LRP1 Regulates the Uptake and Propagation of α-Synuclein

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Research Article

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Abstract

The accumulation and transmission of α-Synuclein (α-Syn) is vital in the pathogenesis of synucleinopathies, including Parkinson's disease (PD), dementia with Lewy body and multiple system atrophy. Clinical progression and severity of synucleinopathies are associated with deposition and spread of protein aggregates. α-Syn spreads between cells in a prion-like manner and causes neurotoxicity. However, the cellular mechanisms underlying misfolded α-Syn accumulation and propagation have not been conclusively determined. Low-density lipoprotein receptor-related protein 1 (LRP1), which is considered as a multifunctional endocytic receptor that is abundantly expressed in neurons, is elevated in the neurons of PD patients. In this study, elevated LRP1 levels were found in PC12 cells treated with extracellular added α-Syn pre-formed fibrils (PFFs) as well as PFFs-injected PD mice. Cytoplasmic α-Syn levels were markedly suppressed by *LRP1* knockdown *in vitro* and *in vivo*. Additionally, LRP1 levels were elevated in cells transfected with full length and N-terminus of α-Syn. Interactions between α-Syn and LRP1 were mediated by lysine residues in the N-terminus of α-Syn. Furthermore, downregulation of LRP1 in mice effectively suppressed the propagation of α-Syn between neurons. These findings indicate that LRP1 regulates the uptake and propagation of α-Syn in the brains and may be a potential target for the treatment of α-Syn aggregation- and propagation-associated diseases.

Introduction

Misfolding and aggregation of α-Synuclein (α-Syn) is a common hallmark of synucleinopathies including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA)[1, 2]. The α-Syn protein is encoded by *SNCA* gene and is composed of 140 amino acids with three main regions[3]. The N-terminus region plays an important role in membrane binding, the NAC region nucleates amyloid fibril formation, while the C-terminus region is a typical inherently disordered region[4, 5]. Physiologically, α-Syn is highly expressed at the pre-synaptic terminals in the nervous system. Under pathological states, α-Syn assembles into well-ordered, β-sheet-rich amyloid fibrils[6]. Previous studies have shown that α-Syn monomer easily aggregates[7]. In the aggregation process, soluble oligomers are first formed, followed by insoluble pre-formed fibrils (PFFs)[8].

Previous data demonstrated the toxicity of α-Syn PFFs, which is associated with their spread[9–11]. The misfolded α-Syn can self-propagate, and progressively spread in a prion-like manner to further induce α-Syn pathology[12]. Indeed, uptake of the α-Syn PFFs by neuronal cells has been observed[13, 14]. α-Syn PFFs recruit endogenous α-Syn and induce the aggregation of α-Syn in adjacent cells, affecting different regions of the brain[15–17]. These events account for the progression and clinical severity of synucleinopathies[18, 19]. Thus, insights into the uptake and propagation of α-Syn are of great help in understanding the pathological progression of synucleinopathies.

Endocytosis is the main pathway that is involved in α-Syn internalization[20], which includes micropinocytosis through cell surface binding of heparan sulfate proteoglycan (HSPG)[21] and receptor-mediated endocytosis by lymphocyte activation gene 3 (LAG3) receptor on the cell surface[22]. α-Syn is

then taken up by exosomes and transferred to connected neurons via axon transport[23]. On the other hand, cell-to-cell transfer of pathological α-Syn aggregates mediated by tunneling nanotubes (TNT) has also been reported[24]. Exogenously added α-Syn PFFs are incorporated into lysosomes of donor cells and then transferred to recipient cells via TNT, where endogenous α-Syn is subsequently recruited to form inclusion bodies[25]. Recent studies have demonstrated that α-Syn is transported into and out of the brain via the blood-brain barrier, and low-density lipoprotein receptor-related protein 1 (LRP1) has been implicated in the α-Syn efflux[26].

LRP1 is a transmembrane protein which belongs to the low-density lipoprotein receptor family[27]. It is principally expressed in neurons, astrocytes, microvascular endothelial cells and is an endocytic and signaling receptor which mediates proliferation and migration of different types of cells[28]. After being processed by furin in the Golgi network, two subunits of LRP1 are produced; extracellular α-subunit which contains four extracellular ligand-binding domains, and the transmembrane and intracellular β-subunit which mediates endocytosis as well as activation of intracellular signaling[29]. Recent studies have highlighted the importance of HSPG in the uptake of α-Syn PFFs, and has been shown to in conjunction with LRP1[30]. The data showed that α-Syn PFFs are taken up via micropinocytosis by binding HSPG embedded on the cell surface[21], which is a critical step in prion-like propagation. Intriguingly, Tau protein shares similar mechanisms of internalization into cells. Tau interacts with α-Syn, which promotes formation of toxic aggregates with distinct molecular conformations[31, 32]. Additionally, LRP1 is a master regulator of tau uptake and propagation. The interaction between tau and LRP1 is mediated by lysine residues in the microtubule-binding repeat region of tau[33]. Besides, LRP1 has been reported to be elevated in neurons of PD patients and is considered as a potential α-Syn transporter[34, 26]. However, data on the role of LRP1 in the uptake and propagation of α-Syn remains limited.

Our data demonstrated that treatment of exogenous α-Syn PFFs in PC12 cells and mice led to an increase in LRP1. Knocking down of LRP1 significantly reduced the uptake and propagation of α-Syn. In addition, there was upregulation of LRP1 in cells transfected with full length and N-terminus of the α-Syn. Analysis of the interaction between lysine residues in α-Syn N-terminus and LRP1 demonstrated that LRP1 robustly regulated the uptake and propagation of α-Syn, thus was a potential target for treatment of diseases related to α-Syn aggregation and propagation.

Materials And Methods

Cell culture

Rat pheochromocytoma PC12 cells and Human Embryonic Kidney 293A (HEK293A) cells were purchased from the Chinese Academy of Sciences. The PC12 cells were cultured in 1640 medium (Gibco, New York, USA) supplemented with 10% horse serum (HS, Biological Industries, NA, USA) and 5% fetal bovine serum (FBS, Gibco, New York, USA). On the other hand, the HEK293A cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco, New York, USA) supplemented with 10% FBS. All cells were

cultured with 100 U/mL penicillin and 100 µg/mL streptomycin (P/S) (Solarbio, Beijing, China) at 37°C in a humidified incubator with 5% CO_2 (Thermo Fisher Scientific, MA, USA).

Cell Viability

8×10^3 per well PC12 cells were seeded on 96-well plates (Thermo Fisher Scientific, MA, USA) and incubated with different concentrations of ultrasound-treated α-Syn PFFs (0, 5, 10, 20 and 50 µg/mL) for 24 h[35]. Thereafter, PC12 cell viability was measured using the Cell Counting Kit-8 (CCK8, Dojindo Laboratories, Kumamoto, Japan) following the manufacturer's instructions. Briefly, at the end of treatment, 100 µL of media containing 10 µL CCK8 was added to each well and incubated in the 5% CO_2 incubator at 37°C for 3 h. The optical density of each well was measured at 450 nm using Max F5 microplate reader (Molecular Devices, CA, USA). The experiment was repeated three times with three replicates in each treatment group.

Lrp1 Gene Knock Down

LRP1 siRNA (sequence of CCGAAATCTGTTCTGGACCAGTTAT), negative control (NC) and positive control (PC) siRNA were purchased from Invitrogen (Thermo Fisher Scientific, MA, USA). The PC12 cells at a density of 5×10^4 cells per square centimeter were cultured overnight in 6-well culture dishes (Thermo Fisher Scientific, MA, USA). The cells were then transfected with siRNA using Lipofectamine RNAi MAX transfection reagent (Thermo Fisher Scientific, MA, USA), following the manufacturer's protocol. The efficiency of interference was detected using western blot and real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Isolation Of Total Rna And Qrt-pcr

Total RNA was isolated from the PC12 cells using the RNAsimple Total RNA Kit (Tiangen, Beijing, China), and then cDNA was synthesized using TIANScript II RT Kit (Tiangen, Beijing, China), as instructed by the manufacturer. Afterwards, the samples were mixed with SYBR Green dye (Applied Biosystems, CA, USA), primers (Sangon Biotech, Shanghai, China) and RNase-free ddH_2O for qRT-PCR. The primer sequences for *LRP1*, *SNCA* and *GAPDH* amplification were as listed in Table 1. The qRT-PCR experiment was performed on an ABI Fast 7500 system (Applied Biosystems, CA, USA). Relative quantification of mRNA expression was determined using the $\Delta\Delta$Ct method. The LRP1 or α-Syn mRNA expression was normalized to that of GAPDH.

