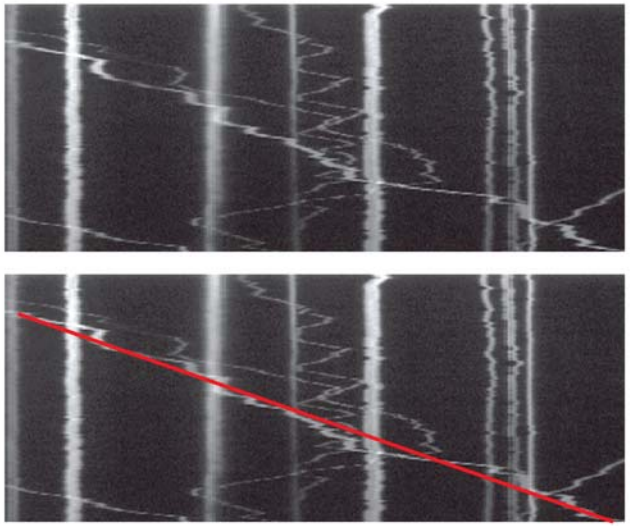
Supplemental Figure 1. Examples of quantitation with sample kymographs. **(A)** To measure average velocity, a line from the beginning of the particle trajectory to the end was drawn, as demonstrated by the red line. Using ImageJ, it was determined that this particle traveled 54 μm over 137 seconds for a velocity of 0.39 μm/sec. This kymograph also shows an example of a particle with multiple reversals and examples of an immobile particles. **(B)** This kymograph shows a particle that paused 2 times during its path.

Supplemental Figure 2. (A) Neurons from wild type (nontransgenic mice) were either mock transfected or transfected with α-synuclein-GFP. Lysates were immunoblotted using an antibody that recognizes total α-syn. α-Syn-GFP appears to be expressed at the same level as endogenous α-syn. **(B)** Neurons from α-syn KO mice were plated, treated with PBS or PFFs and fixed 10 days later. Immunofluorescence was performed for the dendrtitic marker, MAP2, and DAPI to visualize nuclei. There were no obvious alterations in the morphology of neurons from the α-syn KO mice.

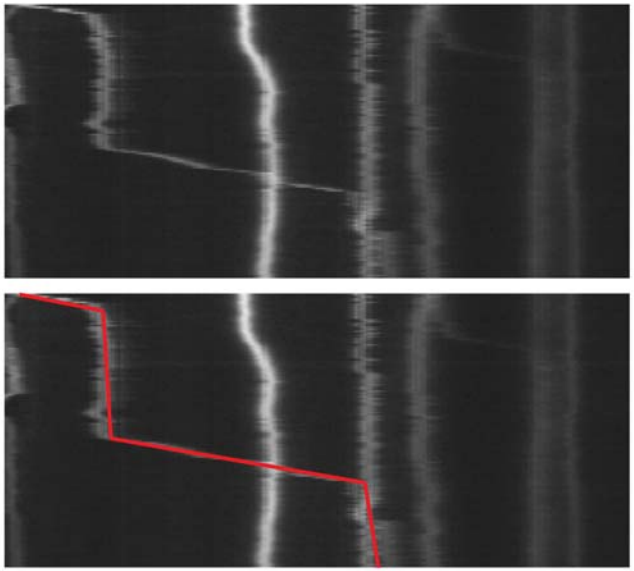
Supplemental Figure 3. Primary hippocampal neurons were transfected with TrkB-GFP and imaged 7 days after PBS or PFF addition. Images were captured every 1 sec for 3 minutes. TrkB: N (number of particles analyzed) = 1062 PBS, N= 582 PFF (59 axons PBS, 35 axons, PFF). There were no significant differences **(A)** Of the mobile particles, the percentages of anterograde and retrograde particles was quantified. There was a significant difference between the percentage of mobile particles between PBS and PFF treated groups for particles traveling in both the anterograde and retrograde directions. There were no significant differences between the

