Stem Cell Reports, Volume *10*

Supplemental Information

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Supplemental Information: Extended Experimental Procedures

Pathological Endogenous α-Synuclein Accumulation in Oligodendrocyte Precursor Cells Potentially Induces Inclusions in Multiple System Atrophy

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Inventory of Supplemental Information

Supplemental Experimental Procedures:

Histopathological Analysis of MSA Patients

For histopathological analysis, formalin-fixed, paraffin-embedded 6-μm-thick sections from the pons of MSA patients were deparaffinized and immunostained. We applied primary antibodies for α-synuclein (α-syn) (1:200, BD Biosciences, 610787) and NG2 (1:200, Merck Millipore, AB5320) and incubated overnight at 4°C. Subsequently to incubation with secondary antibodies (1:200, Alexa Fluor 594 and 647, A21207, A31571) for 1 hour at room temperature, sections were covered with VECTASHIELD mounting medium (Vector Laboratories) with DAPI. For Thioflavin S assessment, sections were incubated with 20 μM Thioflavin S (Sigma Aldrich) in distilled water for 20 min at room temperature before mounting. Images were obtained using a confocal microscopy as described below. Procedures involving the use of human materials were performed in accordance with ethical guidelines set by Kyoto University.

Primary Cell Cultures

Primary oligodendrocyte lineage cell and other glial cell culture

OPCs were prepared as previously described (Maki et al., 2015). Briefly, cerebral cortices from 1- to 2-day-old Sprague Dawley rats (Shimizu Laboratory Supplies Co., Ltd) were dissected, minced, and digested. Dissociated cells were plated in poly-D-lysine-coated 75 cm^2 flasks, and maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 20% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. After the cells were confluent (~10 days), the flasks were shaken for 1 hour on an orbital shaker (220 rpm) at 37°C to remove microglia. The flasks were then changed to new medium and shaken overnight (~20 hours). The medium was collected and plated on non-coated tissue culture dishes for 1 hour at 37°C to eliminate contaminating astrocytes and microglia. The non-adherent cells (OPCs) were collected and replated at a density of 20,000 cells/cm^2 in Neurobasal (NB) medium containing 2 mM glutamine, 1% penicillin/streptomycin, 10 ng/ml PDGF-AA, 10 ng/ml FGF-2 and 2% B27 supplement onto poly-DL-ornithine-coated plates. Four to 6 days after plating, the OPCs were used for the experiments. To differentiate OPCs to mature oligodendrocytes, the culture medium was switched to DMEM containing 1% penicillin/streptomycin, 10 ng/ml CNTF, 15 nM T3 and 2% B27 supplement. Seven days after switching medium, the mature oligodendrocytes were used for the experiments. To obtain astrocytes, non-astrocytic cells were detached from the flasks with mixed glial cells by shaking and removing the medium. Then, astrocytes were dissociated by tripsinization and subsequently replated at a density of 200,000 cells/cm^2.

Primary neuronal cell culture

Cortical neuronal cultures were prepared from 17-day-old Sprague Dawley rat embryos (Shimizu Laboratory Supplies Co., Ltd) using methods described earlier (Maki et al., 2014). Briefly, cortices were dissected and dissociated. Cells were plated on dishes coated with poly-D-lysine in DMEM containing

5% fetal bovine serum and 1% penicillin/streptomycin at a density of 200,000 cells/cm^2. At 24-hour after seeding, the medium was changed to NB medium containing 0.5 mM glutamine, 1% penicillin/streptomycin and 2% B27 supplement. Cultured neurons were used for experiments 14 days after seeding.

Preparation of Recombinant Human α-Syn PFFs

Purification was conducted in accordance with previously established method (Masuda-Suzukake et al., 2013). Human wild-type α-syn cDNA was cloned into the bacterial expression vector pRK172. Transformations and selection were performed using E. coli BL-21(DE3) competent cells (BioDynamics) and ampicillin (100 μg/ml) in Luria-Bertani media. Following overnight incubation of transformed cells in Luria-Bertani media containing ampicillin (100 μg/ml) at 37 °C, the culture was incubated for another 5 hours after 300-fold dilution and then induced with 1mM isopropyl-β-D-thiogalactopyranoside for 5 hours at 37 °C. Bacterial pellets were resuspended in high-salt buffer (1M Tris-HCl, pH 7.5, 1 mM EDTA), heated to 100 °C for 5 min, and centrifuged at 15,000 rpm for 15 min. The supernatants were subjected to chromatography on a Q-Sepharose fast-flow column (GE healthcare) with a gradient of 0 to 0.5 M NaCl in Tris buffer. The proteins were dialyzed overnight against 50 mM Tris-HCl, 150 mM KCl, pH 7.5 and centrifuged at 55,000 rpm at 4°C for 20 min. The supernatants were filtered with 0.2 μm syringe filters (Life Sciences) and diluted in media for experimental use as monomeric α-syn. For PFFs formation, proteins were incubated with constant agitation at 37°C for 3-7 days. α-Syn PFFs were diluted in PBS at 1 μM or 3 μM, sonicated several times (30-60 seconds in total), filtered with 0.2 μm syringe filters (Life Sciences), and diluted in media. For observation with confocal and immunoelectron microscopy, 3 μM and 1 μM α-syn PFFs or monomer were prepared, respectively. The fractions were assayed for the presence of the α-syn proteins by SDS-polyacrylamide gel electrophoresis (PAGE) followed by Coomassie Blue R-250 staining. Protein concentration was determined using the bicinchoninic acid protein assay (Thermo Fisher) and bovine serum albumin as a standard.

Incubation of OPCs with Autophagy-Modifying Drugs

To assess how altered autophagic states affect the endogenous α-syn expression and autophagic markers in OPCs, we incubated OPCs for 24 hours with 10 μM chloroquine (Enzo Life Science), a lysosomal inhibitor, or 500 nM rapamycin (Enzo Life Science), an autophagy inducer.

