

Expanded View Figures

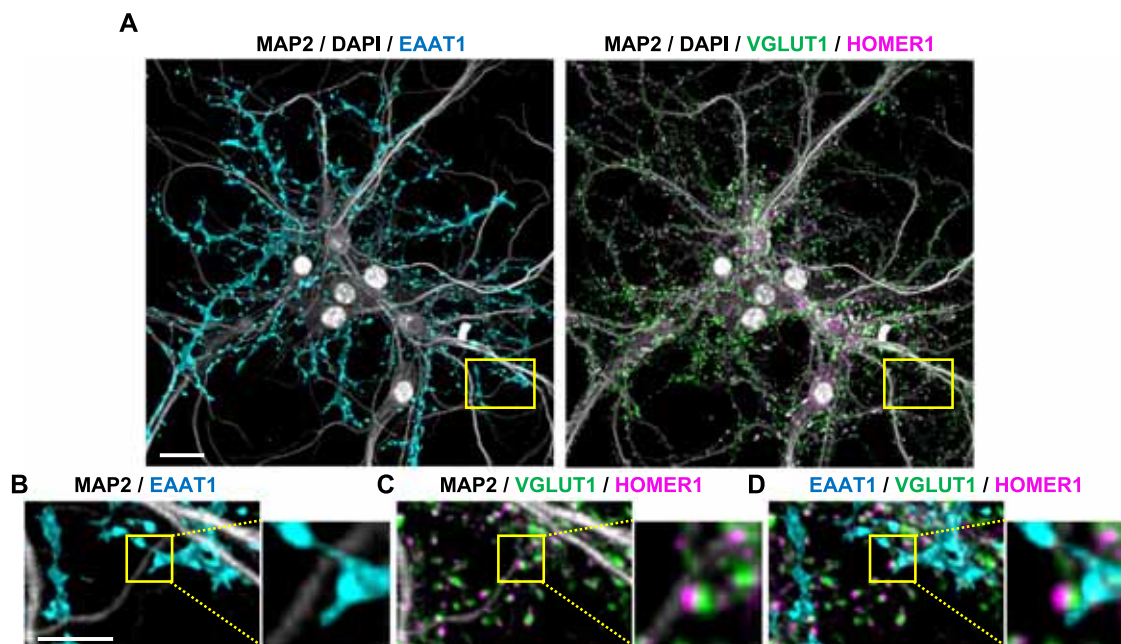


Figure EV1. Characterization of primary mouse hippocampal neuron-astrocyte co-cultures.

(A) Immunofluorescence images of neuron-astrocyte co-cultures. Primary hippocampal neurons derived from embryonic mouse brains were cultured for 10 days in vitro and subsequently co-cultured with astrocytes differentiated from neurospheres for an additional 14 days. Cells were immunostained for MAP2 (neurons, gray), EAAT1 (astrocytes, cyan), VGLUT1 (presynaptic terminals, green), and HOMER1 (postsynaptic sites, magenta). DAPI labels nuclei. Scale bar, 20 μ m. (B–D) Higher-magnification views of the boxed regions in (A), showing representative tripartite synapses. Scale bar, 10 μ m.

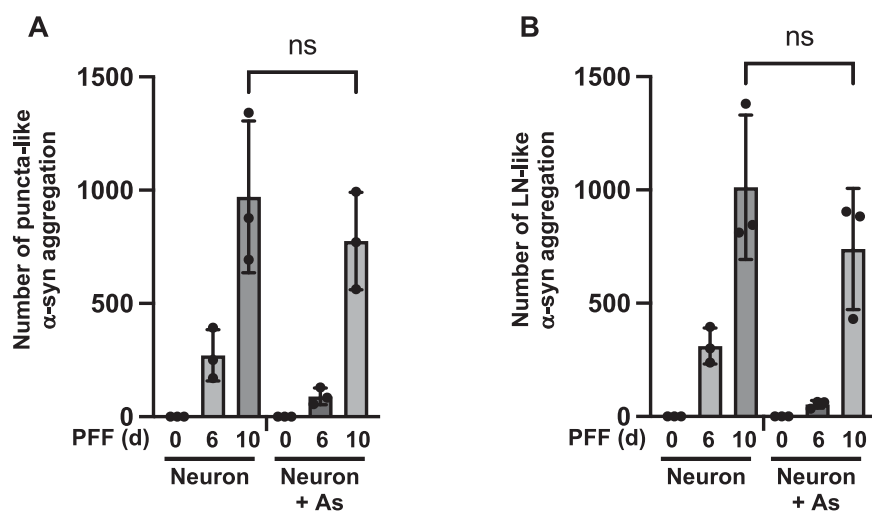


Figure EV2. Quantification of α -syn aggregate numbers under individual conditions.

