



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>China Food
Publishing Co.

SIRT1 Mediates Exercise-Induced Neuroprotection in Parkinson's Disease via Mitochondrial Restoration: Toward Resveratrol as a Dietary Exercise Mimetic

Jingwen Li ^{1,2}, Tingting Liu ², Xiangshu Cheng ², Ke Zhou ¹, Jianshe Wei ^{1,2*}¹ Institute for Sports and Brain Health, School of Physical Education, Henan University, Kaifeng, Henan, 475004, China² Institute for Brain Sciences Research, School of Life Sciences, Henan University, Kaifeng 475004, China

ABSTRACT: Growing evidence indicates that exercise exerts neuroprotective effects in Parkinson's disease (PD). Silent information regulator 1 (SIRT1), a NAD⁺-dependent deacetylase, is a key regulator of mitochondrial function; however, whether exercise-mediated neuroprotection and SIRT1 upregulation converge on a common pathway in PD remains unclear. Here, we demonstrate that exercise alleviates PD pathology by upregulating SIRT1 expression and restoring mitochondrial homeostasis. In MPTP-induced PD mice, both exercise and the SIRT1 agonist resveratrol significantly improved motor and non-motor behaviors, reduced α -synuclein aggregation, and protected dopaminergic neurons in the substantia nigra. Mechanistically, both interventions enhanced SIRT1 expression, thereby rebalancing mitochondrial fission and fusion through the downregulation of fission-related proteins and the upregulation of fusion-related proteins. In parallel, SIRT1 activation promoted mitophagic clearance and improved mitochondrial function, as evidenced by restoration of mitochondrial membrane potential, increased ATP production, and reduced oxidative stress. Notably, these beneficial effects—from behavioral improvement to mitochondrial protection—were completely abolished by the SIRT1 inhibitor EX527, establishing SIRT1 as an essential mediator of exercise-mediated neuroprotection. Collectively, our findings integrate behavioral, humoral, and pharmacological approaches to delineate a coherent SIRT1-mediated pathway and highlight its potential as a target for exercise mimetic therapeutics. Furthermore, the natural polyphenol resveratrol recapitulates these exercise mimetic effects, supporting SIRT1 as a promising therapeutic target and underscoring the potential of dietary bioactives for PD intervention.

1. Introduction

Parkinson's disease (PD) is a prevalent neurodegenerative disorder, characterized pathologically by the progressive degeneration and death of dopaminergic neurons in the substantia nigra (SN) of the midbrain. This neuronal loss gives rise to a range of motor symptoms, including resting tremor, bradykinesia, muscular rigidity, and postural instability. In addition to motor symptoms, PD is often accompanied by non-motor manifestations, such as olfactory dysfunction, sleep disturbances, autonomic dysfunction, and psychiatric disorders ^[1,2]. According to the Global Burden of Disease study, the number of individuals affected by PD is projected to reach 25.2 million globally by 2050, representing a 112% increase compared to 2021. Population aging has been identified as the primary driver of this rapid rise, placing a significant burden on both patients and society ^[3]. Although pharmacological treatments, such as levodopa and dopamine receptor agonists, are

*Corresponding author
Jianshe Wei, MD, PhD, jswei@henu.edu.cn

Received 23 October 2025
Received in revised form 29 November 2025
Accepted 14 January 2026

currently available, these interventions primarily provide symptomatic relief and are insufficient to halt disease progression ^[4]. Consequently, exploring novel therapeutic strategies, particularly those targeting cellular and molecular mechanisms, has become a central focus of research.

Mitochondria, the cell's energy powerhouse, are responsible for generating adenosine triphosphate (ATP) through oxidative phosphorylation, thereby providing the energy required for normal cellular physiological activities ^[5]. In dopaminergic neurons of the SN in PD patients, the activity of mitochondrial respiratory chain complexes is significantly reduced, leading to a decrease in ATP production and an imbalance in energy metabolism ^[6]. Additionally, mitochondrial dysfunction can result in the excessive generation of reactive oxygen species (ROS), causing oxidative stress damage. This, in turn, disrupts the structure and function of biomacromolecules such as proteins, lipids, and nucleic acids, ultimately inducing neuronal apoptosis ^[7,8]. Mitochondrial homeostasis is essential for maintaining neuronal health and function, particularly in dopaminergic neurons ^[9]. This homeostasis depends on three interconnected core processes, including the dynamic balance between mitochondrial fission and fusion, the clearance of damaged mitochondria through mitophagy, and the maintenance of proper energy metabolism and redox balance ^[10,11]. In PD, this tightly regulated network becomes disrupted. An imbalance marked by excessive mitochondrial fission and insufficient fusion results in fragmentation of the mitochondrial network ^[12]. Simultaneously, mitophagy—a key mitochondrial quality-control mechanism—is impaired, leading to the accumulation of dysfunctional mitochondria ^[13]. The combined dysregulation of fission–fusion dynamics and autophagic flux exacerbates mitochondrial dysfunction, as evidenced by reduced energy production and increased oxidative stress, thereby creating a vicious cycle that ultimately drives dopaminergic neuron degeneration ^[14].

Exercise, encompassing various forms of physical activity such as aerobic training, resistance training, and flexibility exercises, has been widely demonstrated to exert beneficial preventive and adjunctive therapeutic effects on a range of chronic diseases ^[15]. In the field of neurological disorders, an increasing body of research has shown that exercise can improve motor function and enhance the quality of life in patients with PD ^[16–18]. However, its underlying mechanisms have not yet been fully elucidated. Studies suggest that exercise may exert neuroprotective effects by regulating mitochondrial biogenesis and improving mitochondrial dynamics ^[19,20]. Silent information regulator 1 (SIRT1), a nicotinamide adenine dinucleotide (NAD⁺)-dependent class III histone deacetylase, plays a pivotal role in regulating a wide range of biological processes, including cellular metabolism, aging, apoptosis, and inflammation ^[21,22]. Within the nervous system, SIRT1 is involved in essential physiological processes such as neuronal survival, differentiation, synaptic plasticity, and neurotransmitter release ^[23,24]. In PD models, the expression and activity of SIRT1 are reduced, while SIRT1 overexpression can attenuate dopaminergic neuronal damage and ameliorate PD symptoms ^[25]. Collectively, although exercise exerts neuroprotective effects and SIRT1 agonists like resveratrol show therapeutic promise, critical gaps remain. These include: (i) whether the benefits of exercise are mediated by circulating factors that converge on SIRT1; and (ii) the precise downstream signaling pathways through which SIRT1 restores mitochondrial dynamics in PD.

Based on the background outlined above, the present study aims to explore the potential mechanisms by which exercise ameliorates PD through stabilizing mitochondrial homeostasis via the SIRT1 pathway. This investigation holds both significant theoretical implications and clinical value. Theoretically, it is expected to uncover a novel mechanism underlying the therapeutic effects of exercise in PD, thereby enriching our understanding of PD pathogenesis and neuroprotective strategies. This could provide a theoretical foundation for the development of new therapeutic targets and pharmacological interventions. Clinically, the findings may offer scientific guidance for the rehabilitation of PD patients, facilitate the design of individualized exercise-based interventions, enhance therapeutic efficacy, improve patients' quality of life, and ultimately reduce the burden on families and society.

2. Materials and methods

2.1 Animals

Adult male C57BL/6 mice (6-8 weeks old, weighing 25-30 g) were purchased from the Huaxing Experimental Animal Breeding Farm in Zhengzhou (Henan, China). The animals were housed in independently ventilated cages at the Brain Science Laboratory of the School of Life Sciences, Henan University. Mice were kept in groups of 3-4 per cage under controlled conditions with an ambient temperature of 21-25°C, relative humidity of 40%-70%, and a 12-hour light/dark cycle. They had ad libitum access to food and water. This experiment was conducted in strict accordance with ethical guidelines for the use of experimental animals and was approved by the Animal Ethics Committee of Henan University (approval number: HUSOM2023-285). C57BL/6 mice were randomly assigned to six groups: the Control group, MPTP-induced group (MPTP), MPTP-induced plus aerobic exercise group (MPTP-Ex), MPTP-induced plus SIRT1 inhibitor EX527 group (MPTP-EX527), MPTP-induced plus aerobic exercise plus SIRT1 inhibitor EX527 group (MPTP-Ex-EX527), and MPTP-induced plus SIRT1 activator resveratrol group (MPTP-RES).

2.2 Exercise protocol and drug administration

To induce PD pathology in mice, we administered MPTP (AbMole, M9049). Except for the Control group, mice in the other groups received daily intraperitoneal injections of 30 mg/kg MPTP for 14 consecutive days, following the protocol established in previous studies ^[26]. Mice in the Control group were administered the same regimen of intraperitoneal injections, but with physiological saline instead of MPTP. For the MPTP-EX527 and MPTP-Ex-EX527 groups, EX527 solution (Aladdin, E129892) was intraperitoneally injected at a dose of 10 mg/kg, starting immediately after the final MPTP injection and continuing for 14 consecutive days ^[27]. For the MPTP-RES group, resveratrol (Aladdin, R107315) was administered by oral gavage at a dose of 50 mg/kg once daily, starting immediately after the final MPTP injection and continuing for 21 consecutive days ^[28].

Mice in the MPTP-Ex and MPTP-Ex-EX527 groups underwent an 8-week aerobic treadmill training regimen, running 5 days per week. In the first week, the mice went through an adaptation phase, with running speeds gradually increasing from 6 to 12 meters per minute, and running times ranging from 10 to 60 minutes.

In the subsequent weeks, the mice ran at a speed of 12 meters per minute for 60 minutes during each training session. Throughout the experiment, the mice were monitored for motor abilities, activity levels, and other behavioral characteristics, as well as for changes in eating, drinking, and fur condition. The exercise protocol was adapted from that used by Schefer et al. ^[29], with modifications based on our research group's preliminary experiments. The goal was to maintain moderate-intensity aerobic exercise, equivalent to 65%-70% of the maximum VO₂.