Differentiation of OPCs Pre-incubated with α-Syn PFFs

1 μM or 3 μM α-syn PFFs were added to OPCs culture when its confluency reaches 60%. Subsequently to 24-hour incubation with α-syn PFFs, cells were washed twice with fresh medium not containing α-syn PFFs. Medium was switched to DMEM containing 1% penicillin/streptomycin, 10 ng/ml CNTF, 15 nM T3 and 2% B27 supplement. After 7 days of incubation, the mature oligodendrocytes were used for the

experiments.

Incubation of Primary Cortical Neurons with Conditioned Medium from OLGs

Primary cortical neuron culture was incubated for 72 hours either 1) with conditioned medium from normal OLGs, 2) with conditioned medium from OLGs differentiated from OPCs preincubated with α-syn PFFs (3 μM), or 3) with neuron medium incubated for 24 hours in no cell plate (serves as control). Conditioned medium was prepared from neuron medium (NB medium containing 0.5 mM glutamine, 1% penicillin /streptomycin and 2% B27 supplement) which were incubated for 24 hours with mature OLGs differentiated from OPCs with or without α-syn PFFs preincubation (as illustrated in Fig. 4A).

Time-Lapse Imaging

Time-lapse imaging was performed with BZ-X710 (Keyence) equipped with an incubator (37°C and 5% CO2) by acquiring images at defined positions every 10 minutes. Images were converted to AVI files.

Cathepsin D activity Assay

The enzymatic activity of cathepsin D in OPCs was measured by cathepsin D assay kit (AnaSpec) according to the manufacturer's instructions. Cathepsin activity was determined by kinetic analysis, which calculates the initial reaction velocity in relative fluorescence units (RFU) per minute. RFU change during the first 5 minutes of the reaction was used for the calculation.

Cytotoxicity and Cell Survival Assay with Media LDH and WST Assay

Cytotoxicity was assessed by media LDH assay kit (Cytotoxicity LDH Assay Kit-WST, Dojindo). LDH is rapidly released into the cell-culture supernatants when the plasma membrane is damaged. 100 μl of the supernatants is incubated with the same amount of substrate mixture from the kit for 30 min. Then the absorbance of the culture medium was measured with a microplate reader at a test wavelength of 490 nm.
Cell proliferation/survival was assessed by WST reduction assay kit (Cell Counting Kit-8, Dojindo). WST assay is a sensitive colorimetric method to detect cell viability. The cells were incubated with 10% WST solution for 1 hour at 37°C. The absorbance of the culture medium was measured at a wavelength of 450 nm and a reference wavelength of 630 nm.

Immunostainings with Confocal Microscopy

After washing the cells twice with PBS, the cells were fixed with 4% PFA for 15 min. After washing twice with PBS, incubation with PBS/0.1%Tween (10 min) and blocking with 3%BSA/PBS (1 hour at room temperature), the cells were incubated with primary antibodies against PDGFRα (1:200, R&D systems, AF1062), MBP (1:200, MBL, PD004 or 1:200, Thermo Fisher Scientific, MA1-10837), α-syn (recognizes human and rat) (1:200, BD Biosciences, 610787), rat α-syn (1:200, CST, 4179S), human

α-syn (1:200, Thermo Fisher Scientific, 180215), TPPP/p25α (1:200, Abcam, ab92305), p62 (200:1, MBL, PM045), Beclin-1 (200:1, Santa Cruz, sc-10086), LC3 (300:1, MBL, PM036), phosphorylated α-syn (1:200, Abcam, ab51253), MAP2 (300:1, Sigma Aldrich, M1406) and NeuN (300:1, Merck Millipore, ABN78) at 4°C overnight. For the validation of endogenous α-syn expression in OPCs, we used Mouse-IgG (200:1, Vector Laboratories, BA-2000) as a primary antibody for negative control. Subsequently, after washing with PBS, they were incubated with secondary antibodies (1:200, Alexa Fluor 488, 594 and 647, A21202, A21203, A31571, A21206, A21207, A31573, A11055, A11058, A21447) for 1 hour at room temperature. After washing with PBS, the cells were covered with VECTASHIELD mounting medium (Vector Laboratories) with DAPI. The cells were observed by Olympus Fluoview FV1000 confocal microscope (Olympus). As for Thioflavin S staining, cells were incubated with 20 μM Thioflavin S (Sigma Aldrich) in distilled water for 20 min at room temperature before mounting. Image analysis and 3D surface reconstruction were performed by FV10-ASW software (Olympus). Sections were imaged at 0.124 μm/pixel resolution in xy dimension and 0.4 μm in z dimension. Regarding the use of the LysoTracker (Life technologies) probes, cells were incubated with probe-containing medium (50 nM) for 30 minutes, before the wash with PBS and fixation. The following immunostaining was conducted as described above.

Immunoelectron Microscopy

Immunoelectron microscopy using ultrathin cryosections was performed as described. Briefly, cells were washed with PBS twice, immersed in 4% PFA with 0.1% glutaraldehyde at 4°C for 2 hours. Following 60 min pre-treatment with 3% BSA in PBS used for blocking agents containing 0.1 % Photo-Flo (EMS), the samples were incubated overnight at 4°C with mouse anti-α-syn antibody (1:200, BD Biosciences, 610787). They were then incubated with Nanogold goat anti-mouse IgG conjugates (1:100, Nanoprobes, 2002) overnight at 4°C. Immunostained sections were fixed with 1% glutaraldehyde in 0.1M PB. To better visualize the particles, the samples were reacted with Silver Enhancement Kit solutions (Nanoprobes) The sections were then washed with 0.1 M PB, placed for 40 min in 0.1 M PB containing 1% osmium tetroxide, dehydrated, and embedded in epoxy resin (Luveak 812; Nacalai Tesque, Kyoto, Japan). After polymerization of the resin, each tissue sample was cut into 70-nm-thick ultrathin sections with a diamond knife on an ultramicrotome (Leica EM UC6 , Heiderberg, Germany), and mounted on coated copper grids (Stork Veco, Eerbeek, The Netherlands). The sections were finally examined with an electron microscope (H-7650; Hitachi, Tokyo, Japan) at 80 kV (Kameda et al., 2012).