(A, B) Quantification of puncta-like (A) and LN-like (B) α -syn aggregates. The number of pS129- α -syn-positive aggregates was quantified across 10 fields of view per condition. Data were presented as mean $\pm$ s.d. from three independent biological replicates. Statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test. ns not significant.

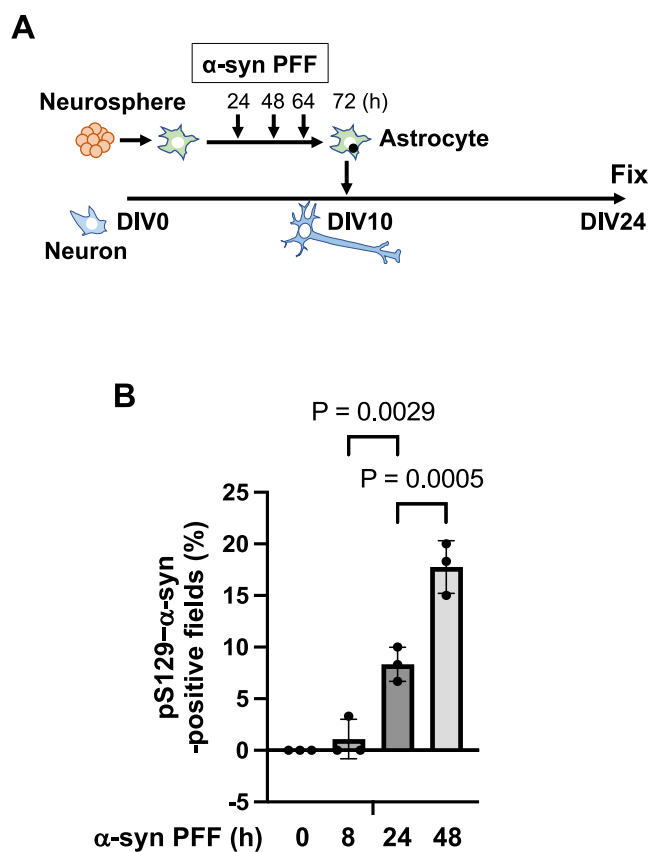


Figure EV3. Prolonged α -syn PFF pretreatment of astrocytes enhances neuronal α -syn aggregation.

(A) Experimental design. Astrocytes differentiated from neurospheres were treated with α -syn PFFs (0.5 μ g/ml) for the indicated durations, passaged, and subsequently co-cultured with naïve neurons from DIV10 to DIV24. (B) Quantification of α -syn aggregate formation in neurons. The percentage of pS129- α -syn-positive fields was calculated ($n = 60$ fields per condition). Data were combined from three independent biological replicates and presented as mean $\pm$ s.d. Statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test.

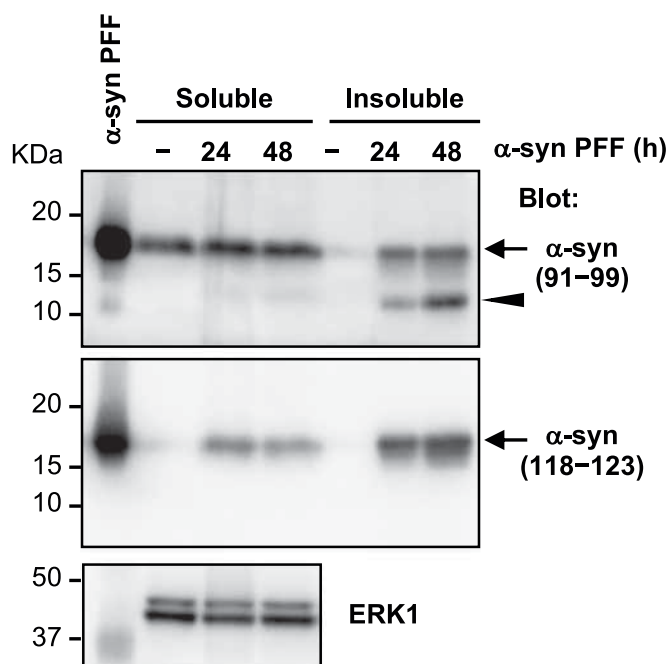


Figure EV4. C-terminal truncation of α -syn PFFs in neurons.

