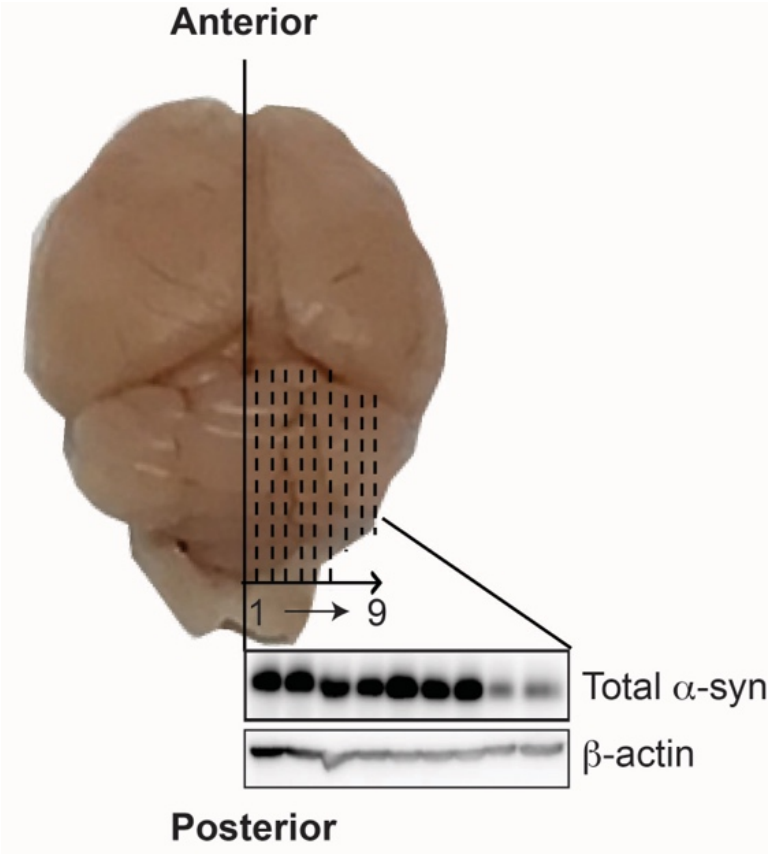
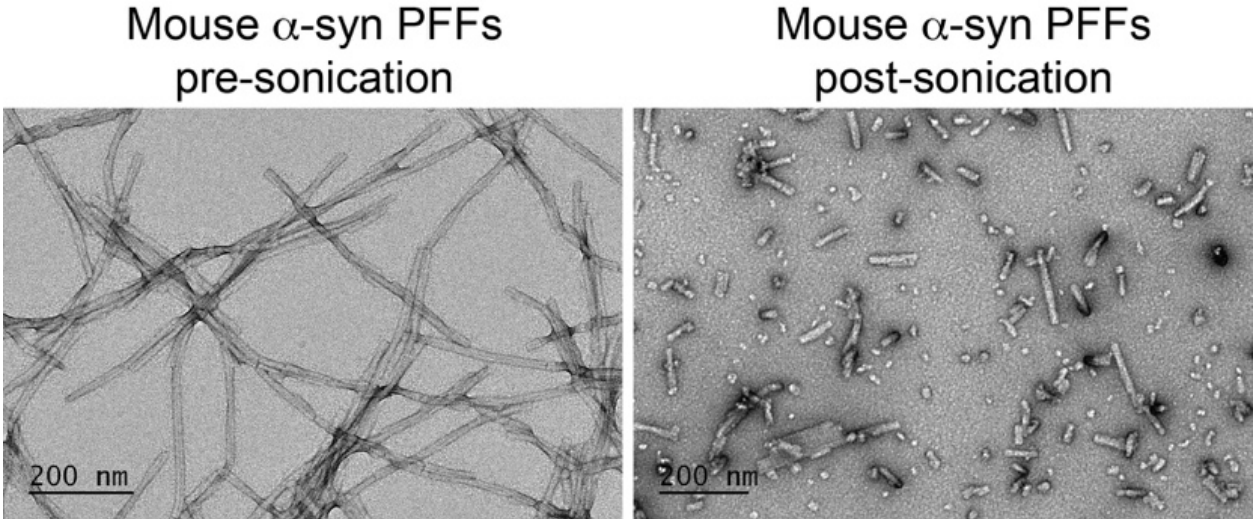
Supplementary Material