2.3 Behavioral Analysis

This study conducted a comprehensive behavioral analysis to systematically assess the core pathological features of PD in mice, including both motor and non-motor symptoms. Motor function was assessed using the gait test (to evaluate bradykinesia and gait abnormalities) and the rotarod test (to assess balance and coordination). Non-motor symptoms were evaluated through the open field test (to assess spontaneous activity and anxiety-like behaviors), the elevated plus maze (specifically for anxiety-like behaviors), and the tail suspension test (to assess depressive-like behaviors).

2.3.1 Gait test

The forelimbs and hindlimbs of the mice were specifically marked, with the forelimb on the same side marked using red non-toxic ink and the hindlimb on the same side marked with blue non-toxic ink. The mice were placed in a narrow gait testing corridor (150 × 7 cm) and allowed to move freely from one end to the other. At the end of the test, a consistent gait position was selected, and the distance between the paw prints was measured using a ruler. To minimize potential measurement bias, all gait assessments were performed by an experimenter blinded to group allocation, and multiple consecutive stride lengths were measured for each mouse and averaged for statistical analysis.

2.3.2 Rotarod test

The rotarod test consists of two components: the rotarod apparatus and an automatic data acquisition and processing system. After setting the parameters and starting the motor, the animal is required to perform motor activity due to the rotation of the rod. The initial speed of the rotarod is set to 4 rpm, with a maximum speed of 40 rpm. The speed increases by 1 rpm every 8 seconds. The duration of each test is 300 seconds. Prior to testing, mice undergo an adaptation training phase. After completing the test, the data are saved for statistical analysis.

2.3.3 Open field test

The arena is a white square box with dimensions of 50 cm × 50 cm × 40 cm. Mice are gently placed at the center of the arena and allowed to explore freely for 5 minutes under illuminated conditions. Prior to each trial, the arena is cleaned with ethanol. After placing the animals in the arena, the experimenter leaves the room. All trials are recorded from a top view using video, and the data are analyzed using Labmaze V3.0 tracking software (Beijing Zhongshi Diche). The total distance traveled and the proportion of time spent in the center area are collected to assess the anxiety levels of the mice.

2.3.4 Tail suspension test

The tail suspension test consists of two components: the tail suspension apparatus and an automatic data acquisition and processing system. A string is tied around the distal 2/3 of the mouse's tail, and the mouse is suspended from the hook of the tail suspension device with its head facing downward, 20 cm above the surface. Behavioral video recordings are captured for 5 minutes using SMART V3.0 behavioral video analysis software. Prior to each test, the internal environment of the apparatus is cleaned. After the test, the data are saved for statistical analysis.

2.3.5 Elevated plus maze test

The tested mice are placed in the central area of the elevated plus maze apparatus. The number of times the mouse enters the open and closed arms of the maze is recorded. Before placing the next mouse, the maze is cleaned with 75% ethanol to maintain a clean internal environment and prevent contamination from feces or urine left by the previous mouse. Statistical analysis is then conducted on the number of entries into the open arms of the maze.

2.4 Cell Culture

MN9D cells were cultured in DMEM (Servicebio, G4520), which contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, under standard culture conditions at 37°C with 5% CO₂ and saturated humidity. After changing the culture medium, cells from all groups except the Control group were treated with the following interventions: MPP⁺ (AbMole, M10041) at a final concentration of 1 mM, exercise/sedentary serum, EX527, resveratrol (Aladdin, R107314), Foxo3a inhibitor AS1842856 (AbMole, M9069), and Drp1 activator Mdivi-1 (Aladdin, M407882). The cells were divided into the following groups: MN9D normal control group (Control), MPP⁺ group (MPP⁺), MPP⁺ plus sedentary serum group (MPP⁺-RstSum), MPP⁺ plus exercise serum group (MPP⁺-RnSum), MPP⁺ plus exercise serum plus EX527 group (MPP⁺-RnSum-EX527), MPP⁺ plus resveratrol group (MPP⁺-RES), MPP⁺ plus resveratrol plus AS1842856 group (MPP⁺-RES-AS), MPP⁺ plus resveratrol plus AS1842856 plus Mdivi-1 group (MPP⁺-RES-AS-Mdivi-1)

2.5 Serum preparation and treatment

Mice in the MPTP and MPTP-Ex groups were euthanized to collect blood. Blood was drawn from the right ventricle and allowed to clot at room temperature for approximately 30 minutes. The clotted blood was then centrifuged at 5000g for 20 minutes. The serum layer was carefully extracted using a pipette, filtered through a 0.22 µm sterile syringe filter, and stored at -80°C.

The MPP⁺ group was treated with MPP⁺ at a final concentration of 1 mM for 24 hours. The MPP⁺-RstSum group was treated with a mixture of 10% sedentary serum, 1% penicillin-streptomycin, and DMEM, along with MPP⁺ for 24 hours. The MPP⁺-RnSum group was treated with a mixture of 10% exercise serum, 1% penicillin-streptomycin, and DMEM, along with MPP⁺ for 24 hours (the 10% serum concentration was supported by a pilot MTT assay; Fig. S1, and was selected as it is a commonly used serum proportion in cell culture and has been widely adopted in previous exercise-conditioned serum incubation studies) [30].

The MPP⁺-RnSum-EX527 group was pretreated with 10 μ M EX527 for 3 hours before treatment with a mixture of 10% exercise serum, 1% penicillin-streptomycin, DMEM, and MPP⁺ for 24 hours [31]. The MPP⁺-RES group was treated with a medium containing 10 μ M resveratrol and 1 mM MPP⁺ for 24 hours [32]. The MPP⁺-RES-AS group was pretreated with 1 μ M AS1842856 for 1 hour before treatment with a medium containing resveratrol and MPP⁺ [33]. The MPP⁺-RES-AS-Mdivi-1 group was pretreated with 1 μ M AS1842856 and 5 μ M Mdivi-1 for 1 hour before treatment with a medium containing resveratrol and MPP⁺ [34].

2.6 Nissl staining

After deep anesthesia, all mice were subjected to left ventricular perfusion with physiological saline to remove blood, followed by fixation with pre-cooled 4% paraformaldehyde. The brain tissue was carefully extracted, post-fixed in 4% paraformaldehyde, and then embedded in paraffin. Continuous coronal sections were prepared using a paraffin embedding machine. To evaluate neuronal integrity in the SN, Nissl staining was performed. Paraffin sections were dewaxed, rehydrated, and incubated with Nissl staining solution (Servicebio, G1036) for 5 minutes. The sections were then rinsed in water until no residual stain remained, dried in a 65°C oven, dehydrated, cleared, and mounted with neutral gum. Neurons in the SN were examined under an optical microscope, and positive cell counts were quantified using ImageJ software.

2.7 Immunohistochemical staining (IHC)

IHC was performed to detect the expression of tyrosine hydroxylase (TH) in the SN. Paraffin sections were subjected to antigen retrieval, endogenous peroxidase blocking, and blocking procedures. TH antibody (Servicebio, GB11181, 1:1000) was applied and incubated overnight at 4°C. HRP-conjugated secondary antibody (Servicebio, GB23303, 1:1000) was applied and incubated at room temperature for 1 hour. The staining was developed using the DAB chromogenic kit (Servicebio, G1212), followed by dehydration, clearing, and neutral gum mounting. Observations under the microscope revealed brown-yellow granules, indicating TH-positive neurons.

2.8 Immunofluorescence (IF)

IF was performed to detect the co-localization of SIRT1 and TOM20 in the SN. Paraffin sections were subjected to antigen retrieval, blocking, and incubation with primary antibodies: SIRT1 (MCE, HY-P80319, 1:200) and TOM20 (Proteintech, 66777-1-Ig, 1:500), followed by overnight incubation at 4°C. The sections were then incubated with a mixed fluorescent secondary antibody (Servicebio, GB25303, GB25301, 1:500) for 1 hour at room temperature, protected from light. After PBS washing, DAPI was applied for 5 minutes to counterstain cell nuclei. Sections were then washed again with PBS and mounted with an anti-fluorescence quenching medium. Fluorescent signals were visualized under a microscope: red (SIRT1), green (TOM20), and blue (DAPI). Signals from different laser channels were merged to generate overlay images. Colocalization analysis of SIRT1 and TOM20 was performed using ImageJ software with the Coloc 2 plugin.

Background subtraction was applied using a rolling ball radius of 50 pixels. Pearson's correlation coefficient was calculated for each region of interest for statistical analysis.

Cells grown on coverslips were fixed with 4% paraformaldehyde at room temperature for 15 minutes, followed by permeabilization and blocking at room temperature. The sections were then incubated with primary antibody against TOM20 overnight at 4°C. After rinsing with PBS, the samples were incubated with the corresponding fluorescent secondary antibody for 1 hour at room temperature in the dark. DAPI solution was applied to counterstain the nuclei, and the coverslips were mounted with an anti-fade mounting medium.

2.9 Subcellular fractionation

Mitochondrial and cytosolic fractions were isolated from mouse brain tissues using a commercial tissue mitochondrial isolation kit (Beyotime, C3606) according to the manufacturer's instructions. Briefly, freshly dissected tissues were homogenized in ice-cold isolation buffer and subjected to differential centrifugation to separate mitochondrial pellets from cytosolic supernatants. The mitochondrial pellets and cytosolic fractions were collected for subsequent protein extraction and Western Blot analysis. TOM20 and GAPDH were used as markers for mitochondrial and cytosolic fractions, respectively.