Immunoblot Analysis

Cells were rinsed twice with PBS and collected into sample buffer containing 50% Tris-Glycine SDS buffer (Novex), 45% RIPA buffer (20 mM HEPES-KOH pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% Nonidet-P40, 1% sodium deoxycholate), 5% 2-mercaptomethanol (Nacalai tesque),.1% phosphatase

inhibitor (Nacalai tesque) and 1% protease inhibitor (Nacalai tesque). Subsequently, samples were heated at 95°C for 5 min, and each sample was loaded onto 5–20% or 15 % polyacrylamide gel (Wako). After electrophoresis and transferring onto a PVDF membrane (Merck Millipore), the membranes were fixed with 4%PFA for 30 min and blocked in Tris buffered saline with 0.1% Tween 20 (TBS-T) containing 5% nonfat dry milk for 60 min at room temperature. Membranes were then incubated overnight at 4°C with primary antibodies for α-syn (recognizes human and rat) (1:1000, BD Biosciences, 610787), rat α-syn (1:1000, CST, 4179S), human α-syn (1:500, Thermo Fisher Scientific, 180215), PDGFRα (1:500, R&D systems, AF1062), MBP (1:1000, Thermo Fisher Scientific, MA1-10837), TPPP/p25α (1:500, Abcam, ab92305) GFAP (1:5000, Thermo Fisher Scientific, 13-0300), NeuN (3000:1, Merck Millipore, ABN78), GAPDH (500:1, Santa Cruz Biotechnology, sc-25778), HSP90α (1:5000, Abcam, ab133491), sodium potassium ATPase (1:5000, Abcam, ab76020), p62 (500:1, MBL, PM045), LC3 (500:1, MBL, PM036), Beclin-1 (500:1, Santa Cruz, sc-48341), cathepsin D (500:1, Santa Cruz Biotechnology, sc-6486), lysine-48-specific ubiquitin (Merck Millipore, 1000:1, 05-1307) or anti-β-actin antibody (1:10000, Sigma Aldrich, A5441), followed by 60 min incubation with secondary goat or donkey anti-IgG HRP antibodies (Santa Cruz Biotechnology, NA9310V, NA9340V, NB7115, NB7357) and visualization by enhanced chemiluminescence (Nacalai tesque). Assessment of Triton-insoluble SDS-soluble fractions was conducted as previously described (Uemura et al., 2015). Cells were homogenized in lysis buffer containing 1% Triton X-100 (150 mM NaCl, 50 mM Tris-HCl, 1% Triton X-100, pH 7.5) and centrifuged at 55,000 rpm at 4°C for 30 min. The supernatants were used for Triton soluble fractions. For SDS-soluble fractions, the pellet was rinsed with the lysis buffer, centrifuged again at 55,000 rpm at 4°C for 30 min followed by removal of the supernatant. Subsequently, the pellet was sonicated in SDS buffer (50 mM Tris-HCl, 2% SDS, pH 7.4) followed by centrifugation at 55,000 rpm at 4°C for 30 min. The supernatant was boiled in sample buffer (1% SDS, 12.5% glycerol, 0.005% bromophenol blue, 2.5% 2-mercaptoethanol, 25 mM Tris-HCl, pH 6.8). Samples containing 20 μg of proteins were loaded onto each lane of 10 % Bis-Tris gels (Novex) for both fractions. The following procedure was performed as mentioned above. Each band was quantified with image J or ImageQuant software (GE healthcare) (Schneider et al., 2012).

Subcellular Fractionation

Trident Membrane Protein Extraction Kit (GeneTex) was used for subcellular fractionation. Extraction of cytosolic and plasma membrane fraction was conducted according to the manufacturer's instructions.

RNA-seq Analysis in OPCs

Library construction and sequencing

RNA-Seq library and sequencing: Agilent SureSelect Strand Specific RNA prep kit (Cat# G9691A) was used with 200ng of total RNA for the construction of cDNA libraries according to the manufacturer's

protocol. All cDNA libraries were sequenced using an Illumina Miseq, producing 76×2 bp paired-end reads with multiplexing.

Bioinformatics analysis

All raw sequencing reads were trimmed using Trimmomatic software (Bolger et al., 2014). Bases and QC assessment of sequencing were generated by FastQC. QC-passed reads were aligned to the Ensembl Rnor 6.0.84 reference genome using Star v2.5.0c (Dobin et al., 2013). The abundance of transcripts was then estimated using an Expectation-Maximization algorithm implemented in the software package Cufflnk v2.2.1 [http://cole-trapnell-lab.github.io/cufflinks/]. Drawing heatmap of the RNA-seq data was performed using R software and the ggplot2 package

Quantitative Real-time PCR

RNA was extracted from cells with RLT lysis buffer (QIAGEN) according to the manufacturer's instructions. RNA concentration was measured by NanoDrop 1000 spectrometer (Thermo Scientific). cDNA was generated with reverse transcription using the PrimeScript RT reagent kit (TaKaRa). The amount of cDNA was quantified with real-time PCR using LightCycler 480 SYBR Green I Master (Roche) and Roche LightCycler 480. The primer sets used in this study is listed in Table S1, thereafter.

Statistical Analysis

All quantitative data were analyzed using Prism 5.0 (Graphpad). Statistical significance was evaluated using a paired t-test or a one-way ANOVA followed by Tukey's honestly significant difference test for multiple comparisons. Data are expressed as mean ± S.D. A p-value of <0.05 was considered statistically significant.

Supplemental Results:

α-Syn PFF Receptor Membrane Proteins in Oligodendrocyte Lineage Cells

A recent report using primary neurons suggested that lymphocyte-activation gene 3 (LAG3) selectively binds to α-syn PFFs and mediates endocytosis as well as cell-to-cell transmission (Mao et al., 2016). Unexpectedly, our RNA-seq analysis suggested that the basal gene expression levels of *Lag3* in OPCs were very low (Fig. S3K). The LAG3 protein expression in OPCs was also relatively low in immunoblot analysis (data not shown), and the pathological function of LAG3 in oligodendrocyte lineage cells remains unclear.