Table 1
Forward and reverse primer sequences for qRT-PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
LRP1	5'-GGCTCTGTGGCTCAAAGGTA-3'	5'-CCTAAAAGTGGGAGCTGGGG-3'
SNCA	5'-CCTCCAACATTTGTCACTTGC-3'	5'-AGCAGGAAAGACAAAAGAGGG-3'
GAPDH	5'-GTTACCAGGGCTGCCTTCTCTTG-3'	5'-CCTTGACTGTGCCGTTGAACTTG-3'

Capping Lysine Residues On α-syn Pffs

Ultrasound-treated α-Syn PFFs were incubated with Sulfo-NHS-Acetate (NHS, Thermo Fisher Scientific, MA, USA) for 1 h to cap the lysine residues. 10 μg/mL of the lysine capped α-Syn PFFs were incubated with PC12 cells. After 24 h, the cells were harvested or fixed for western blot, qRT-PCR and immunofluorescence experiments.

α-syn Plasmid Transfection

Flag-tagged human full length α-Syn plasmid (SNCA), α-Syn N-terminus plasmid (N-SNCA) and α-Syn without N-terminus plasmid (ΔN-SNCA) in *Escherichia coli* were purchased from Sangon (Sangon Biotech, Shanghai, China). The HEK293A cells were pre-seeded on 6-well culture dishes with 1×10^5 cells per well to 70% confluency and then changed with fresh medium. Lipofectamine ^{TM} 3000 regent (Invitrogen, MA, USA) was used to coat and transfect 2 μg of the α-Syn plasmids into the cells. After 48 h, the HEK293A cells were harvested for western blot and immunofluorescence analyses.

Animals And Treatment

C57BL/6 male mice (4-5weeks) weighting 18–22 g were provided by Hunan SJA Laboratory Animal Co., Ltd, China. The mice were given free access to a standard laboratory chow and water, and were maintained in relative humidity and temperature, under a 12-hour light/dark cycle. The animal experiments were approved by the Ethics Committee of the Guilin Medical University (approval No. 2019-0021), and were performed according to the Institutional Animal Guidelines of Guilin Medical University.

6 weeks old mice were randomly divided into PD model group (PFF group) and sham operation group (sham group), with 6 mice in each group. The PD model was established by injecting 2 μL of 1 μg/μL α-Syn PFFs into the right striatum (STR)[36, 16], at AP − 0.5 mm, ML − 2 mm, DV − 3 mm coordinates (relative to Bregma). The injections were performed under standard aseptic surgery conditions. A Hamilton needle was used to inject the α-Syn PFFs into the PD mice while equal volumes of 0.01 M phosphate buffered solution (PBS) were administered into the sham group mice at 0.5 μL/min. After operation, the mice were sterilized, sutured and kept quietly for 4 weeks.

On the other hand, lentivirus encoding either the siRNA targeting LRP1 or a control ‘scramble’ siRNA was injected into the 6-week-old wild-type mice through the tail vein. Eight weeks later, the human-α-Syn lentivirus was stereotactically administered into the STR. After six weeks, the mice were euthanized and then the brain tissues were excised for immunofluorescence and western blot analyses.

Mice Visual Gait Analysis

Visual Gait Analysis System (Xinruan Information Technology, Shanghai, China) was used to evaluate mice movement disorder[37]. The mice were trained for 3 days before the experiment. During detection, the mice were allowed to freely pass through the detection channel of a set length for at least three trials. The internal light source footprint refraction technology was used to perform efficient computer processing on the footprints in a video, and the movement of the mice were evaluated under natural walking. The tests were conducted at concentrated hours during the day while the channel was cleaned with 75% ethanol between the trials to wipe urine and feces to reduce interindividual interference.

Immunohistochemistry And Immunofluorescence

The mice were perfused with 0.9% saline followed by 4% paraformaldehyde[38]. Mice brains were stripped and further fixed for 24 h before dehydration with sucrose. The brains were frozen, sliced into 20 μm thick sections and permeabilized overnight using 0.3% TritonX-100 in PBS (PBST). The sections were then blocked in 2% goat serum for 30 min followed by incubation with mouse tyrosine hydroxylase (TH) antibody (1:2000, Sigma, MO, USA) at 4°C overnight. The samples were processed by a mouse SPN kit (ZSGB-BIO, Beijing, China) and diaminobenzidine (DAB, ZSGB-BIO, Beijing, China) solution. Thereafter, they were dehydrated and mounted and then imaged by a light microscope.

For immunofluorescence assays, the cells on coverslips were washed three times with 0.01 M PBS and fixed with 4% paraformaldehyde for 30 min at room temperature (RT). The cells were then permeabilized with 0.1% PBST for 20 min and blocked with 5% FBS. The cells and brain sections were incubated with the mouse antibody for α-Syn (1:1000, BD Transduction Laboratories, NJ, USA), rabbit antibody for LRP1 (1:2000, Beyotime Biotechnology, Shanghai, China) or mouse antibody for Flag (1:2000, Abmart, Shanghai, China) at 4°C overnight. Thereafter, the samples were incubated with a secondary antibody conjugated to Alexa Fluor 488 or 594 (1:2000, Invitrogen, MA, USA) for 1 h in RT. 4’, 6-diamidino-2-phenylindole (DAPI, 1:5000, Invitrogen, MA, USA) was used to stain the nucleus before mounting and then scanning with a confocal microscope (ZEISS, Oberkochen, Germany).

Western Blot

The cells and brain tissues were homogenized and lysed in RIPA lysis solution (Solarbio, Beijing, China), supplemented with protease and phosphatase inhibitor cocktails (Millipore, MA, USA) for 30 min. The

samples were then centrifuged for 30 min at 12,000 g. The concentration of the supernatant was analyzed using the BCA method before mixing with 5 × loading buffer (Solarbio, Beijing, China). The samples were boiled for 10 min, and then 30 μg of total protein was loaded and separated by a 15% sodium dodecyl sulfate polyacrylamide gel. The samples were transferred to polyvinylidene difluoride membranes (Millipore, MA, USA) and blocked with 5% skimmed milk for 2 h. The membranes were then incubated with rabbit LRP1 antibody (1:5000, Beyotime Biotechnology, Shanghai, China), mouse α-Syn antibody (1:1000, Abcam, Cambridge, UK), mouse Flag antibody (1:2000, Abmart, Shanghai, China), mouse TH antibody (1:2000, Sigma, MO, USA), or mouse β-Actin antibody (1:10,000, Affinity, Melbourne, Australia) overnight at 4℃, followed by incubation with a fluorescent secondary antibody (1:10,000, LI-COR Biosciences, NE, USA) for 1 h. The blots were analyzed by ODYSSEY dual-color infrared fluorescence imaging system (LI-COR Biosciences, NE, USA).

Statistical Analysis

Data were presented as means ± SD, while GraphPad Prism 8.0 software (GraphPad, CA, USA) was used for statistical analyses. Comparison between the two groups was analyzed by unpaired t test. And comparison between three or more groups was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. A $P < 0.05$ was considered statistically significant.

Results

Exogenous α-Syn PFFs increase the expression of LRP1

α-Syn aggregation is a gain-of-toxic-function process that plays a central role in the development of synucleinopathies. Recent *in vitro* studies have demonstrated the neurotoxicity of aberrantly expressed or aggregated α-Syn[36, 9]. In our study, we incubated the PC12 cells with different concentrations of α-Syn PFFs and employed the CCK8 assay to analyze the cell viability. The cell viability was demonstrated with a dose-dependent decline of the α-Syn PFFs. The viability was significantly reduced when the concentration of α-Syn PFFs was 10 μg/mL (Fig. 1A), a concentration that was selected for subsequent experiments. Thereafter, we determined the expression of LRP1 and α-Syn using qRT-PCR, western blot and immunofluorescence assays. Intriguingly, treatment with exogenous α-Syn PFFs increased both mRNA and protein levels of LRP1 (Fig. 1B-D). The confocal microscopy data showed that the PC12 cells can take up both exogenous α-Syn monomer and PFFs, but only the latter caused a significant increase in LRP1 expression (Fig. 1E, F).

Lrp1 Mediates The Uptake Of α-syn Pffs

LRP1 is a major transmembrane receptor expressed in the brain, which is elevated in neurons of PD patients and had been highlighted to be a potential α-Syn transporter[34]. We hypothesized that LRP1 could regulate the uptake of α-Syn PFFs. To prove this, we used *LRP1* siRNA to silence the expression of

LRP1 in PC12 cells. Three different *LRP1* siRNA sequences were transfected into the PC12 cells at different concentrations; 10 nM, 20 nM or 40 nM for 48 h. Both qRT-PCR and western blot analyses showed that 10 nM of siRNA3 had the best LRP1 knockdown effect (Extended data Fig. 1), thus it was selected for the subsequent experiments. On the other hand, western blot and immunofluorescence analyses demonstrated that *LRP1* knockdown reduced the uptake of α-Syn PFFs in the PC12 cells (Fig. 2). These results suggested that *LRP1* knockdown significantly blocked the uptake of α-Syn PFFs.