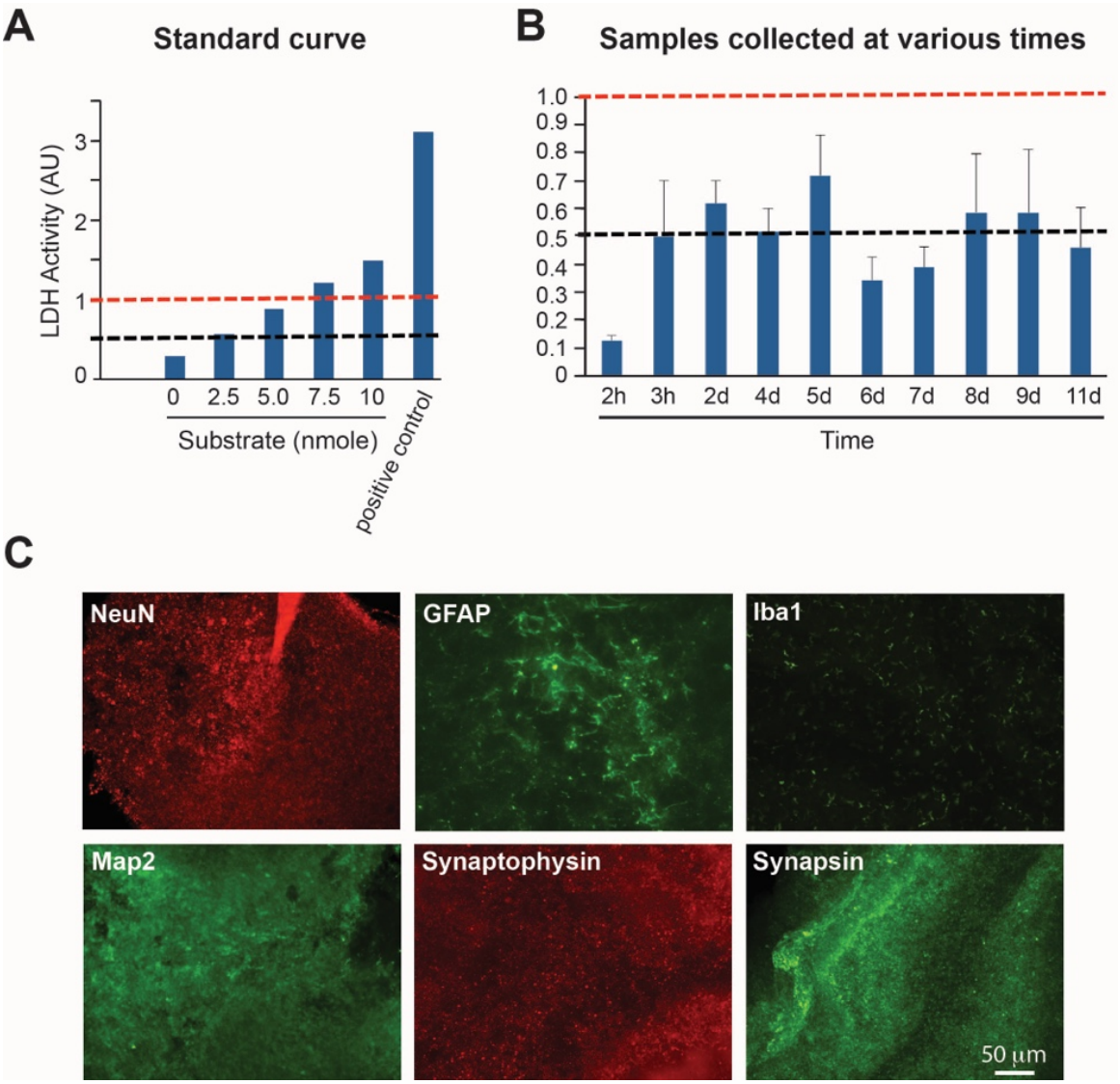
Modeling α-Synucleinopathy in Organotypic Brain Slice Culture with Preformed α-Synuclein Amyloid Fibrils