Supplemental Discussion:

Our studies demonstrated the predominance of α-syn internalization and susceptibility against seeding in OPCs, and indicated the possibility that OPCs are more relevant to the propagation of misfolded α-syn than OLGs. As is previously demonstrated with neurons, internalization of exogenous α-syn in oligodendroglial cells is presumably mediated by endocytosis (Konno et al., 2012; Mao et al., 2016). In fact, according to our RNA-seq analysis, the gene expressions of endocytic proteins such as Rab5a, Rab7a and Rab7b in OPCs seemed to increase after α-syn PFFs application (Fig. S3K). Nevertheless, the gene expressions of possible candidate receptors for misfolded α-syn, such as clathrin and LAG3, remained basically unchanged. A variety of endocytic pathways need to be scrutinized to unravel the seeding mechanisms lying behind our *in vitro* study results. In our study, however, the involvement of endocytic pathway was difficult to confirm, due to the limited tolerability of primary OPCs against the cytotoxicity of dynamin or clathrin inhibitor. In terms of *in vivo* propagation of misfolded α-syn, injection of α-syn PFFs into brains usually leads to α-syn aggregate formation exclusively in neurons. As far as OPCs are concerned, sparse basal expression levels of an α-syn PFF receptor protein, LAG3, may be consistent with these *in vivo* observations.

Supplemental References

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Table S1 Table of Primers Used in This Study

Gene name	Abbreviations	Direction	Sequences
α-synulcien	*Snca*	F	CAACAGTGGCTGAGAAGACC
		R	GAAGGCATTTCATAAGCCTC
Platelet-derived growth factor receptor, α	*Pdgfra*	F	CTAATTCACATTCGGGAAGGTTG
		R	GGACGATGGGCGACTAGAC
Oligodendrocyte transcription factor 2	*Olig2*	F	GACGACATTATGGGCTTTGATGG
		R	GTTTCTGCCTGAACAGTCCAC
Neural/Glial antigen 2	*Cspg4*	F	ATGCCCACTGTAGCCAAAAG
		R	GTGTCACCAGCTAGGCCATT
Nestin	*Nes*	F	CGCCGCTACTTCTTTTCAAC
		R	GCAGCTGGTTTTGCTCTTCT
Myelin basic protein	*Mbp*	F	ACACACAAGAACTACCCACTACGG
		R	AGCTAAATCTGCTGAGGGACAG
Myelin associated glycoprotein	*Mag*	F	ATTCCGAATCTCTGGAGCAC
		R	ACTCAGCCAGCTCCTCTGTC
Glial fibrillary acidic protein	*Gfap*	F	AGAAAACCGCATCACCATTC
		R	GCACACCTCACATCACATCC
Neuron-specific class III β-tublin (Tuj-1)	*Tubb3*	F	ACTTTATCTTCGGTCAGAGTG
		R	CTCACGACATCCAGGACTGA
Monocarboxylate transporter (MCT-1)	*Slc16a1*	F	CTTGTGGCGTGATCCT
		R	GTTTCGGATGTCTCGGG
Brain-derived neurotrophic factor	*Bdnf*	F	ATAGGAGACCCTCCGCAACT
		R	CTGCCATGCATGAAACACTT
Glial cell line-derived neurotrophic factor	*Gdnf*	F	GCGGTTCCTGTGAAGCGGCCGA
		R	TAGATACATCCACACCGTTTAGCGG
Insulin like growth factor 1	*Igf-1*	F	CAGTTCGTGTGTGGACCAAG
		R	GTCTTGGGCATGTCAGTGTG
Glyceraldehyde-3-phosphate dehydrogenase	*Gapdh*	F	TCCCGCTAACATCAAATGGG
		R	CCATCCACAGTCTTCTGAGT