α-syn Pffs Interact With Lrp1 Via Lysine Residues

LRP1 regulates tau uptake and spread, and the interaction between tau and LRP1 is mediated by lysine residues of tau[33]. To investigate whether LRP1 interacts with α-Syn through the lysine residues, NHS was applied to block the lysine residues of α-Syn PFFs. Western blot analyses and immunofluorescence assays demonstrated that, compared with PFF group, there was significant suppression of LRP1 and intracellular α-Syn levels after capping of the lysine residues (Fig. 3). This data demonstrated that capping of lysine residues on α-Syn PFFs effectively decreased the expression of α-Syn and LRP1 in PC12 cells, which revealed that LRP1 might be interacting with α-Syn PFFs via lysine residues.

α-syn N-terminus Mediate The Upregulation Of Lrp1

We have shown that LRP1 mediates the uptake of α-Syn PFFs, and might be interacting with lysine residues of the α-Syn PFFs. Interestingly, most of α-Syn lysine residues are located within the N-terminus region. To analyze whether the lysine residues in the N-terminus of α-Syn mediate the binding to LRP1, SNCA, N-α-Syn, and **Δ**N-SNCA plasmids were transfected into the HEK293A cells for 48 h. We then measured the levels of Flag-tag-α-Syn and LRP1 by western blot and immunofluorescence assays. The data showed that LRP1 was significantly elevated in SNCA and N-SNCA groups while there were no obvious changes in the **Δ**N-SNCA group (Fig. 4). Accordingly, the escalated endogenous α-Syn induced an increase in the expression of LRP1, which was associated with the N-terminus of the α-Syn.

Increased Lrp1 In The α-syn Pffs-induced Pd Model Mice

After stereotactic injection of α-Syn PFFs, the animal visual gait analysis system was used to assess mice motor alterations. Compared with the sham group, α-Syn PFFs injected mice had chaotic footsteps and reduced normal step sequence ratios (Fig. 5A, C). In addition, the average movement speed of α-Syn PFFs injected mice reduced while the tripod support time significantly increased (Fig. 5B, D). These results demonstrated that the α-Syn PFFs-injected mice had significant movement disorder, as shown by DAB staining of the TH-positive neurons. The density of TH in STR and SN reduced significantly in the α-Syn PFFs group (Fig. 5E-H). Similarly, western blot analyses also confirmed the staining of the TH protein (Fig. 6A, C). These data demonstrate successful establishment of α-Syn PFFs-induced mice models of

PD. In both STR and SN, there was significant upregulation of LRP1 in the α-Syn PFFs group accompanied by increased α-Syn, compared to sham mice (Fig. 6).

LRP1 **knockdown reduced the propagation of endogenous and exogenous α-Syn** in vivo

Based on the *in vitro* results, we tried to assess whether LRP1 was also critical for the propagation of α-Syn in the brain. To suppress the expression of *LRP1*, lentivirus carrying *LRP1* siRNA was firstly injected into mice through the tail vein and then reared for 8 weeks. Thereafter, STR stereotactic injections of lentivirus encoding human-*SNCA* or α-Syn PFFs was administered to increase the expression of endogenous and exogenous α-Syn in the mice brain for 6 weeks. The effectiveness of *LRP1* knockdown or *SNCA* overexpression was verified by western blot analysis, which showed reduced expression of LRP1 in STR and SN (Extended Data Fig. 2A-D), and obvious upregulation of α-Syn in the LV-SNCA group (Extended Data Fig. 2E-H). Afterwards, the mice were euthanized and then the propagation of α-Syn from STR to SN was analyzed using immunofluorescence and western blot assays. Immunofluorescence images showed the significant elevation of α-Syn in both STR and SN after injection with human-*SNCA* lentivirus or α-Syn PFFs, while the relative fluorescence intensity was greatly diminished in *LRP1* knockdown mice (Fig. 7A-D and Fig. 8A-D). These results were verified by western blot, which showed that *LRP1* knockdown led to significant reduction of α-Syn in both STR and SN (Fig. 7E-H and Fig. 8E-H). Together, our data indicate that downregulation of LRP1 robustly reduced the propagation of α-Syn from STR to SN in mice.

Discussion

As an endocytic receptor that traffics ligands from the cell surface into the cell, LRP1 has been considered as a potential α-Syn transporter. Previous studies have demonstrated that α-Syn mediates development of synucleinopathies[39, 40]. It has been shown that the aggregation of misfolded α-Syn results in toxicity[9, 41], which is exacerbated by propagation of α-Syn. However, data on the underlying cellular mechanisms of α-Syn uptake and propagation remain elusive. Our data demonstrate that LRP1 effectively regulates the uptake and propagation of α-Syn. In this study, we employed *in vitro* and *in vivo* analyses to investigate the effect of *LRP1* knocking down on the uptake and propagation of α-Syn. Besides, we capped the lysine residues on α-Syn PFFs to explore their interaction with LRP1. In addition, the LRP1 expression was analyzed in cells transfected with full length or N-terminus of the α-Syn. Our results demonstrated that LRP1 regulates the uptake and propagation of α-Syn, and is mediated via the lysine residues in the N-terminus of α-Syn.

Our study demonstrates that exogenous α-Syn PFFs reduced the cell viability in a dose-dependent manner, which was associated with toxicity. It was well documented that the propagation of misfolded α-Syn and α-Syn-mediated toxicity affected many key cellular processes such as mitochondrial functions, calcium buffering and protein clearance[36]. Pathological α-Syn displayed a prion-like behavior, that is, the ability to self-propagate via templated misfolding and intercellular dissemination[42, 43]. Other studies have demonstrated that synuclein pathologies can spread from the host to transplanted cells[44].

In addition, cell lines and primary mouse neurons produced α-Syn inclusions after treatment with α-Syn PFFs[14]. On the other hand, tagged α-Syn was shown to transmit from donor cells to acceptor cells in a cocultured system, forming α-Syn inclusions in the acceptor cells, which indicated that the pathology of α-Syn can be transmitted between cells[45–47]. In addition, data from studies on animal models also demonstrated that trans-neuronal propagation of misfolded α-Syn is involved in the progression of neurodegenerative proteinopathies[48, 49], and the pathology and toxicity were particularly prominent in the dopaminergic neurons of the SN, which resulted in behavioral disorders[50]. Our study also demonstrated that mice injected with α-Syn PFFs showed significant behavioral disorders such as abnormal gait, disturbed step order and sluggish movement. Although the exact mechanism of toxicity is unclear, it might be related to LRP1, a potential transporter of α-Syn.

It has been reported that the expression of LRP1 increases in PD pathogenesis[34]. LRP1 is an endocytic and signaling receptor that binds numerous ligands, which effectively modulates the trafficking of transmembrane receptors[51, 52]. Recent studies have shown that LRP1 is a master regulator of tau uptake and spread[33], and is considered as a potential α-Syn transporter. The current study showed that the levels of LRP1 and α-Syn in STR and SN of PFFs-injected PD mice were significantly increased. Besides, there was increase in the level of α-Syn accompanied with elevated LRP1 in the PC12 cells following treatment with α-Syn PFF. To determine whether LRP1 mediates the internalization of α-Syn, we suppressed the expression of LRP1 in the PC12 cells using siRNA. Our data showed that knockdown of *LRP1* efficiently reduced the uptake of α-Syn in the PC12 cells. Analysis of α-Syn propagation demonstrated that the α-Syn in SN was significantly diminished, especially in the mice injected with lentivirus carrying *LRP1* siRNA in the STR. These data suggested that downregulation of LRP1 significantly blocked the uptake and propagation of α-Syn.