2.10 Assessment of mitochondrial function

2.10.1 Mitochondrial membrane potential measurement (JC-1 staining)

Mitochondrial membrane potential in isolated tissue mitochondria: Mitochondrial membrane potential was assessed using the fluorescent probe JC-1 (Servicebio, G1515). Mitochondria were isolated from the mouse SN as described for subcellular fractionation (Section 2.9). The mitochondrial pellet was resuspended in assay buffer and incubated with JC-1 at 37°C in the dark for 30 minutes. After incubation, samples were washed twice with assay buffer to remove unincorporated dye. Fluorescence was measured under a fluorescence microscope. JC-1 forms red fluorescent aggregates (Ex/Em: 585/590 nm) in polarized mitochondria and green fluorescent monomers (Ex/Em: 514/529 nm) upon depolarization. Mitochondrial membrane potential was expressed as the ratio of red to green fluorescence intensity, with a decreased ratio indicating mitochondrial depolarization.

Mitochondrial membrane potential in cultured cells: Cells were seeded in black-walled, clear-bottom 24-well plates and incubated with JC-1 in serum-free medium at 37 °C for 30 minutes, protected from light. Cells were then gently washed twice with pre-warmed PBS. Cells were imaged under a fluorescence microscope. The red/green fluorescence intensity ratio from multiple random fields was calculated using ImageJ software to quantify mitochondrial membrane potential.

2.10.2 ATP quantification

ATP levels were quantified using a colorimetric assay kit (Elabscience, E-BC-K157-M). Briefly, tissue (SN) or cell (MN9D) samples were homogenized/lysed in the kit's extracting solution, heat-denatured, and centrifuged. The supernatant was incubated with the reaction working solution at 37 °C for 30 minutes. After

protein precipitation and centrifugation, the supernatant was mixed with chromogenic reagents, and absorbance was measured at 636 nm.

2.10.3 Intracellular ROS measurement

Intracellular ROS levels were detected using the fluorescent probe 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) (Servicebio, G1706). Treated MN9D cells, seeded in black-walled 96-well plates, were incubated with DCFH-DA diluted in serum-free medium at 37°C for 30 minutes in the dark. Subsequently, cells were washed three times with warm PBS to remove extracellular probe. The labeled cells in the well plates were directly transferred to a fluorescence microscope for observation.

2.11 Transmission Electron Microscopy (TEM)

Tissue from the SN of mice was rapidly extracted and cut into 1 mm³ pieces, which were then fixed overnight in electron microscopy fixative (Servicebio, G1102) at 4°C. After rinsing with PBS, the samples were fixed in 1% osmium tetroxide for 2 hours. The tissues were subsequently dehydrated through a graded ethanol series, replaced with acetone, infiltrated with epoxy resin, and polymerized. Ultrathin sections (60–80 nm) were prepared, stained with uranyl acetate and lead citrate for double electron staining, and examined under a transmission electron microscope. Images of the mitochondrial ultrastructure in the SN were captured.

2.12 Western Blot (WB)

After anesthetizing the mice, the SN from each group was harvested. Protein samples were extracted using RIPA lysis buffer (Servicebio, G2002) containing PMSF, and the supernatant was mixed with sample loading buffer (Servicebio, G2075) and boiled at 95°C. The proteins were separated by electrophoresis on a gel, transferred to a PVDF membrane under constant current, and blocked for 1 hour at 37°C. Primary antibodies used included anti-TH (Servicebio, GB11181, 1:1000), anti- α -synuclein (α -syn) (Servicebio, GB11773, 1:1000), anti-DAT (Servicebio, GB111215, 1:1000), anti-SIRT1 (MCE, HY-P80319, 1:200), anti-Mfn1 (Proteintech, 13798-1-AP, 1:8000), anti-Mfn2 (Proteintech, 12186-1-AP, 1:8000), anti-Opa1 (Proteintech, 27733-1-AP, 1:8000), anti-Mff (Proteintech, 17090-1-AP, 1:10000), anti-Fis1 (Proteintech, 10956-1-AP, 1:8000), anti-Drp1 (Proteintech, 12957-1-AP, 1:8000), anti-Foxo3a (Servicebio, GB11092, 1:1000), anti-Pink1 (Affinity, DF7742, 1:2000), anti-Parkin (Affinity, AF0235, 1:2000), anti-p62 (Servicebio, GB11239, 1:1500), anti-LC3B (Affinity, AF4650, 1:2000), anti-GAPDH (Servicebio, GB11002, 1:2000), anti-TOM20 (Servicebio, GB151481, 1:2000) and anti- β -Tubulin (Affinity, T0023, 1:2000). The membrane was then incubated with HRP-conjugated secondary antibody (Servicebio, GB23303, 1:3000) for 1 hour. Immunoblotting was performed using an ECL reagent kit, and the data were analyzed using ImageJ software.

2.13 Proteomic analysis

Proteins were extracted from the SN tissues of Control, MPTP, and MPTP-RES mice using RIPA buffer supplemented with protease inhibitors. Protein concentrations were determined using a BCA assay, after which 100 μ g of protein from each sample was reduced, alkylated, and digested overnight with trypsin following SP3 cleanup. The resulting peptides were analyzed by nano-LC-MS/MS using an Orbitrap Astral

mass spectrometer operated in data-independent acquisition mode. Differentially expressed proteins (DEPs) were identified and visualized using a volcano plot, with thresholds set at an absolute fold change ≥ 1.5 and adjusted P value of < 0.05 . Functional enrichment analyses of the DEPs were subsequently performed using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases.

2.14 Statistical analysis

Statistical analyses were performed using GraphPad Prism software (GraphPad Software, USA). Data are presented as mean $\pm$ standard deviation (SD). The Shapiro-Wilk test was used to assess the normality of data distribution within each group. Variance homogeneity for unpaired t-tests between two groups was evaluated using the F-test. For comparisons among three or more groups, variance homogeneity was assessed using the Brown–Forsythe test, followed by one-way analysis of variance (ANOVA) and Tukey's post hoc test for multiple comparisons. Statistical significance was defined as $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

3. Results

3.1 Exercise improves behavioral performance and dopaminergic neuronal pathology in PD mice

To investigate the effects of exercise on both motor and non-motor functions in PD mice, we established an MPTP-induced mouse model and subjected the animals to 8 weeks of moderate-intensity treadmill exercise (Fig. 1A). MPTP-induced mice exhibited significant motor impairments, including reduced stride length and shorter latency to fall in the rotarod test. Exercise intervention markedly improved both stride length and rotarod performance compared with the sedentary MPTP group (Fig. 1B–C). Exercise also exerted beneficial effects on depressive- and anxiety-like behaviors. In the tail suspension test, the exercise group demonstrated significantly reduced immobility time relative to the MPTP group, suggesting an attenuation of depressive-like behavior (Fig. 1D). In the open field test, exercised mice spent more time in and made more frequent entries into the central area, indicating reduced anxiety-like behavior (Fig. 1E–G). Similarly, in the elevated plus maze, exercise-treated mice entered the open arms more frequently, further supporting the anxiolytic effects of exercise (Fig. 1H–I).

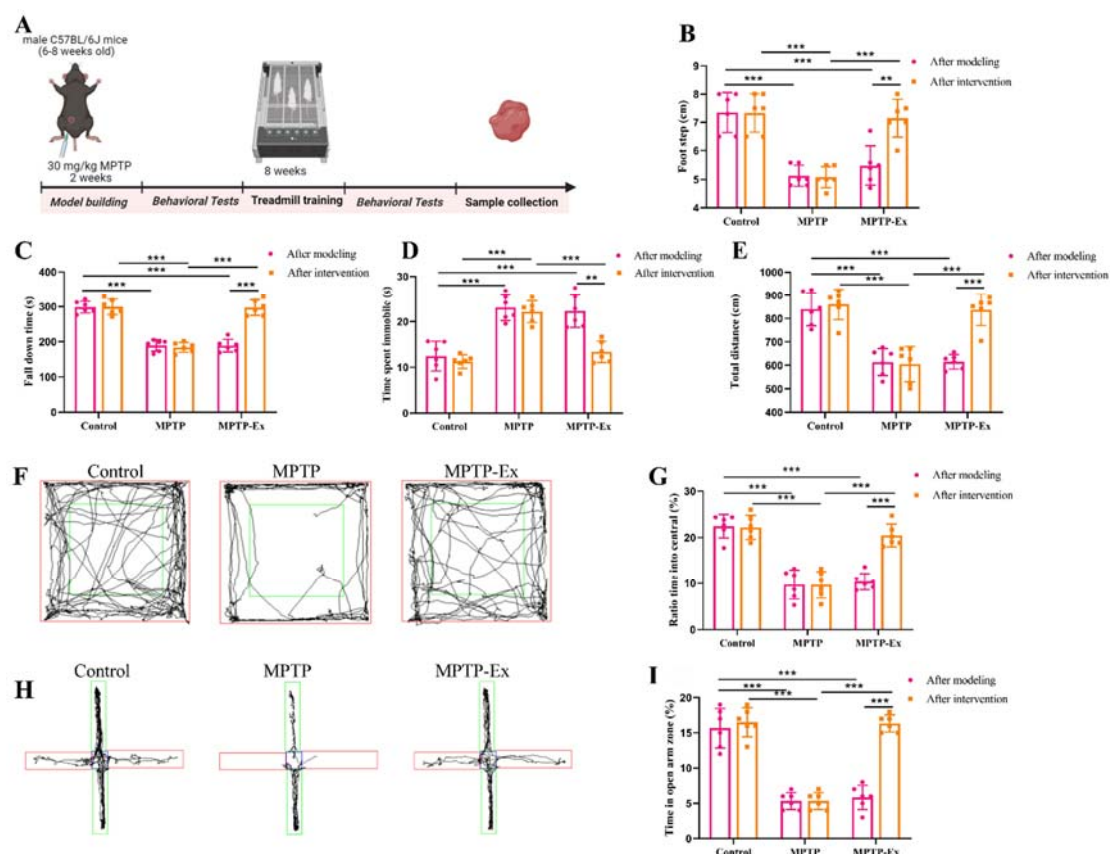


Figure 1. Exercise improves motor and non-motor dysfunction in MPTP-induced PD mice. (A) Schematic diagram of the experimental protocol. (B) Stride distance of mice. (C) Time spent on the rotarod by mice. (D) Total immobility time of mice in the tail suspension test. (E) Total distance traveled by mice in the open field test. (F) Representative movement track of mice in the open field. (G) Percentage of times mice entered the central area of the open field. (H) Representative movement track of mice in the elevated plus maze. (I) Quantitative analysis of the percentage of time spent in the open arms of the elevated plus maze by mice in each group. $n = 6$, $**P < 0.01$, $***P < 0.001$.