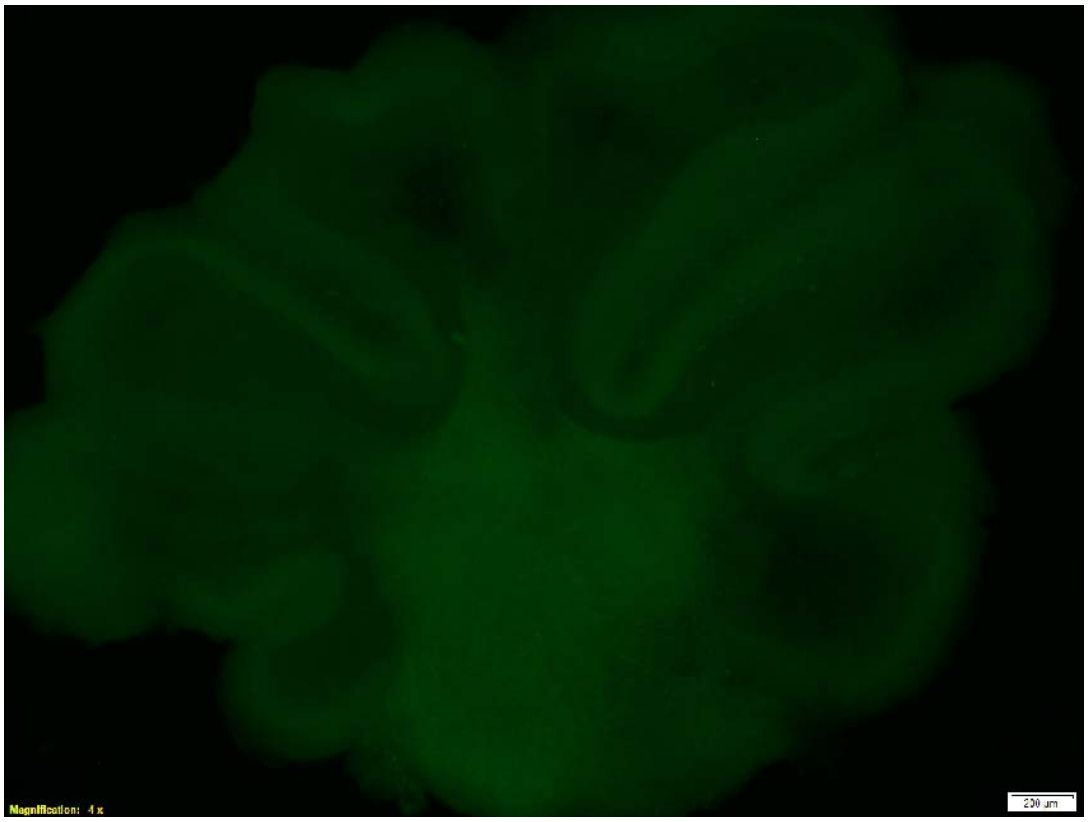
Supplementary Figure 1. The level of endogenous α-syn in sagittal cerebellar slices. Sagittal cerebellar slices were prepared as indicated and numbered from 1 to 9 (medial to lateral). The amount of α-syn and β-actin in each slice was determined by immunoblot analysis.



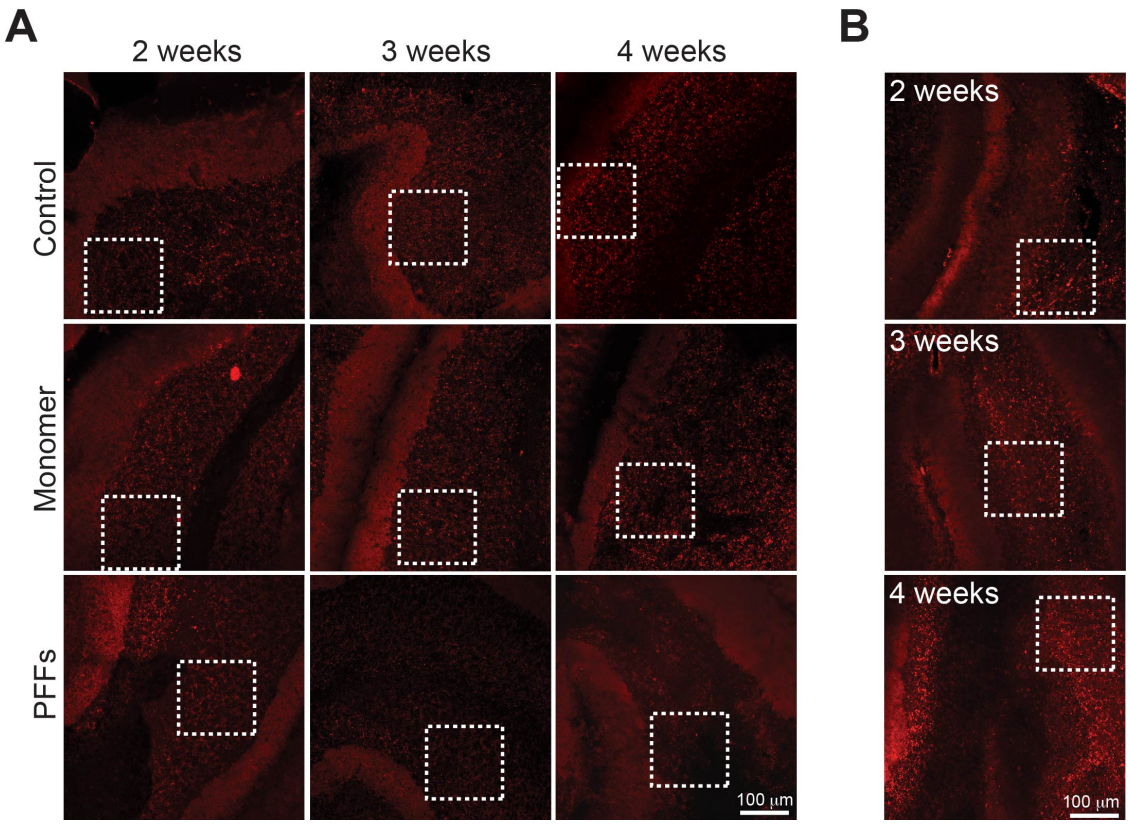
Supplementary Figure 2. Electron microscopic images of mouse α-syn PFFs before and after sonication as indicated. Scale bar represents 200 nm.