Further analysis showed that capping of lysine residues on α-Syn PFFs prevented their internalization in the PC12 cells, which indicated that the lysine residues on α-Syn PFFs were critical for their uptake by the cells. In addition, the expression of LRP1 was significantly decreased with the reduced uptake of α-Syn PFFs after capping with lysine residues. This was solely attributed to the capping of lysine residues on α-Syn PFFs, which reduces their binding to LRP1, giving rise to minimal entrance of α-Syn PFFs into the cell, thus less formation of α-Syn pathologies in the cells[34, 53]. Available evidence has shown that LRP1 contains cysteine-rich complement-type repeats, which are known to bind and internalize their ligands[54, 55]. Furthermore, the complement-type repeats can coordinate Ca^{2+} and interact with lysine residues on ligands through salt bridges[56]. What makes sense is that α-Syn has a high lysine content; out of the total 140 amino acids, 15 (about 10%) are lysine amino acids, 11 of which are located within the N-terminus region (the lysine content of the N-terminus region is around 18%). We then hypothesized that the N-terminus region of α-Syn may be principally involved in LRP1-mediated internalization. We transfected the recombinant human full length α-Syn, N-α-Syn or **Δ**N-α-Syn plasmid into the cells, and showed that only the full length α-Syn and N-α-Syn could significantly increase the amount of LRP1. These results revealed that the interaction between α-Syn and LRP1 is mediated by lysine residues in the N-terminus of α-Syn.

Conclusion

In summary, our data demonstrated that LRP1 mediates the uptake of α-Syn via binding to the lysine residues in N-terminus of α-Syn. Besides, downregulation of LRP1 effectively reduced the propagation of α-Syn between neurons. Taken together, a model illustrating that LRP1 regulates the uptake and propagation of α-Syn via lysine residues in the N-terminus of α-Syn was proposed (Fig. 9). Our study demonstrated that targeting neuronal LRP1 may lead to significant reduction of α-Syn propagation, which could be a potential novel therapeutic target for α-Syn-related neurodegenerative diseases.

Declarations

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Competing Interests The authors declared that there is no conflict of interest.

Author Contributions Participated in research design: Min Chen, Qinghua Li, Chengwei Liu. Conducted experiments: Caixia Peng, Hanjiang Luo. Performed data analysis: Min Chen, Hanjiang Luo, Caixia Peng, Jia Liu. Writing-original draft: Caixia Peng, Hanjiang Luo. Writing-review and editing: Min Chen, Qinghua Li, Chengwei Liu. All authors revised the manuscript. All authors read and approved the final manuscript.

Data Availability The data will be available on reasonable request to the corresponding author.

Ethical approval All the experiments in this study were performed according to the guidelines of the Experimentation Ethics Committee of the Guilin Medical University, China (approval No. 2019-0021).

Consent to participate Not applicable.

Consent to publish Not applicable.

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Figures

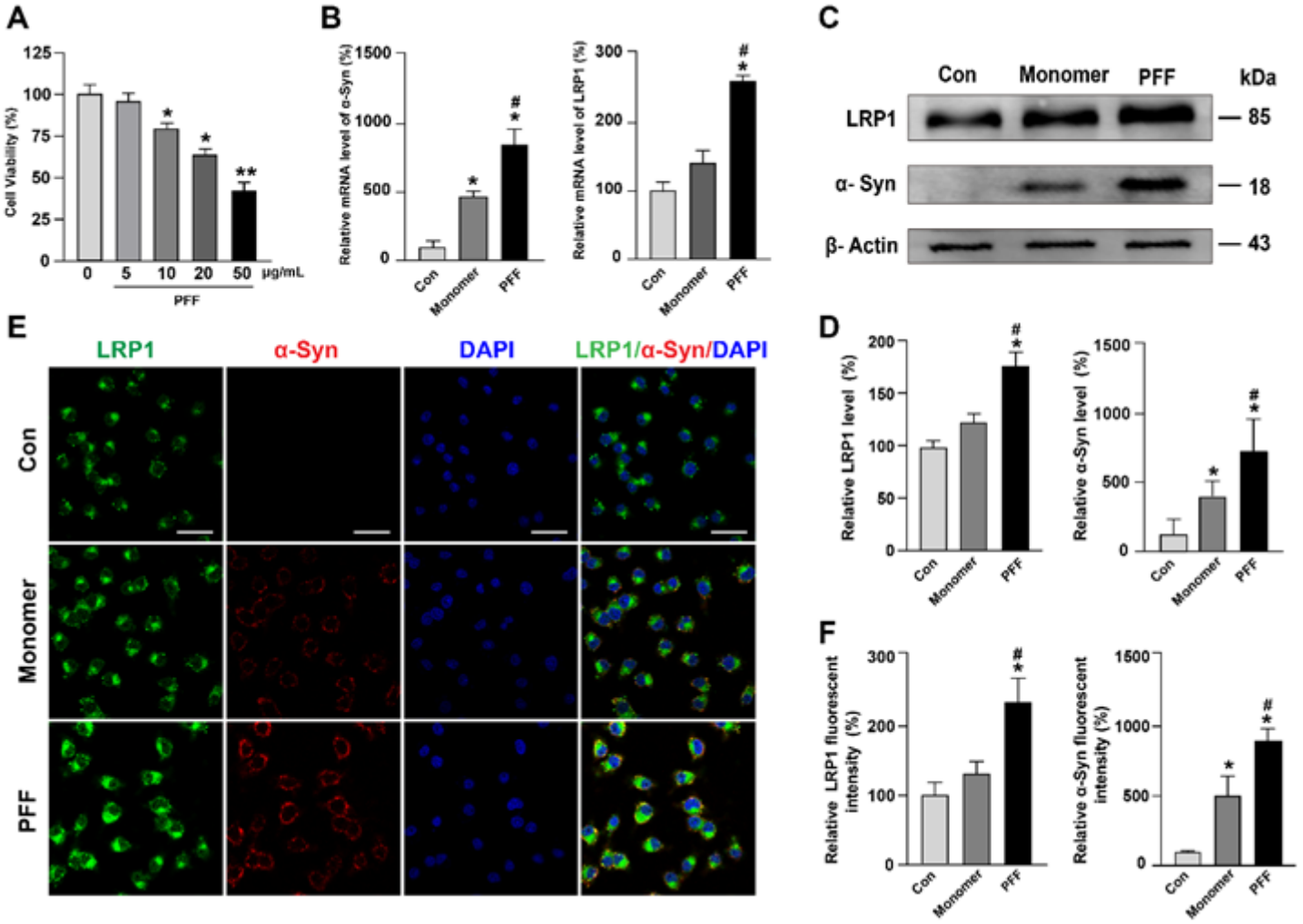

Figure 1

Exogenous α-Syn PFFs upregulate the expression of LRP1in PC12 cells.**A**.Viability ofthe PC12 cells incubated with different doses (0, 5, 10, 20 and 50μg/mL) ofα-Syn PFFs for 24h. Compared with 0μg/mL group, *p<0.05 and ** p<0.01, n = 3. **B**. qRT-PCR analysisof α-Syn and LRP1 levelsin thePC12 cells treated with 10μg/mLα-Syn monomer or PFFs for 24h.**C**.Western blot analysis of LRP1 and α-Syn levels in the PC12 cells. **D**. Densitometric analysis ofthe expression of LRP1 and α-Syn proteins.**E**. Immunofluorescenceresults showing the expression of LRP1 (green),α-Syn (red) and DAPI (blue),scale bar = 400μm. **F**. Quantitative immunofluorescence intensity analysis of the expression ofLRP1 and α-Syn.Compared with control (Con) group, *p<0.05 and ** p <0.01;compared with Monomer group,$^{\#}p$<0.05 and $^{\#\#}p$<0.01, n = 3.