Nissl staining revealed a significant reduction in the number of neurons in the SN of MPTP-induced mice, consistent with neuronal degeneration. The remaining neurons exhibited pathological features such as cell body shrinkage, nuclear condensation, and loss of Nissl bodies, which are indicative of neuronal damage. In contrast, neurons in the MPTP-Ex group exhibited significantly less degeneration, with their morphology remaining relatively intact, suggesting exercise provides neuroprotective effects against PD-related neuronal degeneration (Fig. 2A–B). IHC for TH, a key marker for dopaminergic neurons, revealed a significant decrease in the number of TH-positive neurons in the SN of MPTP mice, indicating dopaminergic neuronal damage. However, the number of TH-positive neurons was significantly higher in the MPTP-Ex group compared to the MPTP group, suggesting that exercise helps maintain the survival and function of dopaminergic neurons (Fig. 2C–D). WB analysis further confirmed these findings, showing that TH protein expression in the SN of MPTP mice was significantly downregulated, while exercise intervention restored TH expression (Fig. 2E–F). Additionally, α -syn, a pathological protein involved in PD, exhibited significant aggregation in the SN of MPTP mice, with expression levels markedly higher than those in the Control group. Exercise significantly reduced the abnormal expression of α -syn, suggesting that exercise may exert neuroprotective effects by alleviating pathological protein aggregation (Fig. 2E, G). Furthermore, the expression level of the dopamine transporter (DAT)—a protein responsible for the reuptake of dopamine from

the synaptic cleft into dopaminergic neurons—was significantly reduced in MPTP mice. In contrast, DAT expression was notably increased in the MPTP-Ex group, indicating that exercise contributes to the restoration of dopamine reuptake capacity, which is essential for proper dopaminergic signaling and neuronal health (Fig. 2E, H).

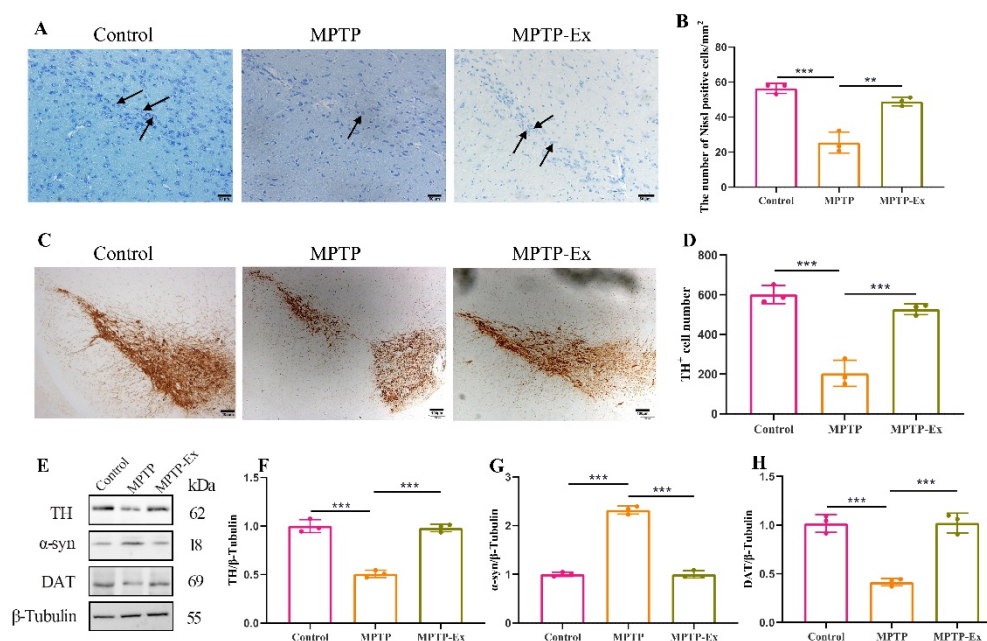


Figure 2. Exercise mitigates dopaminergic neuronal damage and pathological protein aggregation in the SN of PD mice. (A) Representative images of Nissl-stained sections of the SN from Control, MPTP, and MPTP-Ex mice. Nissl staining highlights the morphology of neurons. Arrows indicate normal, intact neurons. Scale bar: 50 μ m. (B) Quantitative analysis of the number of surviving neurons in the SN across groups. (C) Representative images of TH IHC in the SN, illustrating dopaminergic neuron integrity across groups. Scale bar: 100 μ m. (D) Quantitative analysis of TH-positive neurons in the SN across groups. (E) Representative WB bands showing the expression of TH, α -syn, and DAT proteins in SN tissue. (F–H) Quantitative analysis of TH (F), α -syn (G), and DAT (H) protein expression levels across groups. $n = 3$, ** $P < 0.01$, *** $P < 0.001$.

3.2 Exercise restores SIRT1 expression, enhances its association with mitochondria, and improves mitochondrial functional integrity in the SN of PD

To elucidate the mechanisms underlying the neuroprotective effects of exercise in PD and the involvement of SIRT1, we first assessed SIRT1 protein expression in the SN using WB analysis. The results demonstrated that SIRT1 expression was significantly reduced in MPTP-induced PD mice, whereas exercise intervention markedly restored SIRT1 protein levels (Fig. 3A–B). Given the central role of mitochondria in PD pathogenesis, we next investigated whether exercise influences the association of SIRT1 with mitochondria. Double IF staining was performed to evaluate the spatial relationship between SIRT1 and the mitochondrial marker TOM20. In MPTP-induced PD mice, colocalization of SIRT1 with TOM20 was significantly decreased, indicating a reduced association of SIRT1 with mitochondria. In contrast, exercise intervention significantly enhanced SIRT1–TOM20 colocalization in the SN compared with the MPTP group (Fig. 3C–D).

Given that alterations in SIRT1–TOM20 colocalization may be influenced by changes in mitochondrial abundance, we complemented the IF analysis with biochemical and functional assessments. Subcellular fractionation was performed to isolate mitochondrial and cytosolic fractions from SN tissue. WB analysis

revealed that, under basal conditions, SIRT1 was comparably distributed between the mitochondrial and cytoplasmic fractions in the Control group. Following MPTP administration, SIRT1 levels were significantly reduced in both fractions. Notably, exercise intervention markedly restored SIRT1 expression in the mitochondrial fraction, whereas SIRT1 levels in the cytoplasmic fraction were only partially recovered (Fig. 3E–F). The purity of the mitochondrial and cytoplasmic fractions was validated using TOM20 and GAPDH as specific markers, respectively (Fig. S2). The degree of cytosolic contamination in the mitochondrial fractions, assessed by the GAPDH-mito/GAPDH-cyto ratio, remained consistently low across all experimental groups, confirming the technical rigor and reproducibility of the isolation protocol (Fig. S2A). Notably, a significant MPTP-induced increase in the mitochondrial outer membrane protein TOM20 was detected within the cytosolic fraction. This increase was not attributable to an isolation artifact but instead reflects pathological mitochondrial damage and the release of outer membrane components. Exercise intervention significantly attenuated this aberrant localization of TOM20 (Fig. S2B), providing direct biochemical evidence supporting the exercise-mediated preservation of mitochondrial structural integrity.

To further determine whether the altered association of SIRT1 with mitochondria was accompanied by changes in mitochondrial functional status, we measured ATP levels in SN tissue and assessed mitochondrial membrane potential in isolated SN-derived mitochondria. MPTP administration resulted in a significant reduction in ATP levels in SN tissue, indicating impaired cellular energy homeostasis (Fig. 3G). In parallel, JC-1 staining of isolated mitochondria revealed a pronounced decrease in the red/green fluorescence ratio in the MPTP group, characterized by a loss of red fluorescence from JC-1 aggregates and a concomitant increase in green fluorescence from JC-1 monomers, indicative of substantial mitochondrial membrane depolarization (Fig. 3H–I). In contrast, exercise intervention significantly restored ATP levels in SN tissue and increased the JC-1 red/green fluorescence ratio compared with MPTP mice, reflecting recovery of mitochondrial membrane potential (Fig. 3G–I). Notably, these improvements in cellular energy status and mitochondrial membrane potential coincided with the preferential restoration of mitochondria-associated SIRT1, supporting the interpretation that changes in SIRT1–TOM20 colocalization reflect alterations in mitochondrial functional status rather than differences in mitochondrial abundance. Collectively, these findings demonstrate that exercise not only restores overall SIRT1 expression in the SN but also reinforces its mitochondrial association, accompanied by marked improvements in mitochondrial function and energy-related parameters in PD mice.