F; forward, R; reverse

Supplemental Figure S1

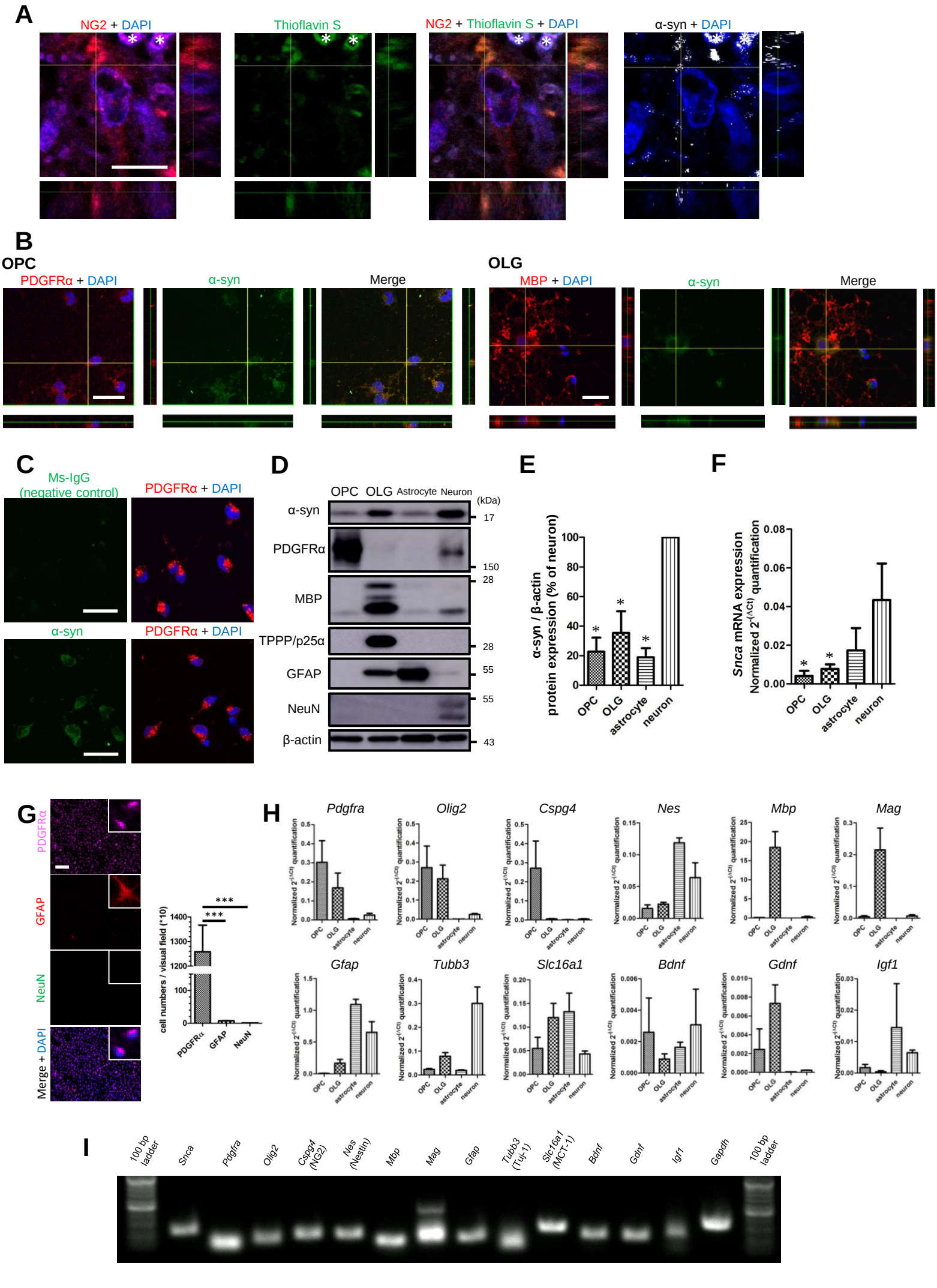

Figure S1 Identification of α-Syn Accumulation in OPCs of an MSA Brain, and *In Vitro*α-Syn Expression in Primary Oligodendrocyte Lineage Cell Culture

MSA Brain

(A) The $NG2^+$ OPC contains Thioflavin S-immunopositive aggregates, which are immunoreactive to α-syn in the pons of an MSA patient. The images were acquired by confocal microscopy. The white asterisks indicate erythrocytes. The scale bar represents 10μm.

Primary Oligodendrocyte Lineage Cells

(B) Confocal microscopy reveals α-syn immunoreactivity in both the cytoplasm of OPCs and OLGs. The scale bars represent 20 μm.

(C) The α-syn immunoreactivity in OPCs is guaranteed by validating the difference between immunostaining results with mouse-IgG and anti-α-syn antibody (mouse-derived). The scale bars represent 20 μm.

(D) Immunoblot analysis with an anti-α-syn antibody and antibodies against each cell-type marker illustrates α-syn expression in oligodendrocyte lineage cells as well as sufficiently high cell purity of each primary culture.

(E) Quantification of α-syn expression in each cell type by immunoblot analysis verifies the appreciable levels of α-syn protein expressions in oligodendrocyte lineage cells. Relative α-syn expressions in glial cells with respect to that in neurons are illustrated. Mean±SEM; n=3, respectively; independent cultures; one-way ANOVA, *$p<0.05$ (compared with neuronal expression).

(F) Quantitative real-time PCR analysis confirms the *Snca* mRNA expression in each cell-type culture. Mean±SEM; n=6 for OPCs and OLGs, n=3 for astrocytes and neurons; independent cultures; one-way ANOVA, *$p<0.05$ (compared with neuronal expression).

(G) The purity of OPC culture is validated by immunostaining using antibodies against PDGFRα, GFAP, and NeuN. The scale bar represents 100μm. Cell numbers per each ×175 magnified visual field were quantified. Mean±SEM; n=3, respectively; one-way ANOVA, ***$p<0.001$.

(H) Quantitative real-time PCR shows each cell marker transcript and neuromodulating factors. Mean±SEM; n=6 for OPCs and OLGs, n=3 for astrocytes and neurons, independent cultures.

(I) Validation of primers used for quantitative real-time PCR is shown.