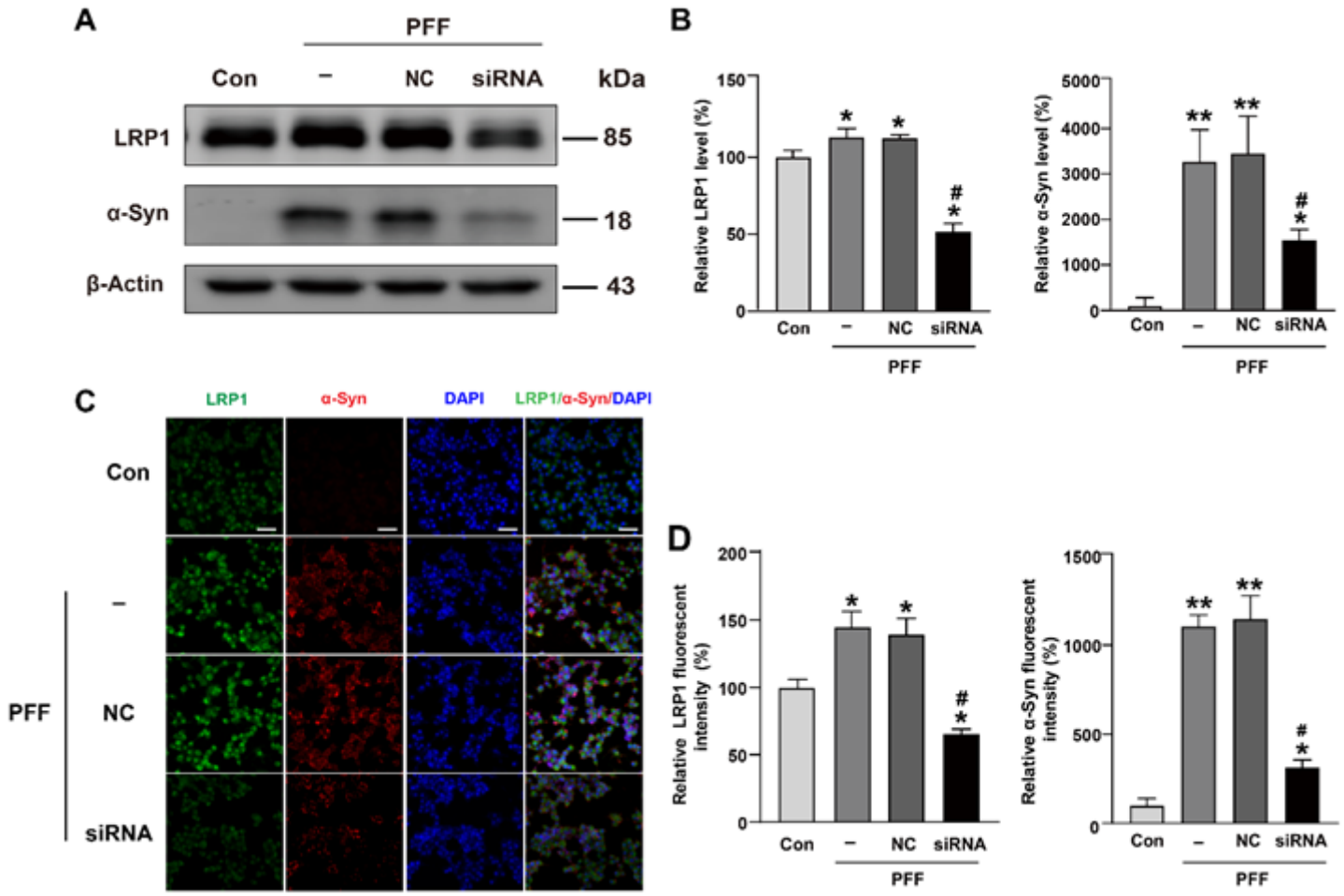

Figure 2

LRP1 mediates the uptake of α-Syn PFFsby PC12 cells.**A**.Western blot analysis demonstrating the expression of LRP1 and α-Syn in PC12 cells with or without *LRP1* knockdown (10nM siRNA)after incubation with 10μg/mLα-Syn PFFs for 24 h. **B**. Densitometric analysis of the LRP1 and α-Syn proteins.**C**.Immunofluorescenceresults of the LRP1 (green), α-Syn (red) and DAPI (blue),scale bar =

200μm. **D**. Quantitative immunofluorescence intensity analysis of the LRP1 and α-Syn. Compared with the Con group, *p<0.05 and ** p <0.01; compared with PFF/- group,#p<0.05, n = 3.

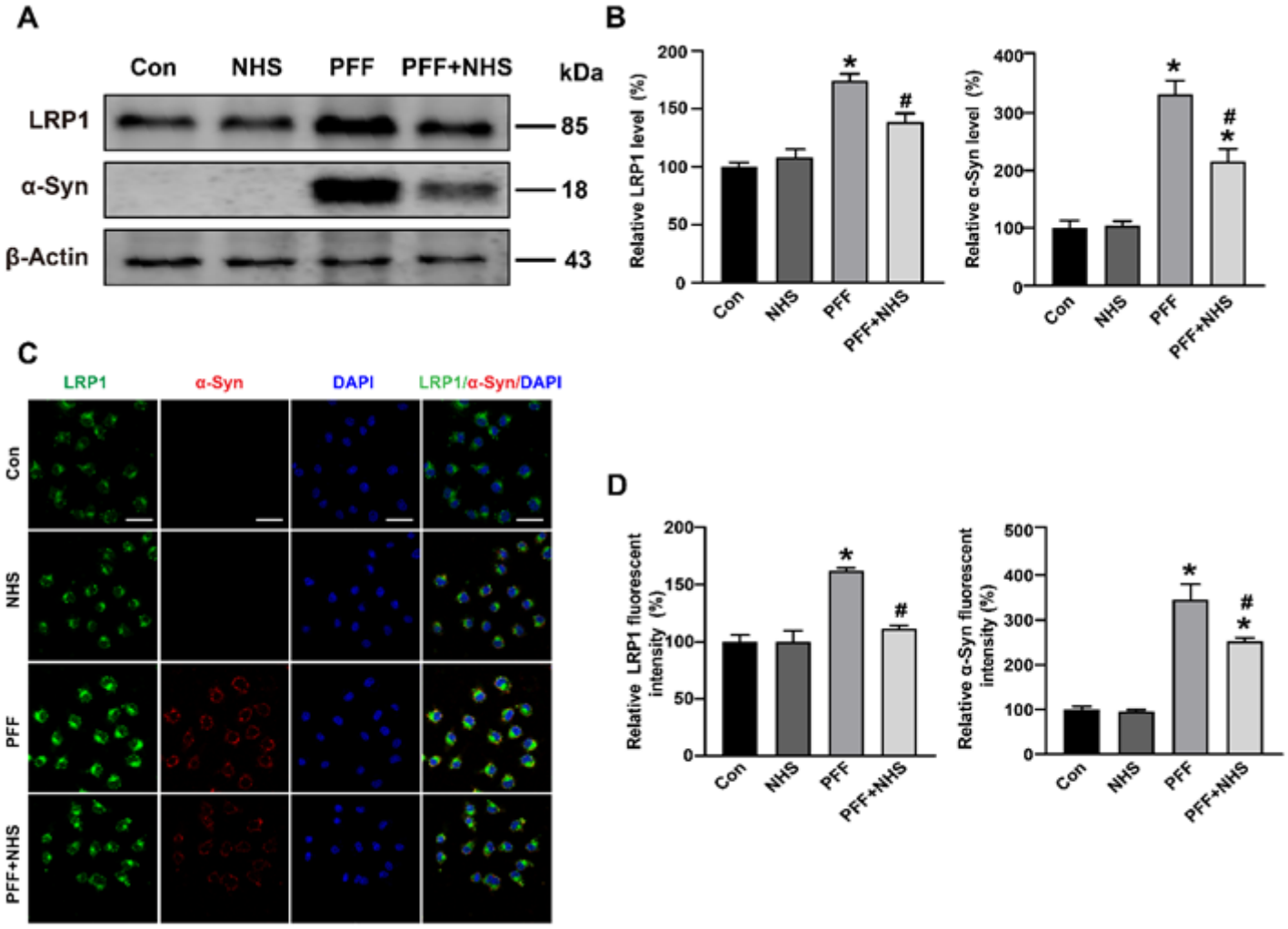

Figure 3

α-Syn PFFs interact with LRP1 via lysine residues. **A**. Western blot analysis of LRP1 and α-Syn levels in PC12 cells after addition of exogenous α-Syn PFFs (10μg/mL)with or without capping of lysine residues for 24h. **B**. Densitometric analysis of the expression of LRP1 and α-Syn proteins.**C**.Immunofluorescenceanalysis of LRP1 (green), α-Syn (red) and DAPI (blue), scale bar = 400 μm. **D**. Quantitative immunofluorescence intensity analysis of LRP1 and α-Syn. Compared with Con group, * p< 0.05; compared with PFFgroup,# p< 0.05, n = 3.