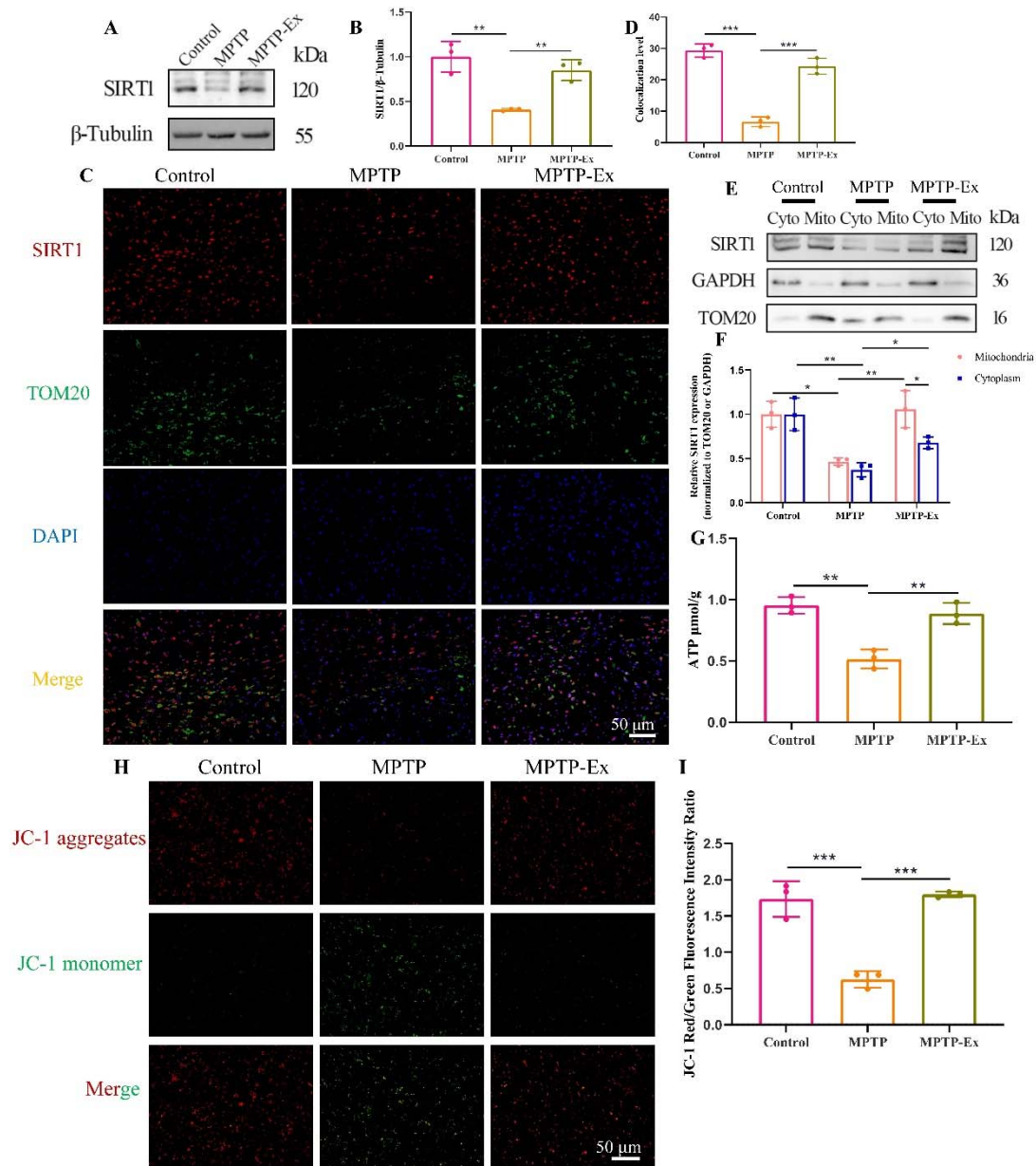


Figure 3. Exercise restores SIRT1 expression, enhances its association with mitochondria, and improves mitochondrial functional integrity in the SN of PD mice. (A) Representative WB images showing SIRT1 protein expression in SN tissue from Control, MPTP, and MPTP-Ex mice. (B) Quantitative analysis of SIRT1 protein levels normalized to β -Tubulin. (C) Representative double IF images showing SIRT1 (red) and the mitochondrial marker TOM20 (green) in the SN. Nuclei were counterstained with DAPI (blue). TOM20 is an outer mitochondrial membrane protein commonly used as a mitochondrial marker. (D) Quantification of SIRT1–TOM20 colocalization, expressed as Pearson's correlation coefficient, reflecting the spatial association between SIRT1 and mitochondria. (E) Representative WB images of SIRT1 in mitochondrial and cytosolic fractions isolated from SN tissue. (F) Quantification of SIRT1 levels in mitochondrial and cytosolic fractions. SIRT1 levels in the mitochondrial fraction were normalized to TOM20, whereas those in the cytosolic fraction were normalized to GAPDH. TOM20 and GAPDH were used as specific markers to verify the purity of mitochondrial and cytosolic fractions, respectively. (G) ATP levels measured in SN tissue homogenates, reflecting cellular energy status. (H) Representative JC-1 fluorescence images of isolated SN-derived mitochondria. JC-1 is a membrane potential-sensitive dye that forms red fluorescent aggregates in polarized mitochondria and green fluorescent monomers in depolarized mitochondria. (I) Quantification of mitochondrial membrane potential, expressed as the ratio of red to green JC-1 fluorescence intensity, reflecting mitochondrial membrane polarization. Scale bar: 50 μ m. $n = 3$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

3.3 SIRT1 inhibition abolishes the neuroprotective and mitochondria-stabilizing effects of exercise in PD mice

To determine whether SIRT1 is required for the neuroprotective effects of exercise in PD mice, we employed the selective SIRT1 inhibitor EX527 (Fig. 4A). Compared with MPTP mice, pharmacological

inhibition of SIRT1 significantly exacerbated MPTP-induced motor and non-motor deficits, indicating that SIRT1 plays a critical role in maintaining behavioral function under PD conditions (Fig. S3). As expected, exercise intervention markedly improved behavioral performance in MPTP-induced mice. Importantly, co-administration of EX527 completely abolished these exercise-mediated behavioral improvements, demonstrating that SIRT1 is necessary for the beneficial effects of exercise (Fig. S3).

Consistent with the behavioral findings, IHC and WB analyses revealed that pharmacological inhibition of SIRT1 markedly exacerbated dopaminergic neurodegeneration. Mice treated with MPTP in combination with EX527 exhibited a pronounced reduction in the dopaminergic marker TH, accompanied by a significant decrease in the number of TH-positive neurons in the SN (Fig. 4D–I). Nissl staining further confirmed that neuronal loss in the SN was aggravated following SIRT1 inhibition (Fig. 4B–C). Exercise intervention significantly attenuated MPTP-induced dopaminergic neuronal loss. However, this neuroprotective effect was abolished by EX527 treatment, as evidenced by the loss of exercise-mediated improvements in TH expression and neuronal survival (Fig. 4B–I). Collectively, these findings indicate that SIRT1 is required for the neuroprotective effects of exercise in PD mice.

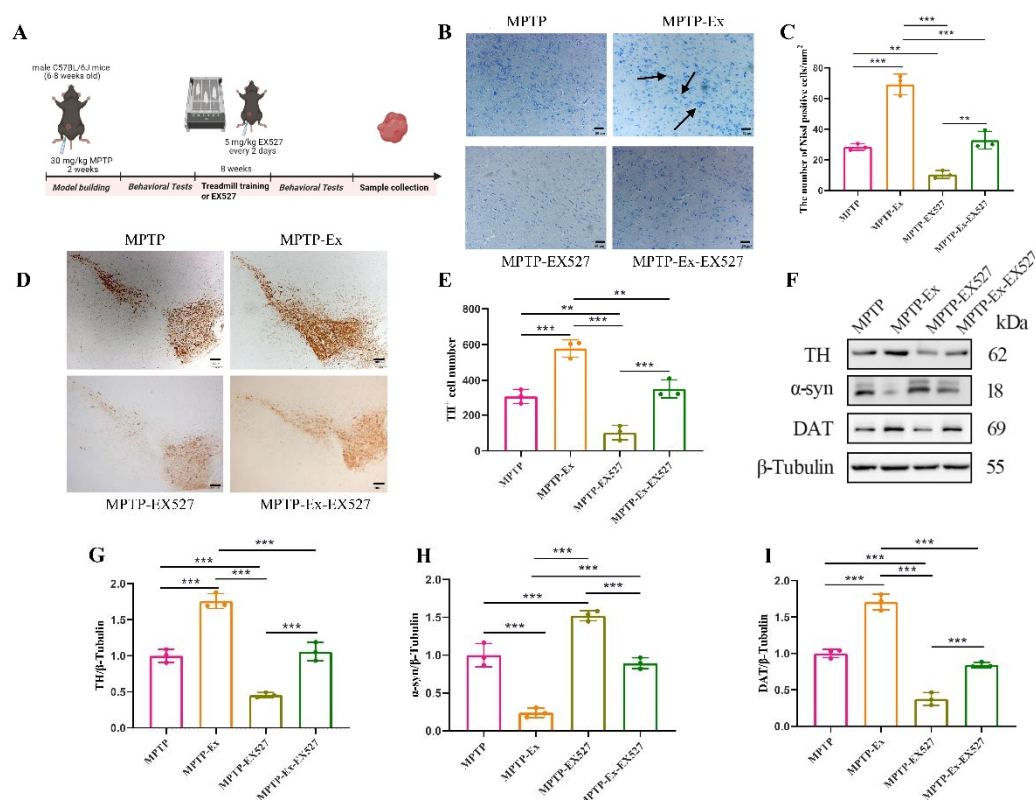


Figure 4. Pharmacological inhibition of SIRT1 abolishes exercise-mediated neuroprotection against MPTP-induced dopaminergic neurodegeneration in the SN. (A) Schematic illustration of the experimental design. Mice were randomly assigned to the MPTP, MPTP-Ex, MPTP-EX527 and MPTP-Ex-EX527 groups. (B) Representative Nissl-stained coronal sections of the SN showing neuronal morphology in each group. Arrows indicate intact, morphologically normal neurons. Scale bar: 50 μ m. (C) Quantification of surviving Nissl-positive neurons in the SN across groups. (D) Representative IHC staining for TH in the SN. Scale bar: 100 μ m. (E) Quantitative analysis of TH-positive neurons in the SN across groups. (F) Representative WB images showing protein expression levels of TH, α -syn, and DAT in SN tissue. (G–I) Quantification of protein levels of TH (G), α -syn (H), and DAT (I), normalized to β -Tubulin. $n = 3$, $**P < 0.01$, $***P < 0.001$.