Primary hippocampal neurons were treated with α -syn PFFs (0.5 μ g/ml) at DIV20 for the indicated durations. Soluble and insoluble fractions prepared from cell lysates were subjected to immunoblot analysis using antibodies recognizing either the central region (amino acids 91-99) or the C-terminal region (amino acids 118-123) of α -syn. Arrows indicate full-length α -syn, and the arrowhead indicates a C-terminally truncated fragment. Note that the antibody against the C-terminal region (amino acids 118-123) specifically recognizes human α -syn PFFs, but not endogenous mouse α -syn. ERK1 serves as a loading control.

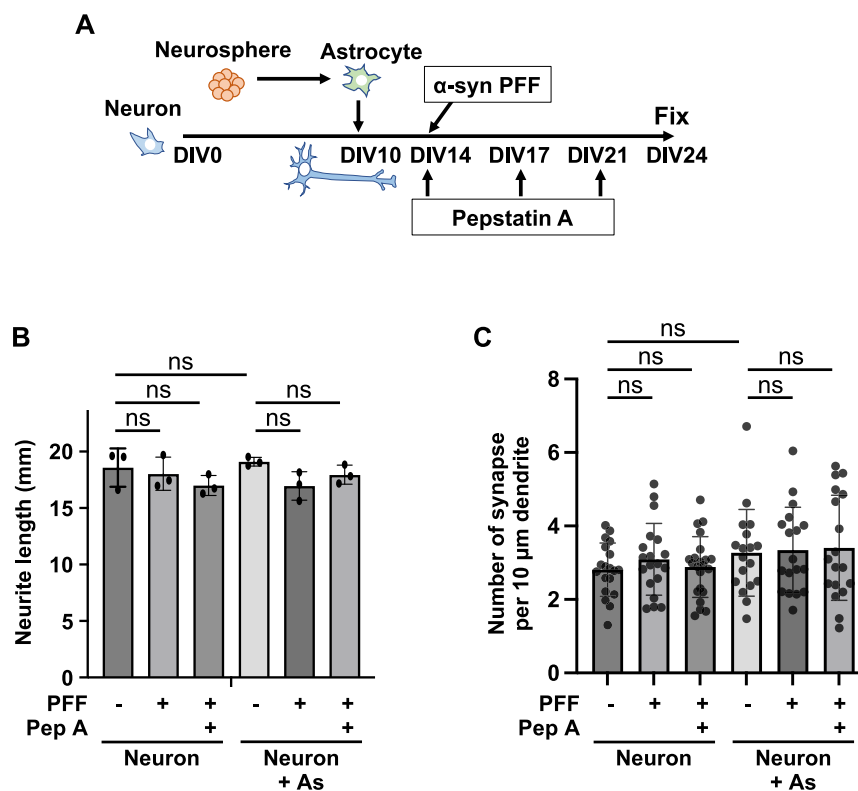


Figure EV5. Effects of α -syn PFF and Pep A treatments on neurite length and synapse density.

(A) Experimental design. Neuron-only cultures or neuron-astrocyte co-cultures were treated with or without α -syn PFFs (0.5 μ g/ml) for 10 days in the presence or absence of Pep A (10 μ M), which was replenished every 3–4 days. Following fixation, cells were immunostained for MAP2 (dendrites), VGLUT1 (presynaptic marker), and HOMER1 (postsynaptic marker). (B) Quantification of MAP2-positive neurite length. Neurite length based on MAP2-positive signals was measured across three fields of view per condition. Data were presented as mean $\pm$ s.d. from three independent experiments. Statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test. ns not significant. (C) Quantification of synapse density. Synaptic puncta were defined as closely apposed VGLUT1-positive presynaptic puncta and HOMER1-positive postsynaptic puncta located along MAP2-positive neurites. Synapse density was expressed as the number of synaptic puncta per 10 μ m of dendrite length (total analyzed dendrite length >50 μ m per neuron; $n > 18$ neurons). No significant differences were observed among the experimental groups. Data were presented as mean $\pm$ s.d. Statistical significance was assessed using the Kruskal-Wallis test followed by Dunn's multiple-comparison test. ns not significant.