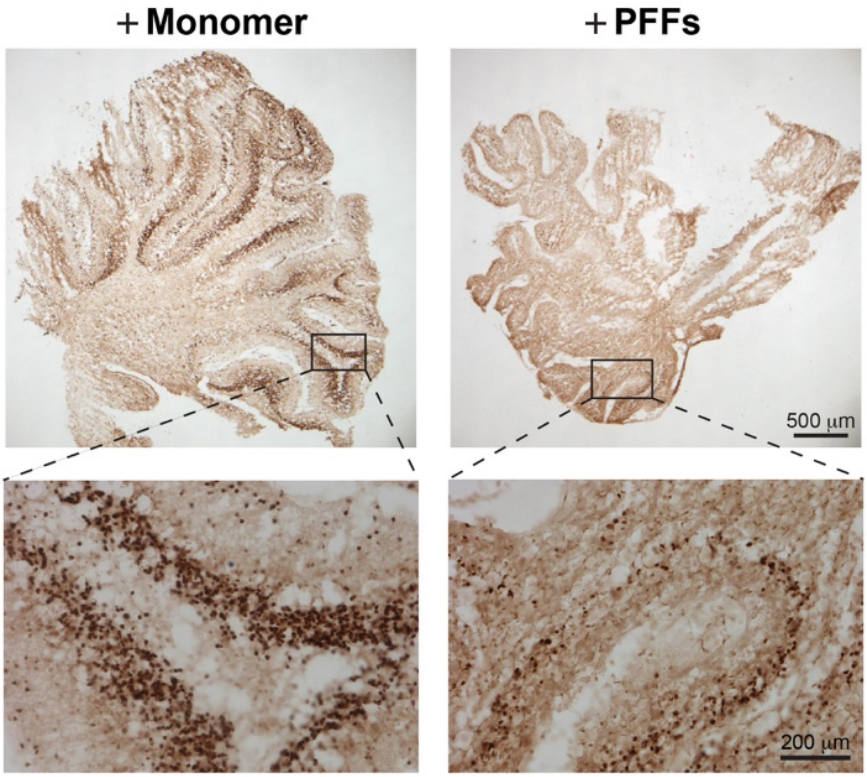
Supplementary Figure 3. Viability of cerebellar slice culture. A) Standard curve of LDH activity assay in arbitrary unit (AU). The red and black dashed lines indicated 1 and 0.5 AU, respectively. B) The LDH activity was measured in media collected from 2 hours (h) to 11 days (d) in culture. Three replicates of each time point were used for the analysis and error bar represents standard error. The red and black dashed lines indicated 1 and 0.5 AU, respectively. C) Immunofluorescence staining was performed with antibodies against NeuN, GFAP, Iba1, Map2, Synaptophysin, and Synapsin as indicated. Scale bar represents 50 µm.



Supplementary Figure 4. A representative image of pα-syn staining of α-syn-null cerebellar slice treated with α-syn monomer for 4 weeks. Scale bar represents 200 µm.



Supplementary Figure 5. Lower magnification images of synaptophysin stain. Cerebellar slices prepared from wild-type (A) and α-syn-null mouse (B) were untreated (control) or treated with α-syn monomer or PFFs for 2, 3, and 4 weeks as indicated. Synaptophysin was stained with an anti-synaptophysin antibody. Squares indicated areas used for image analysis shown in Figure 5. Scale bar represents 100 μm.



Supplementary Figure 6. Decreased NeuN staining in rat cerebellar slices treated with PFFs. Slices were treated with α-syn monomer or PFFs as indicated. Four weeks after the treatment, slices were subject to immunohistochemical staining of NeuN.