Next, we examined whether the exacerbated neuropathology induced by SIRT1 inhibition was associated with impaired mitochondrial homeostasis. TEM revealed severe mitochondrial ultrastructural damage in the

MPTP-EX527 group, characterized by pronounced mitochondrial swelling, vacuolization, and disruption of cristae architecture. In contrast, mitochondria in the MPTP-Ex group exhibited relatively preserved morphology, with reduced swelling and more intact cristae structure. Notably, following SIRT1 inhibition, the mitochondrial protective effects of exercise were abolished (Fig. 5A).

At the molecular level, we examined key proteins regulating mitochondrial dynamics, a process that preserves mitochondrial morphology and function through coordinated fission and fusion. Dynamin-related protein 1 (Drp1) is a cytosolic GTPase that mediates mitochondrial fission upon recruitment to the outer mitochondrial membrane. Fission protein 1 (Fis1) and mitochondrial fission factor (Mff) act as outer mitochondrial membrane adaptor proteins that facilitate the recruitment and activation of Drp1. In contrast, mitochondrial fusion is governed by mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), which mediate outer mitochondrial membrane fusion, and optic atrophy protein 1 (Opa1), which regulates inner mitochondrial membrane fusion and cristae organization. WB analysis revealed that MPTP administration induced a pronounced imbalance in mitochondrial dynamics, characterized by increased expression of the fission-related proteins Drp1, Fis1, and Mff and decreased expression of the fusion-related proteins Mfn1, Mfn2, and Opa1. This imbalance was most pronounced in the MPTP-EX527 group. Exercise intervention significantly reversed the MPTP-induced disruption of mitochondrial dynamics, as evidenced by downregulation of the fission-related proteins Drp1, Fis1, and Mff and upregulation of the fusion-related proteins Mfn1, Mfn2, and Opa1. In contrast, co-administration of EX527 abolished these exercise-mediated effects (Fig. 5B–J).

Because mitochondrial dynamics are tightly linked to mitochondrial quality control—particularly the selective elimination of damaged mitochondria—we further examined key mitophagy-related proteins, including Pink1, Parkin, p62, and LC3B. Pink1 and Parkin function as upstream sensors and mediators of mitochondrial damage, initiating selective mitophagy through ubiquitin-dependent signaling. p62 serves as an autophagic adaptor that recognizes ubiquitinated cargo, whereas LC3B, particularly its lipidated form LC3B-II, reflects autophagosome membrane formation and maturation. Together with mitochondrial dynamics-related proteins, these markers provide an integrated assessment of mitochondrial quality control, spanning damage recognition to mitochondrial clearance. Disruption of mitophagy signaling was most pronounced in the MPTP-EX527 group, as evidenced by aberrant expression of Pink1, Parkin, p62, and LC3B. Exercise intervention partially reversed the MPTP-induced impairment of mitophagy, as reflected by normalization of Pink1/Parkin signaling and p62 and LC3B levels, indicating improved mitochondrial quality control. In contrast, SIRT1 inhibition abolished these exercise-mediated effects, resulting in persistent dysregulation of mitophagy in the MPTP-Ex-EX527 group (Fig. 5B, L–O).

Given the central role of SIRT1 in mediating exercise-induced mitochondrial protection, we examined whether different interventions directly affected SIRT1 expression. WB analysis revealed that SIRT1 protein levels were significantly reduced in the MPTP group, whereas exercise intervention markedly restored SIRT1 expression in MPTP mice. In contrast, pharmacological inhibition of SIRT1 with EX527 resulted in a

pronounced suppression of SIRT1 protein levels in both the MPTP-EX527 and MPTP-Ex-EX527 groups (Fig. 5C–D). Collectively, these results identify SIRT1 as a primary molecular target modulated by MPTP toxicity and exercise intervention, thereby providing a mechanistic basis for subsequent analyses of downstream signaling pathways.

To further investigate signaling mechanisms associated with exercise intervention, we examined the SIRT1–Foxo3a pathway. Foxo3a is a well-characterized downstream transcription factor of SIRT1 and has been implicated in the regulation of mitochondrial function and neuronal stress responses. WB analysis showed that pharmacological inhibition of SIRT1 with EX527 significantly reduced Foxo3a expression in MPTP mice. Exercise intervention significantly increased Foxo3a protein levels in the SN of MPTP mice; however, this exercise-mediated upregulation of Foxo3a was abolished by co-treatment with EX527 (Fig. 5B, K). Collectively, these findings indicate that Foxo3a expression is tightly regulated by SIRT1 under MPTP-induced neurotoxic conditions.

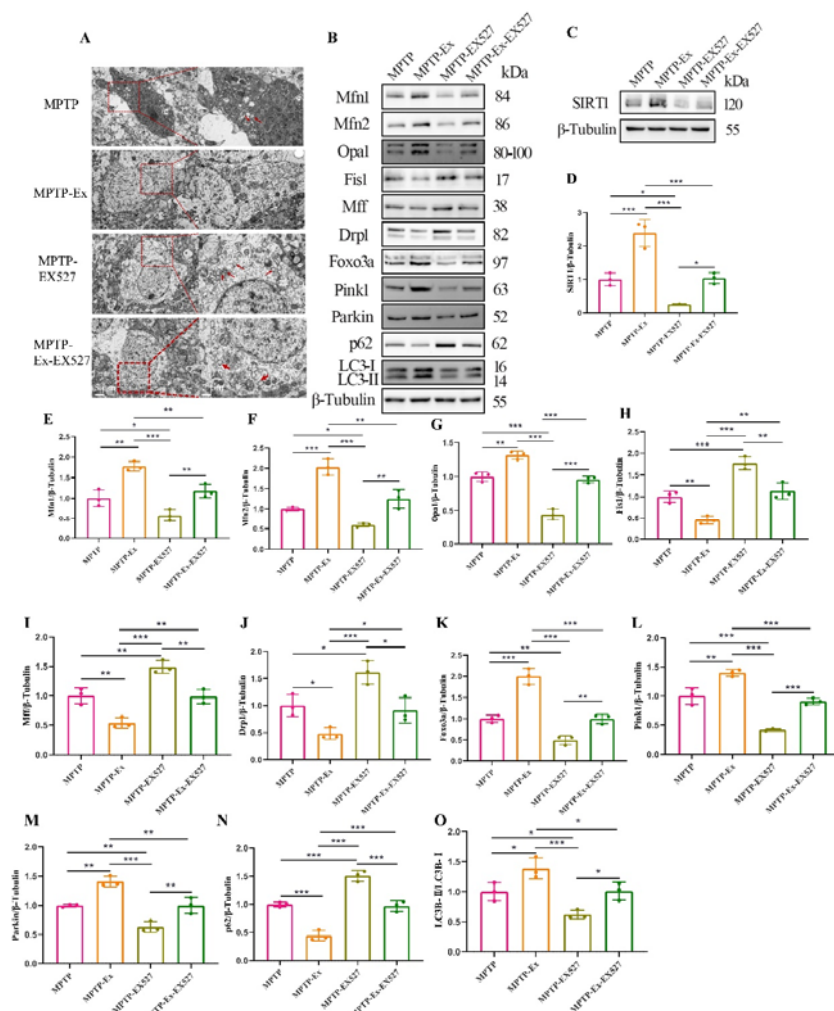


Figure 5. SIRT1 inhibition abolishes exercise-mediated improvements in mitochondrial dynamics, and mitophagy in the MPTP model. (A) Representative TEM images showing mitochondrial ultrastructure in the SN across groups. Red arrows indicate damaged mitochondria characterized by cristae disruption and vacuolization. Scale bars: 5 μm, and 2 μm. (B) Representative WB images showing the expression of mitochondrial dynamics-related proteins, including fission proteins (Drp1, Fis1, and Mff) and fusion proteins (Mfn1, Mfn2, and Opa1), as well as mitophagy-related proteins (Pink1, Parkin, p62, and LC3B), and Foxo3a. (C) Representative WB images of SIRT1. (D) Quantitative analysis of SIRT1 protein expression across groups. (E–O) Quantification of protein levels of Mfn1 (E), Mfn2 (F), Opa1 (G), Fis1 (H), Mff (I), Drp1 (J), Foxo3a (K), Pink1 (L), Parkin (M), p62 (N), and LC3B (O), normalized to β-Tubulin. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

To determine whether these structural and molecular alterations were accompanied by functional changes, we assessed mitochondrial-related functional parameters. ATP levels in SN tissue were significantly reduced following MPTP administration, indicating impaired cellular energy homeostasis. Exercise intervention significantly restored ATP levels, whereas inhibition of SIRT1 abolished this effect (Fig. 6C). In parallel, mitochondrial membrane potential was evaluated using JC-1 staining in isolated SN-derived mitochondria. MPTP exposure resulted in a marked decrease in the JC-1 red/green fluorescence ratio, indicative of mitochondrial depolarization. Exercise intervention significantly restored mitochondrial membrane potential, whereas EX527 prevented this recovery (Fig. 6A–B).

Collectively, these findings indicate that inhibition of SIRT1 abolishes the exercise-mediated preservation of mitochondrial homeostasis, encompassing mitochondrial dynamics, mitophagy-related quality control, and mitochondrial functional integrity. The loss of these protective mechanisms is associated with exacerbated mitochondrial damage and increased neuronal vulnerability in PD mice.