2 groups for **(B)** number of particles per 50 μm length of axon, **(C)** number of pauses, or **(D)** number of reversals. **(E)** A Poisson Regression on velocities binned with 10 cutpoints was not statistically significant between PBS and PFF groups for anterograde TrkB-GFP velocities (Wald $\chi^2 = 0.258$, p = NS). The right panel presents the median and interquartile ranges of the velocities of the mobile GFP-Rab7 particles. The Mann Whitney test did not produce significant differences for anterograde velocities. **(F)** Poisson Regression on retrograde velocities binned with 10 cutpoints was not statistically significant between PBS and PFF groups for anterograde TrkB-GFP velocities (Wald $\chi^2 = 1.54$, p = NS). The right panel presents the median and interquartile ranges of the velocities of the mobile TrkB-GFP particles.

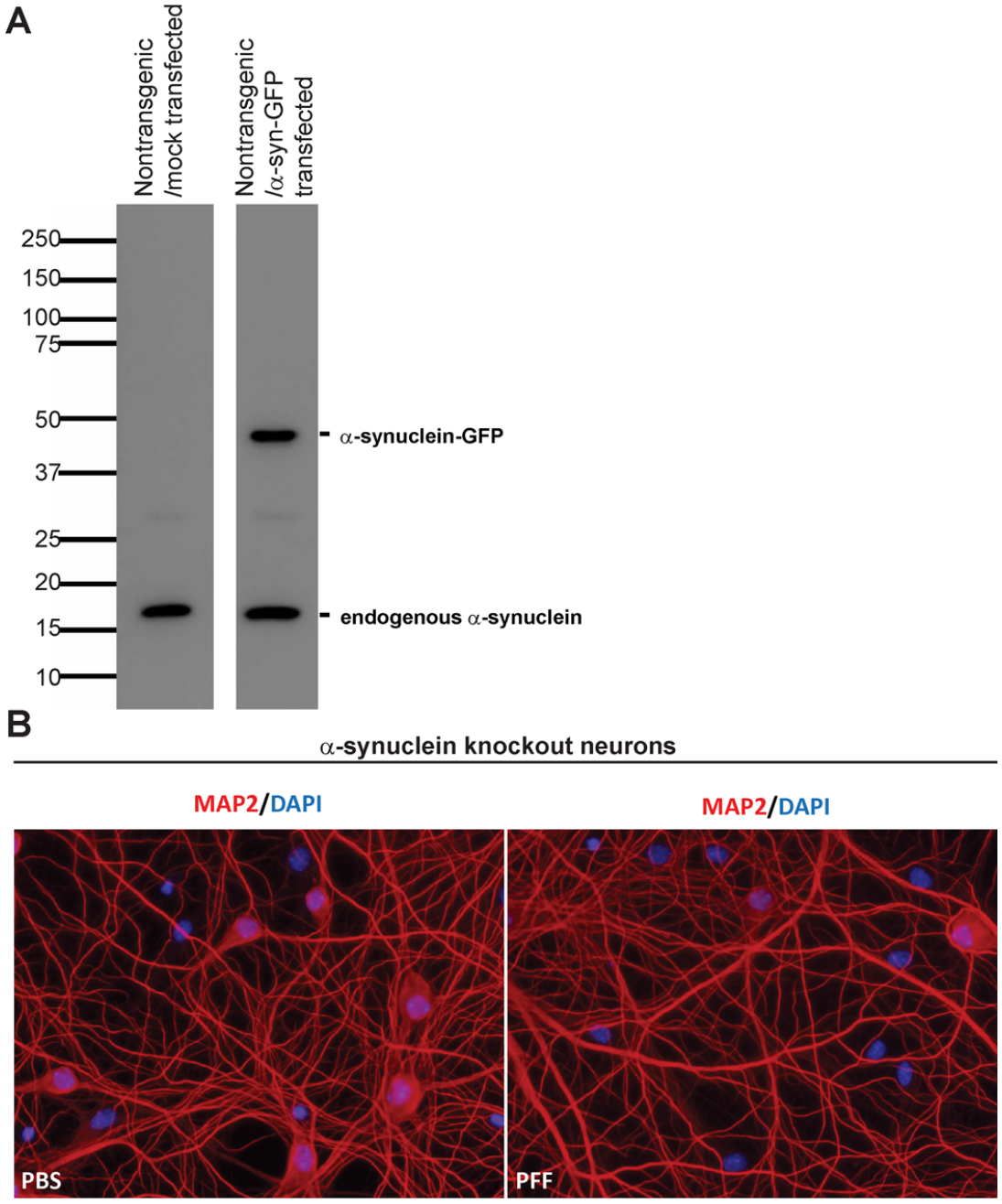
A



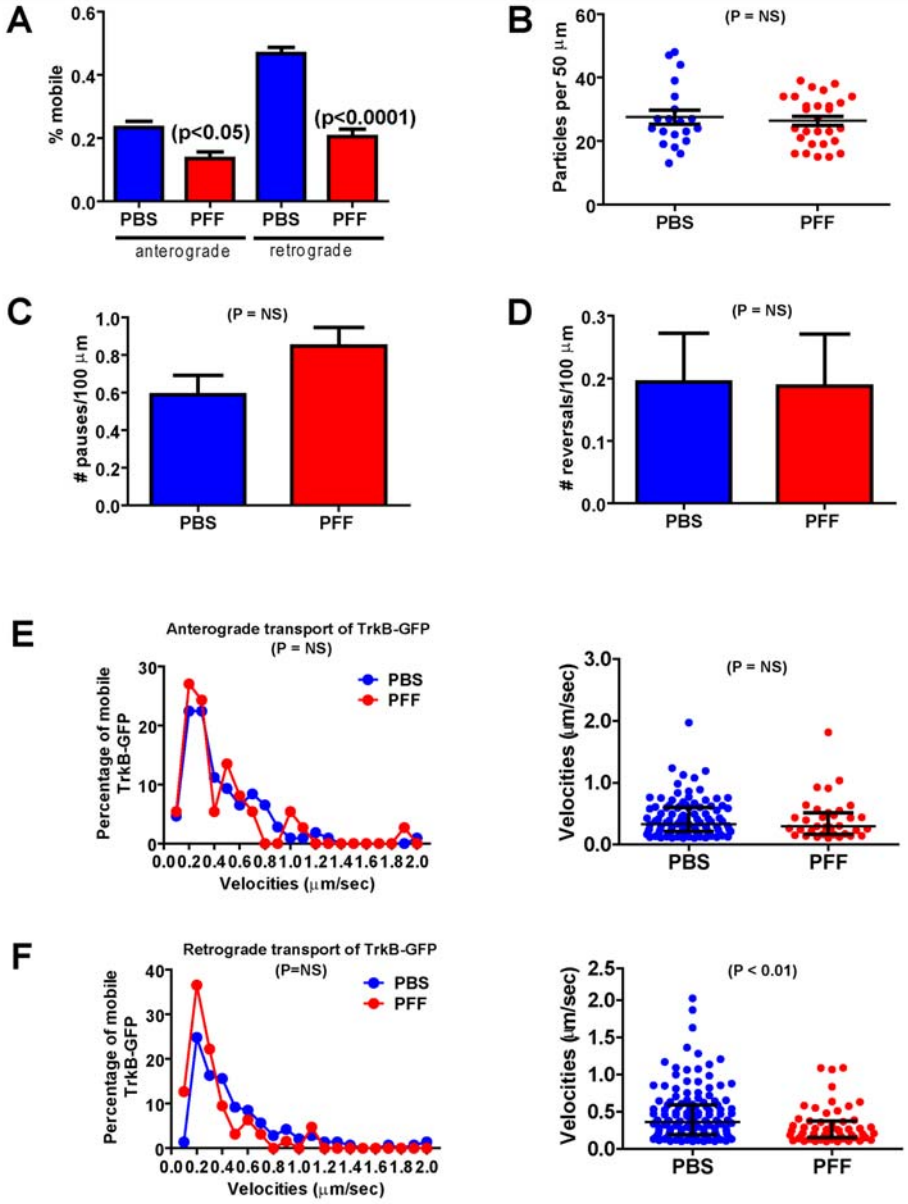
B



Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3