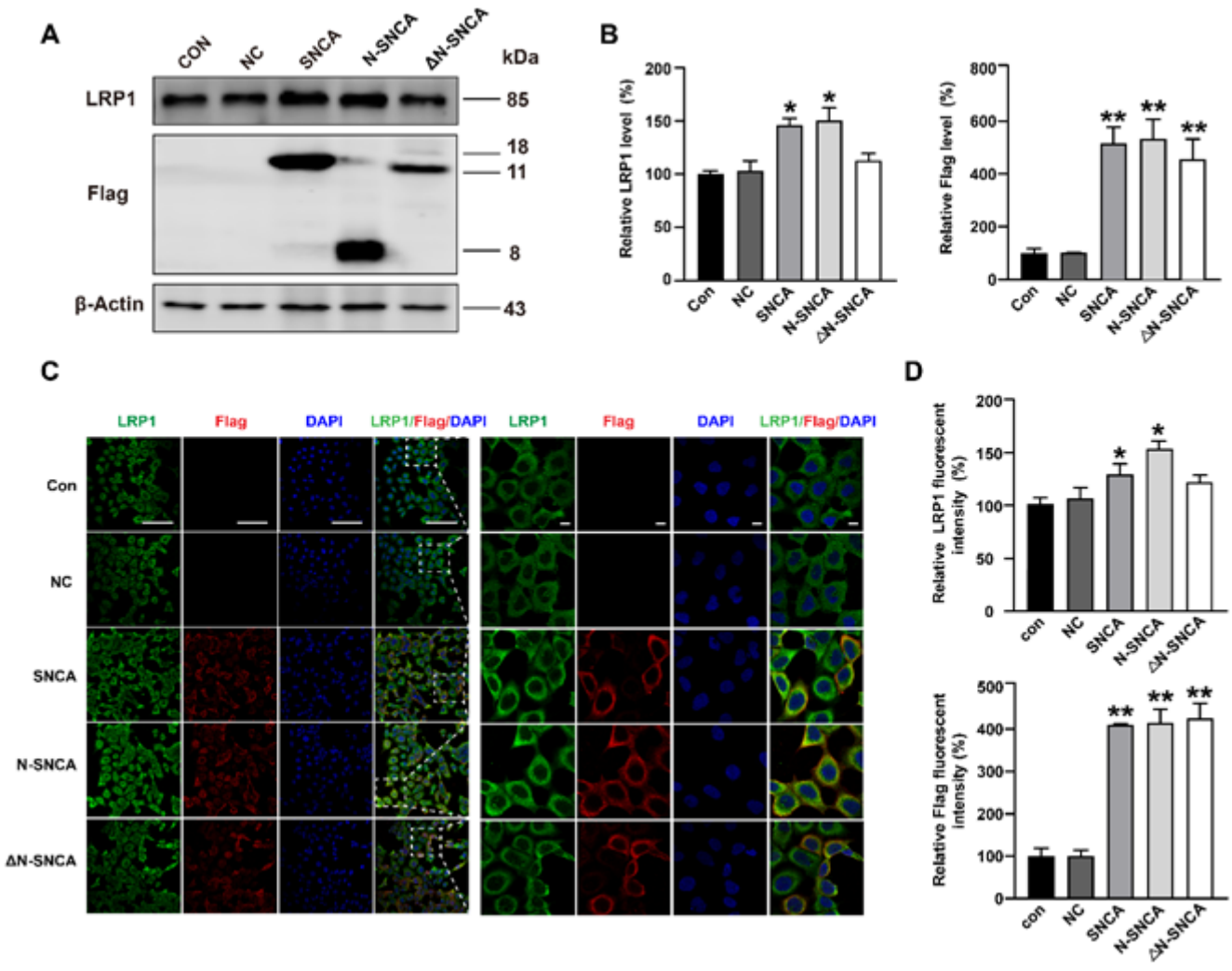

Figure 4

α-Syn N-terminus mediates the increase of LRP1.**A**. Western blot analysis of theLRP1 and Flag-tag-α-Synprotein expression in HEK293A cells(1×10^5) after transfection with Flag-tagged full length α-Syn plasmid (2μg), N-α-Syn plasmid (2μg)or ΔN-α-Syn plasmid (2μg) for 48h. **B**. Densitometric analysis of the levels of LRP1and Flag-tag-α-Syn proteins.**C**.Immunofluorescencestaining ofLRP1 (green), Flag-tag-α-Syn (red) and DAPI (blue), scale bar = 200 μm. **D**. Quantitative immunofluorescence intensity analysis of LRP1 and Flag-tag-α-Syn. Compared with Con group, * $p< 0.05$ and ** $p < 0.01$, n = 3.

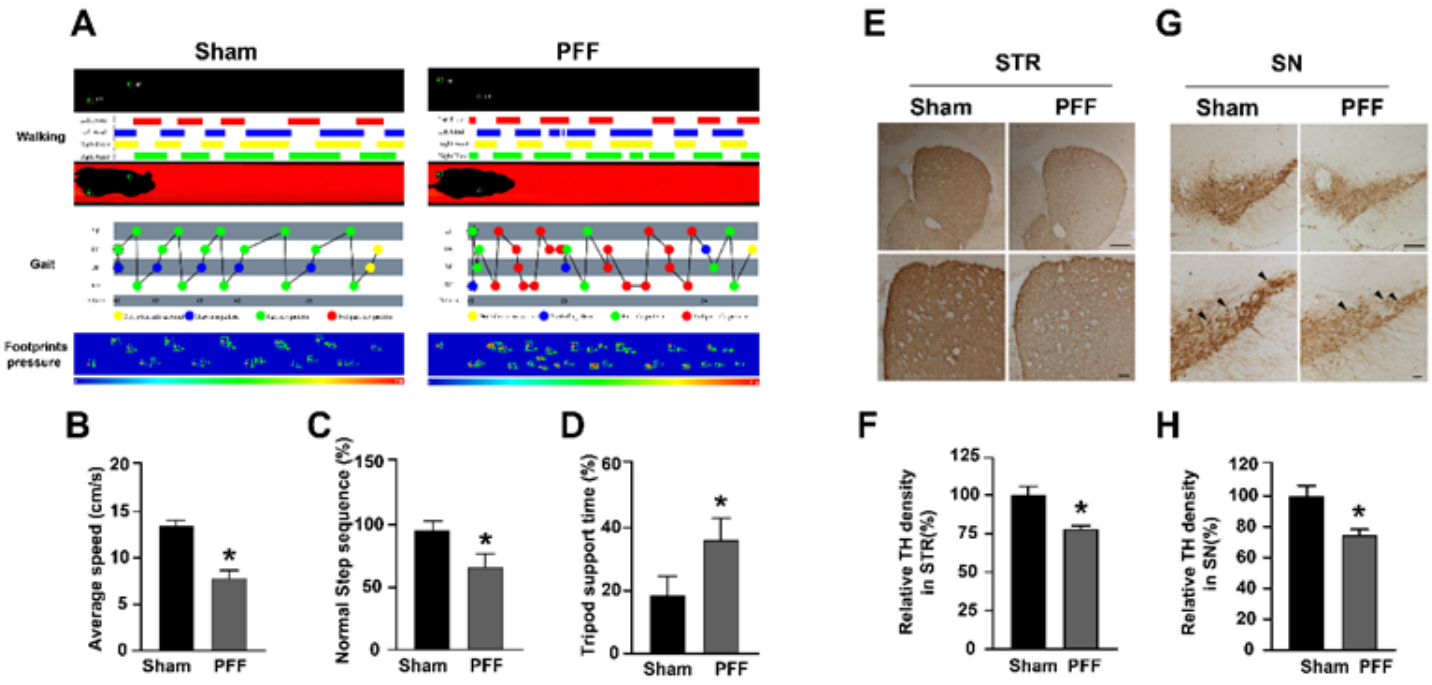

Figure 5

α-Syn PFFs-induced PD mice models. **A**. Visual gait analysis of walking, gait and footprints pressure images in mice after 2 μg of α-Syn PFFs or equal volumes of PBS stereotactic injections for 4 weeks. **B**. Average speed analysis in mice. **C**. Normal step sequence analysis in mice. **D**. Tripod support time analysis in mice. **E**. Immunohistochemistry results of TH in STR, scale bar = 100 μm. **F**. Quantitative immunohistochemistry density analysis of TH in STR. **G**. Immunohistochemistry results of TH in SN, scale bar = 200 μm. **H**. Quantitative immunohistochemistry density analysis of TH in SN.Compared with Sham group, * $p< 0.05$, n = 3.