Supplemental Figure S2

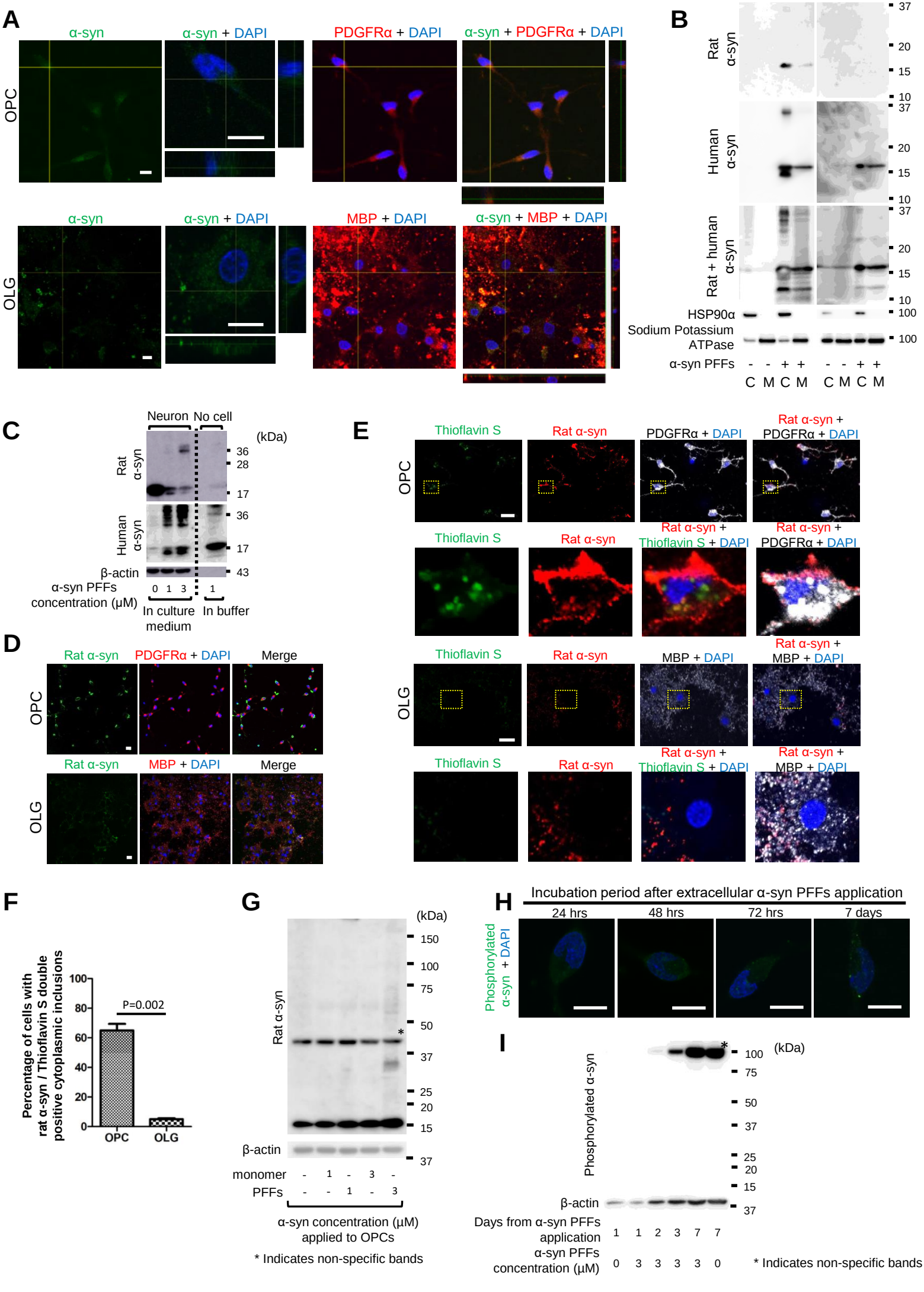

Figure S2 Characterization of Cytoplasmic Inclusions in Oligodendroglial Cells; Extracellular α-Syn PFFs Trigger the Aggregation of Endogenous Rat α-Syn Predominantly in OPCs

(A) Confocal microscopy of OPCs and OLGs incubated with recombinant human α-syn monomer (3 μM) shows no evident cytoplasmic α-syn accumulation. The primary antibody used for the immunostaining recognizes both exogenous human and endogenous rat α-syn. Each scale bar represents 10 μm.

(B) Subcellular fractionation of OPCs and OLGs shows cytosolic accumulation of endogenous rat and exogenous human α-syn in OPCs in response to 24-hour incubation with recombinant human α-syn pre-formed fibrils (α-syn PFFs). Dimerization of endogenous rat α-syn is also observed in the cytosolic fraction in OPCs. C = cytoplasmic fraction; M = plasma membrane fraction.

(C) Immunoblot analysis of primary rat neurons verifies the specific detection of endogenous α-syn by the rat-specific anti-α-syn antibody. The far right lane represents 1 μM of α-syn PFFs without cell lysates, which is recognized only with human-specific anti-α-syn antibody guaranteeing the specificity of each antibody. Endogenous rat α-syn in neurons is also multimerized by 24-hour incubation with α-syn PFFs.

(D) Enhanced immunoreactivity of endogenous rat α-syn is observed predominantly in α-syn PFFs-treated OPCs labeled with PDGFRα. OLGs are labeled with MBP. The scale bar represents 20 μm.

(E) Magnified views of α-syn PFFs-treated oligodendroglial cells by confocal microscopy reveal Thioflavin S-positive inclusions extensively observed in OPCs, but not in OLGs. Each magnified oligodendroglial cell corresponds to the cells surrounded by the dotted yellow lines. Each scale bar represents 20 μm.

(F) Percentages of cells containing endogenous rat α-syn/Thioflavin S double-positive inclusions are quantified in OPC and OLG culture. The intracellular localization of each inclusion is confirmed by each cell marker, PDGFRα or MBP. Mean±SEM; n=3, respectively; independent cultures; one-way ANOVA.

(G) Immunoblot analysis of OPCs incubated with monomeric or fibrillar recombinant human α-syn shows different response of endogenous rat α-syn expressions. Multimerization is not observed with the application of monomeric α-syn.

(H) (I) Phosphorylated α-syn is not detected within a timeframe of 7 days after α-syn PFFs (3 μM) application to OPCs. Each scale bar represents 10 μm.