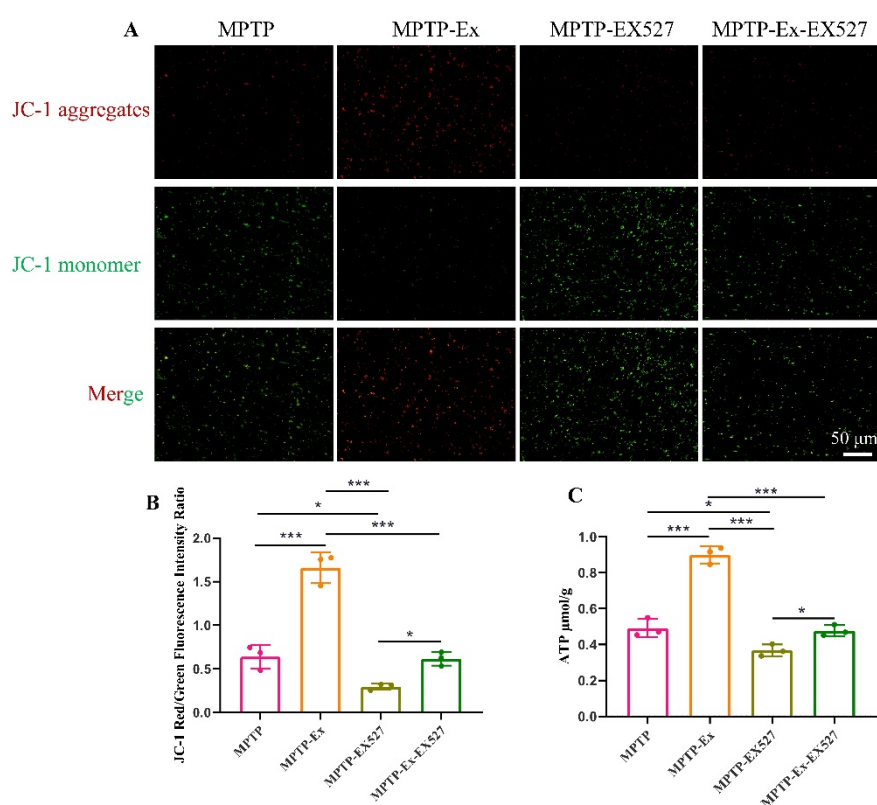


Figure 6. Inhibition of SIRT1 abolished the exercise-induced recovery of mitochondrial membrane potential and the enhancement of ATP production in the MPTP model. (A) Representative JC-1 fluorescence images of mitochondria isolated from the SN. (B) Quantification of mitochondrial membrane potential, expressed as the ratio of red to green JC-1 fluorescence intensity, which reflects mitochondrial membrane polarization. (C) ATP levels measured in homogenates of SN tissue. Scale bar: 50 μm. $n = 3$, $*P < 0.05$, $***P < 0.001$.

3.4 Exercise serum recapitulates the beneficial effects of exercise by improving mitochondrial homeostasis in a SIRT1-dependent manner *in vitro*.

To verify whether bioactive factors in the serum following exercise can induce neuroprotective effects and whether these effects are SIRT1-dependent, we collected serum from exercise and sedentary mice from previous experiments for *in vitro* cell culture assays. First, we assessed the impact of exercise serum on

dopaminergic neuronal markers and α -syn pathology in MPP⁺-induced cells. WB analysis showed that, compared to the Control group, MPP⁺ exposure significantly reduced the protein expression levels of TH and DAT and accompanied by abnormal accumulation of α -syn. Treatment with exercise serum significantly reversed these pathological alterations, as evidenced by a robust restoration of TH and DAT expression and a marked reduction in α -syn aggregation. In contrast, serum from sedentary failed to confer significant neuroprotective effects. Importantly, pharmacological inhibition of SIRT1 with EX527 completely abolished the beneficial effects of exercise serum, indicating that its neuroprotective actions are critically dependent on SIRT1 (Fig. 7A–D).

To further elucidate the underlying molecular mechanisms, we investigated SIRT1-associated signaling pathways and mitochondrial homeostasis. WB analysis demonstrated that treatment with exercise serum significantly upregulated SIRT1 protein expression and concomitantly increased the expression of its downstream transcription factor Foxo3a (Fig. 7F–H, O). Consistent with upregulation of the SIRT1–Foxo3a pathways, exercise serum effectively corrected the MPP⁺-induced imbalance in mitochondrial dynamics, as evidenced by reduced expression of mitochondrial fission-related proteins (Drp1, Fis1, Mff) and increased expression of fusion-related proteins (Mfn1, Mfn2, and Opa1) (Fig. 7H–N). In parallel, exercise serum markedly improved mitophagy-related quality control, as reflected by normalization of Pink1/Parkin signaling and of p62 and LC3B expression levels (Fig. 7H, P–S). In contrast, pharmacological inhibition of SIRT1 with EX527 significantly attenuated the regulatory effects of exercise serum on SIRT1, Foxo3a, mitochondrial dynamics proteins, and mitophagy-related markers, resulting in persistent disruption of mitochondrial homeostasis (Fig. 7F–S).

To visually validate the effects of exercise serum on mitochondrial integrity, TOM20 staining was performed. Cells exposed to MPP⁺ exhibited pronounced mitochondrial fragmentation and disruption of the mitochondrial network. Treatment with exercise serum substantially improved mitochondrial morphology, restoring a more elongated and interconnected network. However, co-treatment with EX527 partially reversed these protective effects, resulting in increased mitochondrial fragmentation and network disorganization (Fig. 7E).

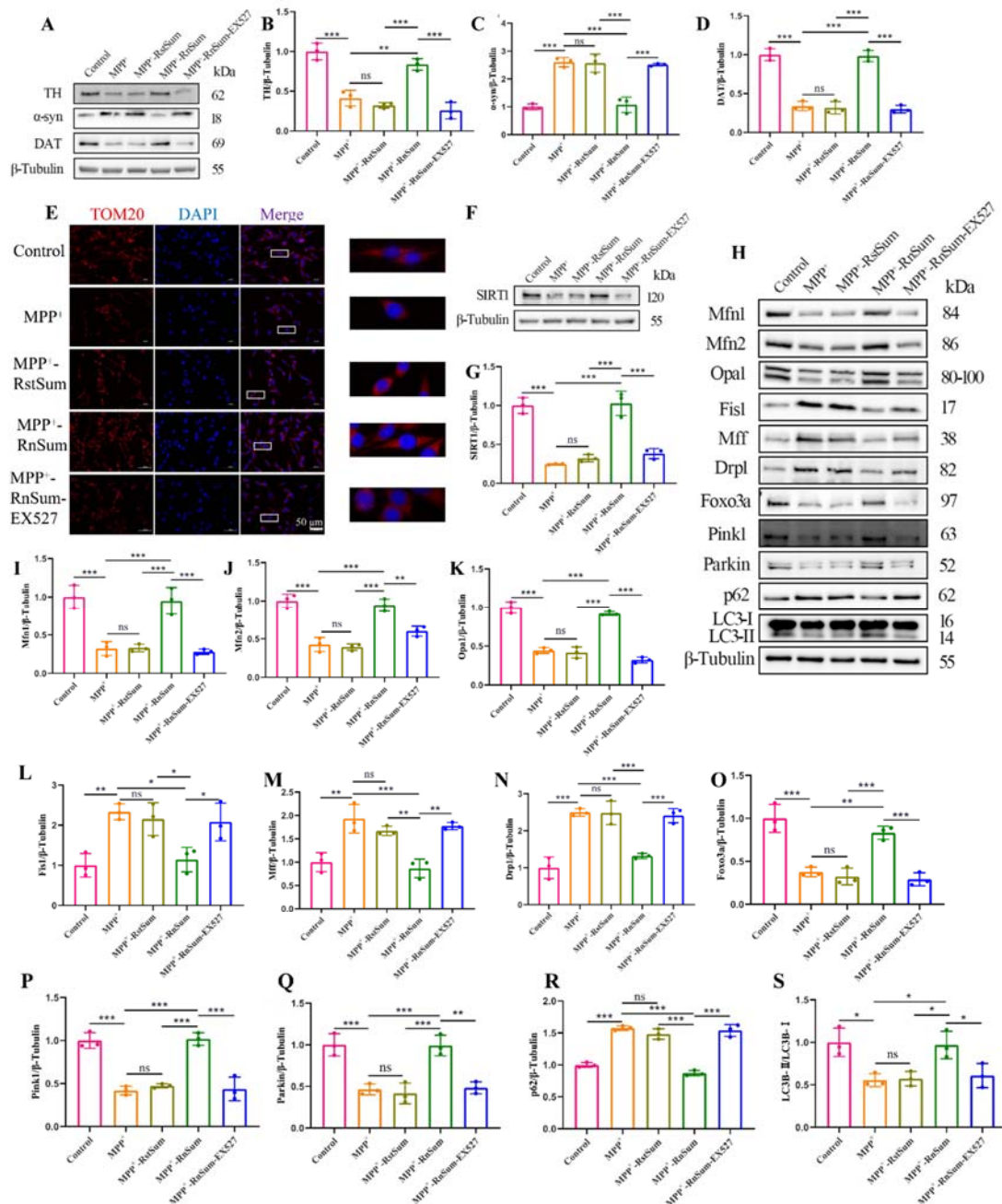


Figure 7. Exercise serum promotes mitochondrial homeostasis and neuronal survival in MPP⁺-injured neurons in a SIRT1-dependent manner. Cells were exposed to MPP⁺ to induce dopaminergic neuronal injury and were subsequently treated with serum collected from exercise or sedentary mice, in the presence or absence of the SIRT1 inhibitor EX527. (A) Representative WB images showing protein expression levels of TH, α -syn, and DAT in cells. (B-D) Quantification of protein levels of TH (B), α -syn (C), and DAT (D), normalized to β -Tubulin. (E) Representative TOM20 IF images showing mitochondrial network morphology in each group. Scale bar: 50 μ m. (F) Representative WB images of SIRT1. (G) Quantitative analysis of SIRT1 protein expression across groups, normalized to β -Tubulin. (H) Representative WB images showing the expression of mitochondrial dynamics-related proteins, including fission proteins (Drp1, Fis1, and Mff) and fusion proteins (Mfn1, Mfn2, and Opa1), as well as mitophagy-related proteins (Pink1, Parkin, p62, and LC3B), and Foxo3a. (I-S) Quantification of protein levels of Mfn1 (I), Mfn2 (J), Opa1 (K), Fis1 (L), Mff (M), Drp1 (N), Foxo3a (O), Pink1 (P), Parkin (Q), p62 (R), and LC3B (S), normalized to β -Tubulin. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = no significance.