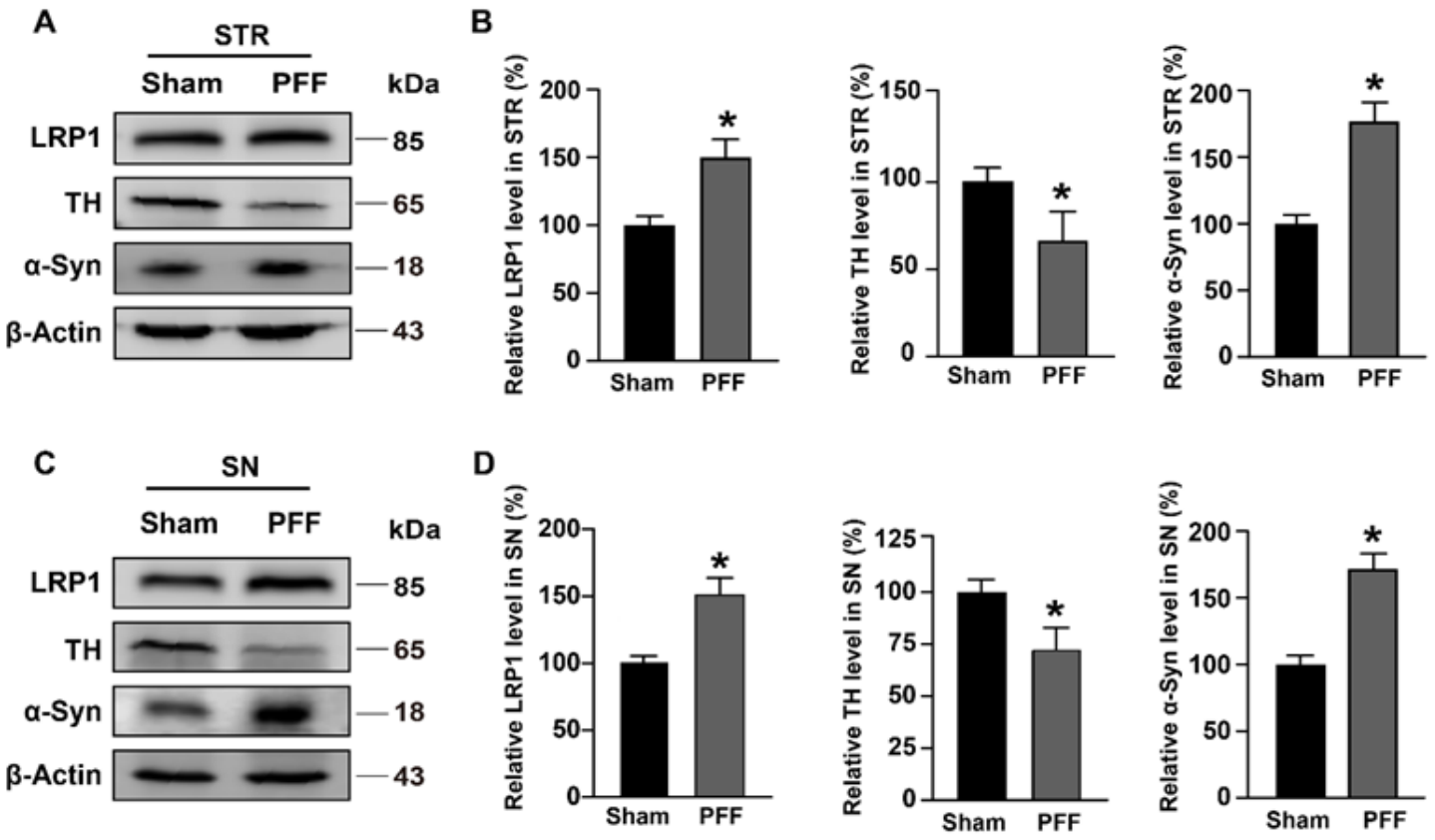

Figure 6

Elevated LRP1 levels in α-Syn PFFs-induced PD mice models. **A**. Western blot analysis of LRP1, TH and α-Syn levels in mice STR after 2 μg of α-Syn PFFs or equal volumes of PBS stereotactic injection for 4 weeks. **B**. Densitometric analysis of LRP1, TH and α-Syn protein levels in mice STR. **C**. Western blot analysis of LRP1, TH and α-Syn levels in mice SN. **D**. Densitometric analysis of LRP1, TH and α-Syn protein levels in mice SN. Compared with Sham group, * $p < 0.05$, n = 3.

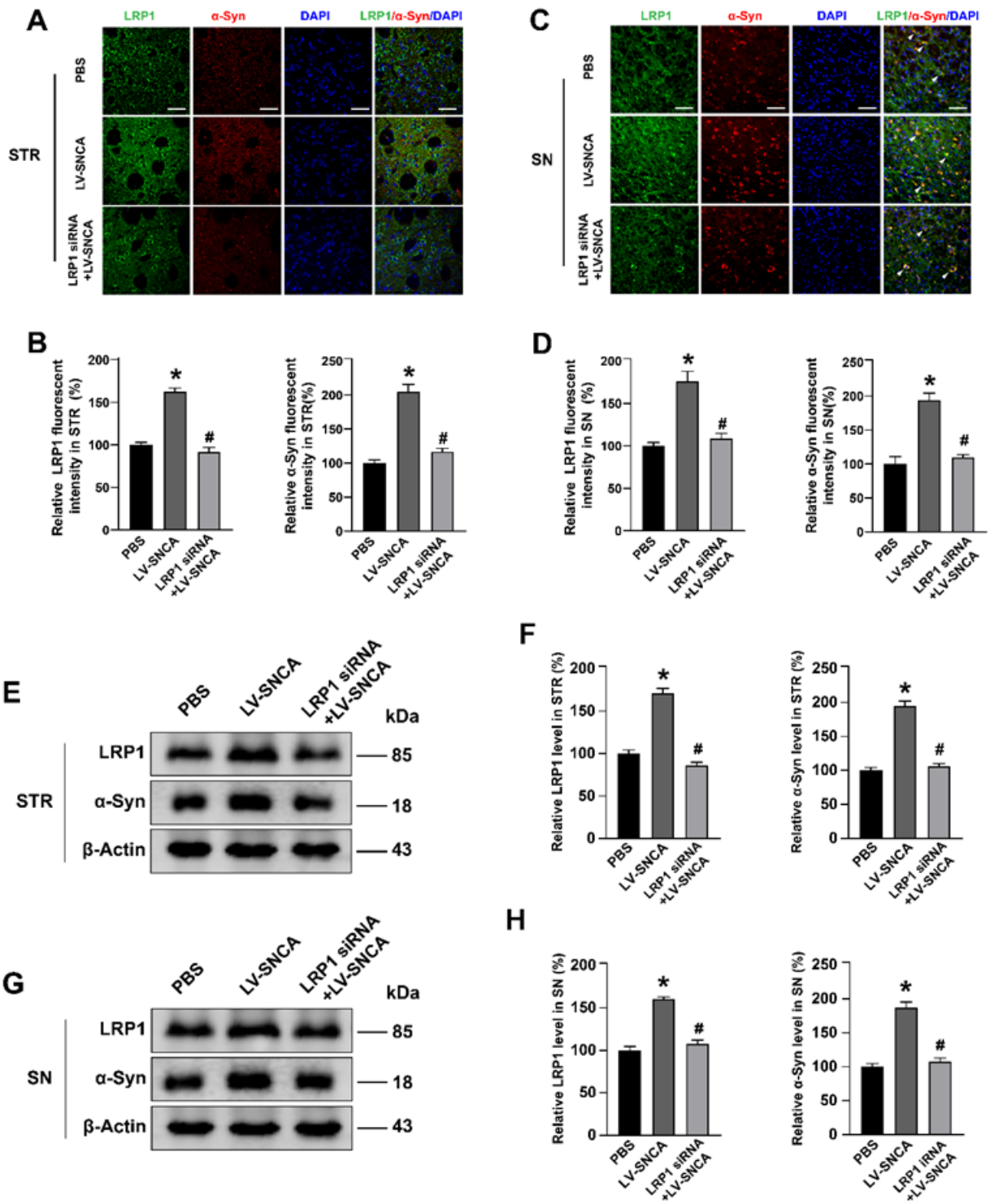

Figure 7

*LRP1*suppression reduced the propagation of endogenous α-Syn *in vivo*.**A**. Tissue immunofluorescencedata showing the expression of LRP1 (green), α-Syn (red) and DAPI (blue) in mice STRafter stereotactic injections of human-α-Syn lentivirus for 6 weeks with or without *LRP1*silencing, scale bar = 200 μm. **B**. Quantitative immunofluorescence intensity analysis of LRP1 and α-Syn in mice STR.**C**. Tissue immunofluorescenceresults of LRP1 (green), α-Syn (red) and DAPI (blue) in mice SN, scale

bar = 200 μm. **D**. Quantitative immunofluorescence intensity analysis of LRP1 and α-Syn in mice SN. **E**. Western blot analysis of LRP1 and α-Syn levels in mice STR. **F**. Densitometric analysis ofLRP1 and α-Syn levels in mice STR.**G**. Western blot analysis of LRP1 and α-Syn levels in mice SN. **H**. Densitometric analysis of LRP1 and α-Syn levels in mice SN. Compared with PBS group, * $p< 0.05$; compared with LV-SNCA group,$^{\#}$ $p< 0.05$, n = 3.