Supplemental Figure S3

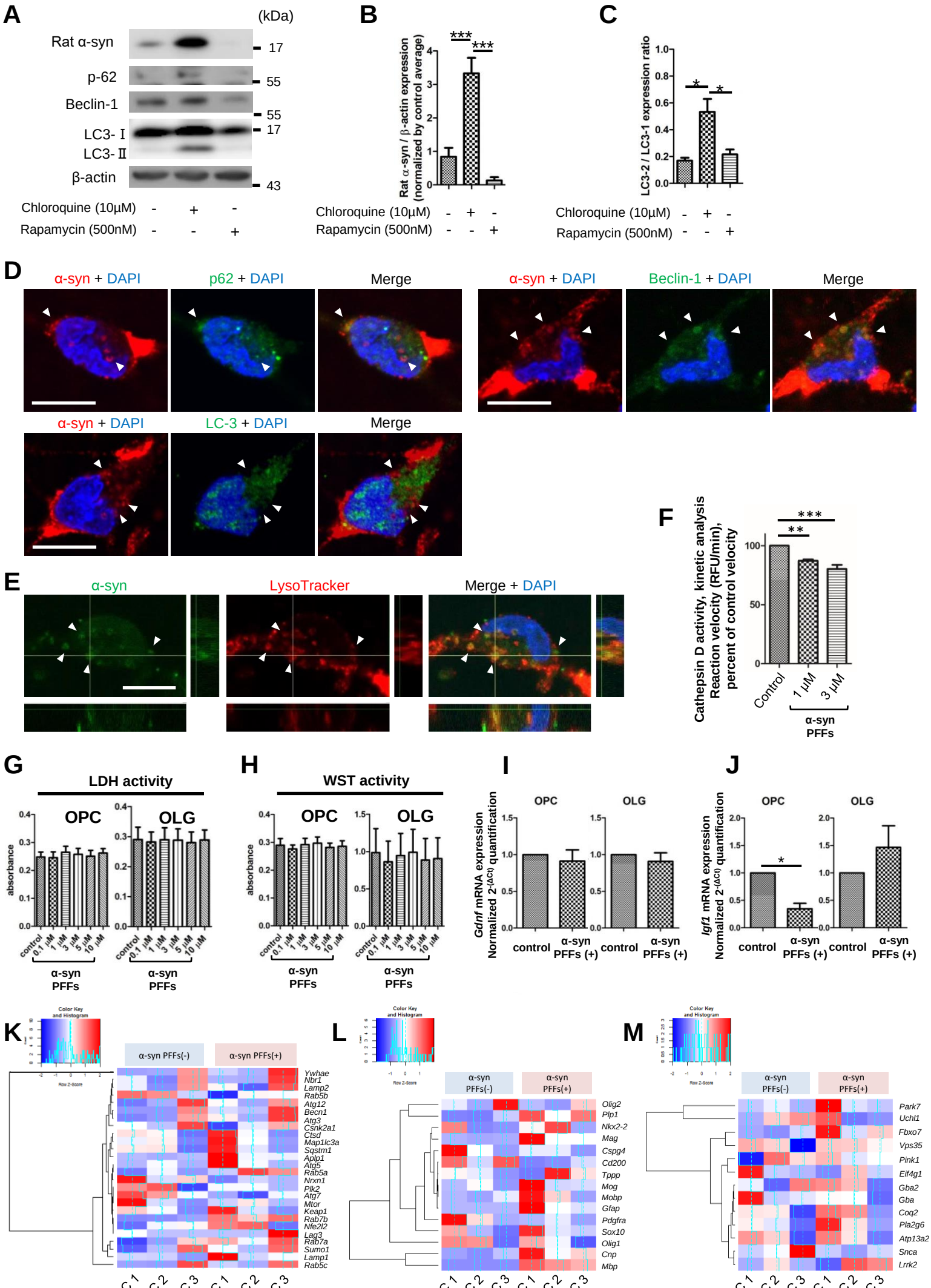

Figure S3 Autophagic Response and Functional Influence Induced by α-Syn PFFs Application to OPCs

(A) Immunoblot analysis of OPCs after 24-hour incubation with chloroquine (10 μM) or rapamycin (500 nM). Chloroquine induced increased expressions of endogenous rat α-syn, p-62, Beclin-1, and LC3-II, while rapamycin induced suppression of these protein expressions.

(B) (C) Quantification of (B) the endogenous rat α-syn expression and (C) the LC3-II / LC3-I ratio is displayed. Mean±SEM; n=4, respectively; independent cultures; one-way ANOVA. *$p<0.05$, ***$p<0.001$.

(D) Immunoblot analysis of α-syn PFFs-treated OPCs labeled with antibodies against autophagic markers, p62, Beclin-1, and LC3, shows the interaction of α-syn with these autophagic markers (as indicated by white arrowheads). Each scale bar represents 10 μm.

(E) Lysosomal localization of α-syn is revealed by LysoTracker (as indicated by white arrowheads) 24 hours after α-syn PFFs application to OPCs. The scale bar represents 10 μm.

(F) The alteration of cathepsin D activity in OPCs 24 hours after α-syn PFFs application is shown. Each enzymatic activity is indicated by the initial reaction velocity (% of control velocity), which was calculated from the change of relative fluorescence units (RFU) during the first 5 minutes of the reaction. Mean±SEM; n=5, respectively; independent cultures; one-way ANOVA. **$p<0.01$, ***$p<0.001$.

(G) (H) LDH as well as WST activity in culture medium indicates that α-syn PFFs application does not induce acute cell death in OPCs or OLGs during a time frame of 24 hours. Mean±SEM; n=5, respectively; independent cultures.

(I) (J) Quantitative real-time PCR analysis shows mRNA expression of *Gdnf* and *Igf-1* in α-syn PFFs (3 μM)-treated oligodendrocyte lineage cells. Mean±SEM; n=6, respectively; independent cultures; paired t-test, *$p<0.05$.

(K) (L) (M) RNA-seq analysis of α-syn PFFs-treated OPCs reveals altered expression of transcripts related to (K) proteolysis and protein trafficking, (L) phenotypic markers, and (M) risk genes for familial Parkinson's disease and MSA. Each pair of OPC culture samples (OPC1, OPC2, and OPC3) was allocated for the two groups with and without α-syn PFFs (3 μM) application.