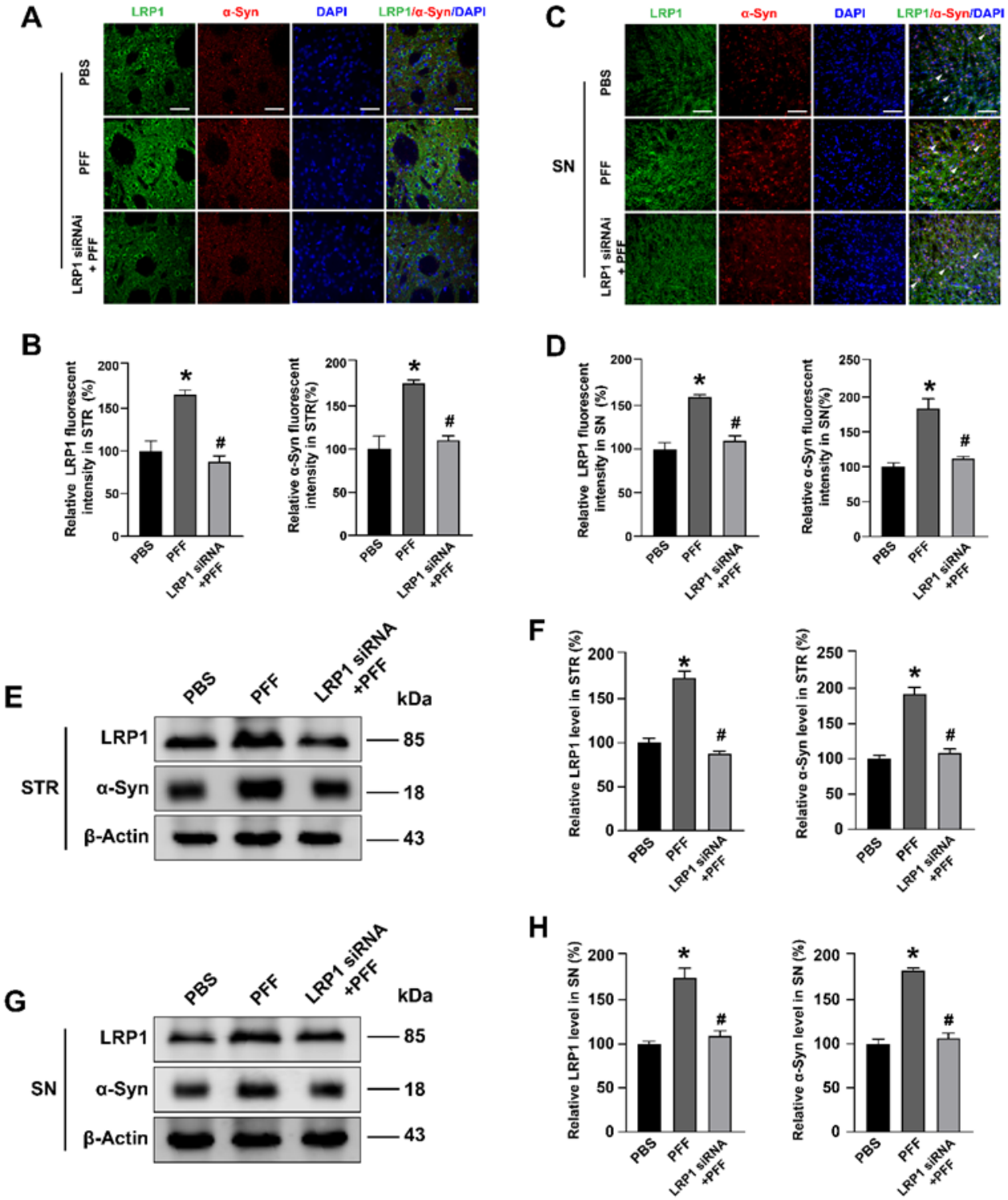

Figure 8

LRP1 suppression reduced the propagation of exogenous α-Syn *in vivo*. **A**. Tissue immunofluorescencestaining of LRP1 (green), α-Syn (red) and DAPI (blue) in mice STR after stereotactic injections of α-Syn PFFs for 6 weeks with or without *LRP1* knock down, scale bar = 200 μm. **B**. Quantitative immunofluorescence intensity analysis of LRP1 and α-Syn in mice STR. **C**. Tissue immunofluorescencestaining of LRP1 (green), α-Syn (red) and DAPI (blue) in mice SN, scale bar = 200 μm. **D**. Quantitative immunofluorescence intensity analysis of LRP1 and α-Syn in mice SN. **E**. Western blot analysis of LRP1 and α-Syn levels in mice STR. **F**. Densitometric analysis of LRP1 and α-Syn levels in mice STR. **G**. Western blot analysis of LRP1 and α-Syn levels in miceSN. **H**. Densitometric analysis of LRP1 and α-Syn levels in mice SN. Compared with PBS group, * $p< 0.05$; compared with PFF group,# $p< 0.05$, n = 3.

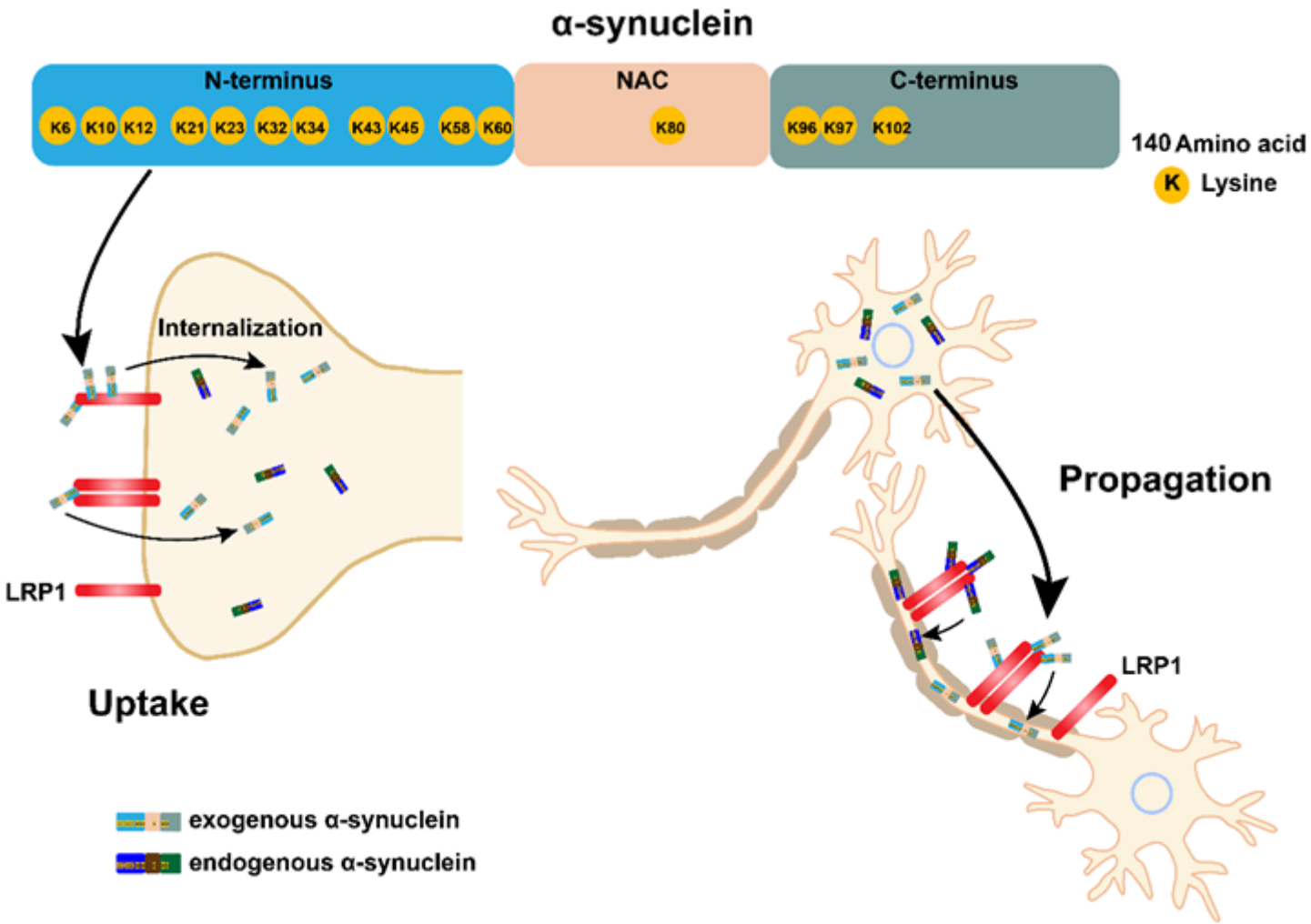

Figure 9

LRP1 regulates the uptake and propagation of α-Syn via lysine residues in the N-terminus of α-Syn.

Supplementary Files

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