Supplemental Figure S4

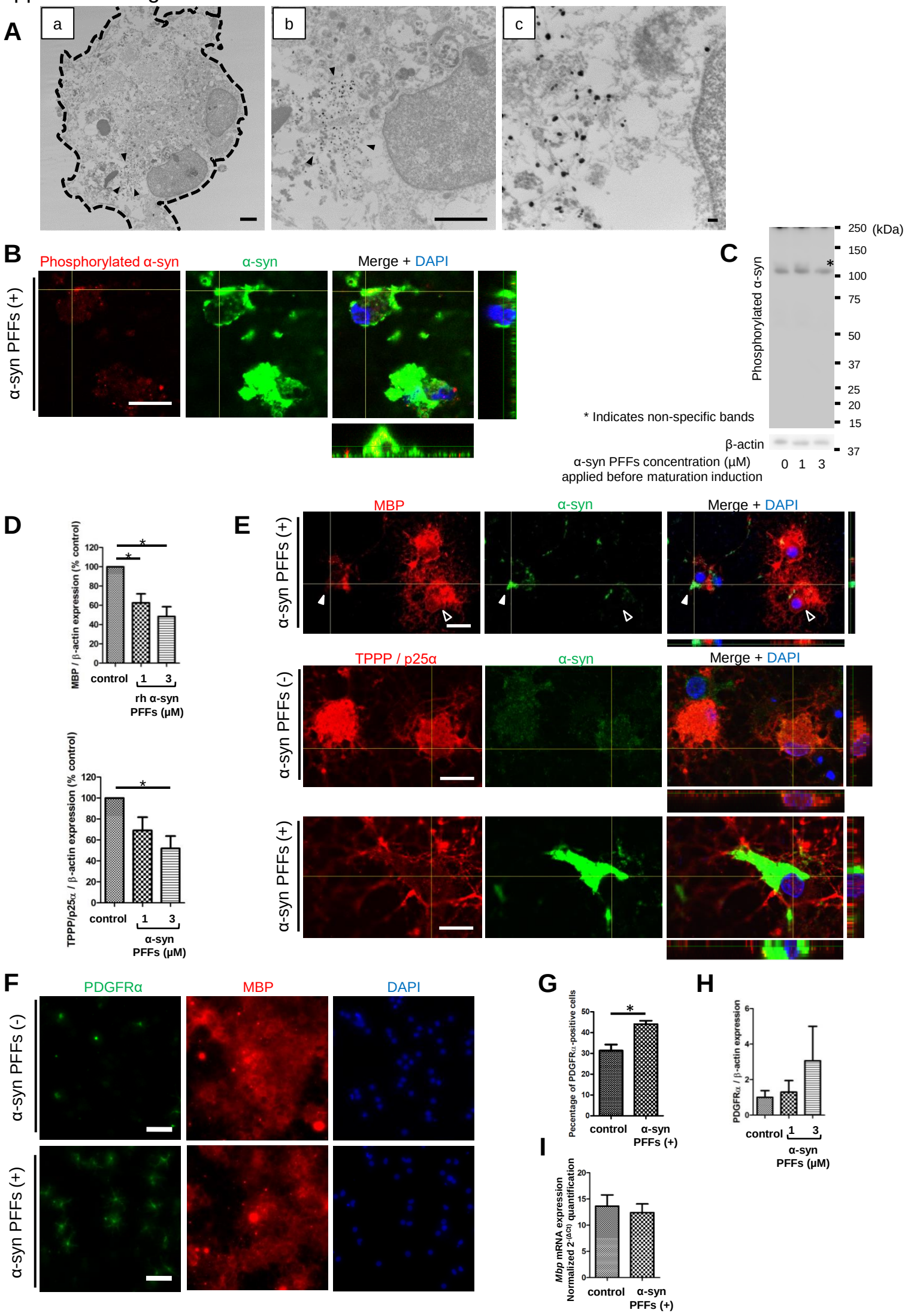

Figure S4 Decreased Expression of Myelin-associated Proteins in OLGs Derived from α-Syn PFFs-treated OPCs

(A) Immunoelectron microscopy of mature OLGs differentiated from α-syn PFFs (1 μM)-pretreated OPCs shows intracellular localization of fibrillary α-syn. The primary antibody recognizes both rat and human α-syn. a),b) Immunogold labeling of α-syn in OLGs shows intracellular α-syn localization (arrow heads). The scale bar represents a) 2 μm and b) 500 nm, respectively. The cell membrane is traced with dotted lines. c) Magnified image of gold particles associated with fibril-like structures adjacent to the nucleus. The scale bar represents 100 nm.

(B) (C) α-Syn aggregates in OLGs derived from α-syn PFFs-treated OPCs are vaguely immunoreactive to phosphorylated α-syn antibody (B). The scale bar represents 20 μm. The expression of phosphorylated α-syn is, however, not clearly confirmed by immunoblot analysis (C).

(D) Quantification by immunoblot analysis in OLGs derived from α-syn PFFs-treated OPCs shows significant reductions of MBP and TPPP/p25α protein expression. Mean±SEM; n=4, respectively; independent cultures; one-way ANOVA, *$p<0.05$.

(E) Confocal microscopy reveals that MBP expression in OLGs containing α-syn aggregates is markedly decreased (white arrow heads) in contrast to OLGs not containing aggregates (void arrow heads). The scale bar represents 20 μm. TPPP/p25α expression is also affected by α-syn PFFs application. Each scale bar represents 20 μm.

(F) Distribution of cells labeled with PDGFRα and MBP is shown. OLG culture derived from α-syn PFFs (1 μM)-treated OPCs contains more PDGFRα-positive cells, compared with normal OLG culture. Each scale bar represents 50 μm.

(G) Quantification of the percentage of PDGFRα-positive cells suggests the inhibition of OLG maturation due to α-Syn PFFs (1 μM) application. Mean±SEM; n=3, respectively; independent cultures; paired t-test, *$p<0.05$.

(H) Quantification of immunoblot analysis of OLG culture derived from α-syn PFFs-treated OPCs shows increasing trend of PDGFRα protein expression (normalized by β-actin expression). Mean±SEM; n=4, respectively; independent cultures; paired t-test.

(I) Quantification of *Mbp* mRNA expression in these OLGs reveals downward trend of *Mbp* gene expression induced by α-syn PFFs (3 μM) application. Mean±SEM; n=4, respectively; independent cultures; paired t-test.

Supplemental Movie S1 Time-Lapse Video of Primary OPC Culture Incubated with Proliferation Medium

Primary OPC culture was incubated for 62 hours with proliferation medium containing PDGF-AA and FGF-2. Images were acquired at defined positions every 10 minutes.

Supplemental Movie S2 Time-Lapse Video of Primary OPC Culture Incubated with Differentiation Medium

Primary OPC culture was incubated for 62 hours with differentiation medium containing CNTF and T3. Images were acquired at defined positions every 10 minutes.