We next examined whether these molecular and structural improvements were accompanied by functional restoration of mitochondria. Exposure to MPP⁺ significantly reduced mitochondrial membrane potential, as evidenced by a decreased JC-1 red/green fluorescence ratio, and markedly lowered intracellular ATP levels, indicating impaired mitochondrial bioenergetic capacity. Treatment with exercise serum

significantly restored mitochondrial membrane potential and ATP production, whereas pharmacological inhibition of SIRT1 with EX527 abolished these exercise serum-mediated functional improvements (Fig. 8C–E). In addition, intracellular ROS levels were measured to assess mitochondrial oxidative stress, as mitochondrial dysfunction is a major source of ROS overproduction and a key contributor to MPTP-induced neuronal damage. MPP⁺ exposure resulted in excessive ROS accumulation, whereas exercise serum treatment significantly reduced ROS levels. This antioxidant effect of exercise serum was largely abrogated by SIRT1 inhibition, further supporting a critical role for SIRT1 in mediating the mitochondrial protective effects of exercise serum (Fig. 8A–B).

Collectively, these *in vitro* findings demonstrate that exercise serum contains circulating neuroprotective factors capable of recapitulating the beneficial effects of exercise by preserving dopaminergic neuronal integrity, reducing α -syn aggregation, restoring mitochondrial dynamics and mitophagy-related quality control, improving mitochondrial function, and attenuating oxidative stress. These protective effects are closely associated with the SIRT1–Foxo3a pathway and are effectively abolished by pharmacological inhibition of SIRT1.

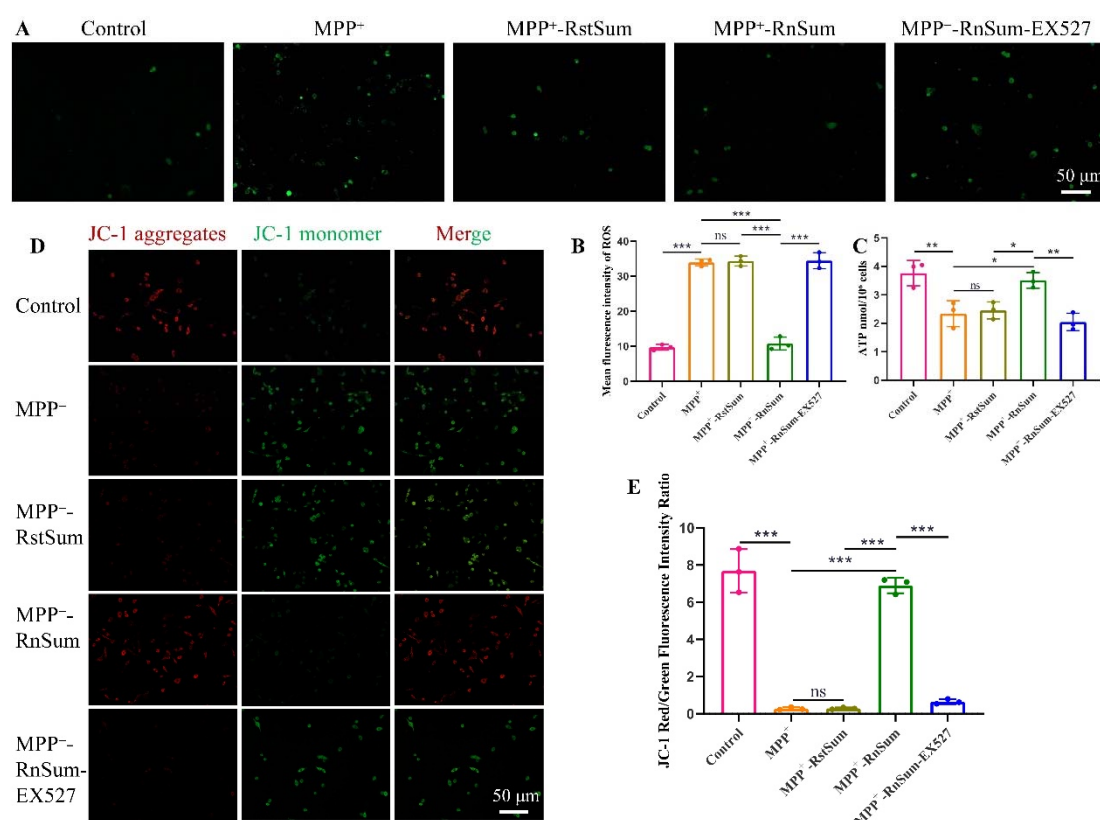


Figure 8. Exercise serum restores mitochondrial function and attenuates oxidative stress in MPP⁺-induced neuronal injury in a SIRT1-dependent manner. (A) Representative fluorescence images illustrating intracellular ROS levels detected using the DCFH-DA fluorescent probe. (B) Quantitative analysis of intracellular ROS fluorescence intensity. (C) Representative JC-1 fluorescence images of cells. (D) Quantification of mitochondrial membrane potential, expressed as the ratio of red to green JC-1 fluorescence intensity. (E) Intracellular ATP levels measured in cells. Scale bar: 50 μ m. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns = no significance.

3.5 The SIRT1 agonist resveratrol mimics exercise-mediated neuroprotection by improving mitochondrial homeostasis

To further investigate whether pharmacological modulation of SIRT1 could alleviate PD-related pathology *in vivo*, we administered a selective SIRT1 agonist to MPTP mice (Fig. 9A). Compared with the MPTP group, treatment with the SIRT1 agonist markedly ameliorated MPTP-induced motor and non-motor deficits, indicating that pharmacological targeting of SIRT1 is beneficial for preserving behavioral function under PD-related conditions (Fig. S4).

Consistent with these functional improvements, IHC and WB analyses revealed that resveratrol significantly attenuated MPTP-induced neurotoxicity. Compared with the MPTP group, the MPTP-RES group exhibited a significant increase in the number of TH⁺ neurons in the SN, as well as elevated protein levels of TH and DAT, accompanied by a marked reduction in pathological α -syn aggregation (Fig. 9D–I). Nissl staining further confirmed the neuroprotective effect of resveratrol on neuronal survival (Fig. 9B–C).

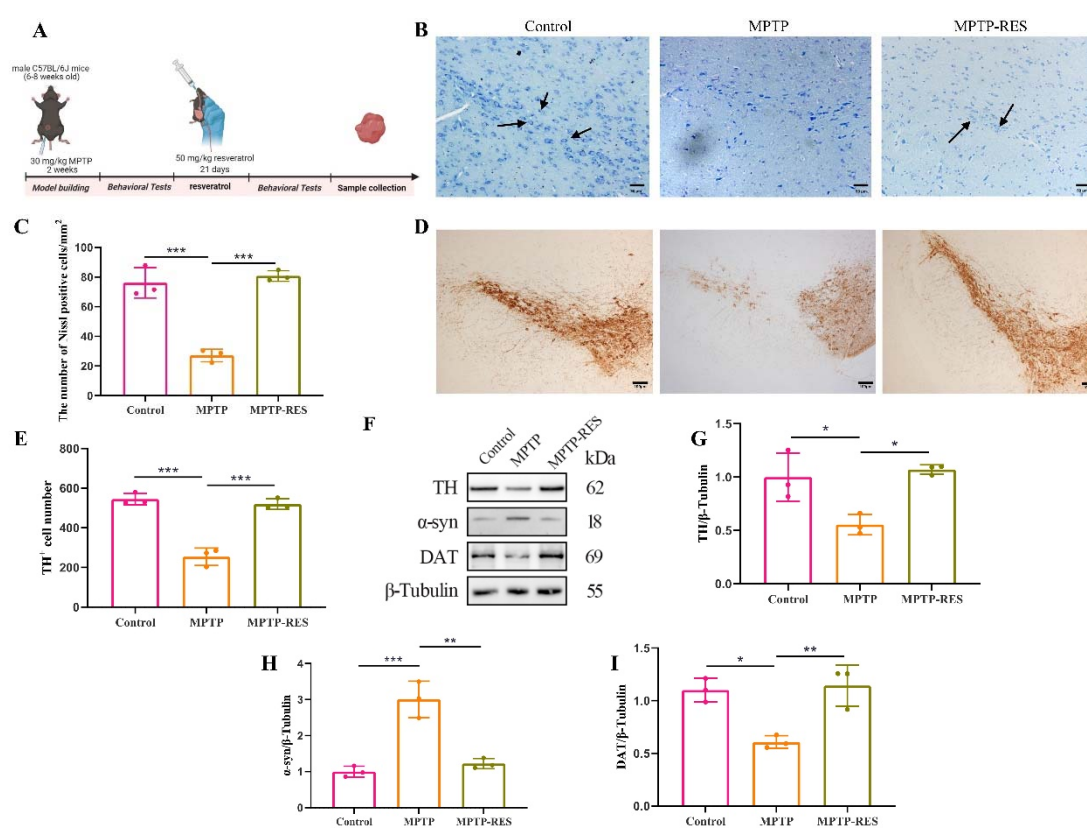


Figure 9. The SIRT1 agonist resveratrol attenuates MPTP-induced neuropathological damage in PD mice. (A) Schematic diagram of the experimental protocol. (B) Representative Nissl-stained neuropathology of the SN. Scale bar: 50 μ m. Arrows indicate normal neurons. (C) Quantitative analysis of surviving neurons in the SN. (D) Representative TH IHC sections of the SN. Scale bar: 100 μ m. (E) Quantitative analysis of TH-positive neurons in the SN. (F) Representative WB images of TH, α -syn, and DAT proteins. (G–I) Quantitative analysis of TH (G), α -syn (H), and DAT (I) protein expression levels, normalized to β -Tubulin. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Next, we examined whether pharmacological targeting of SIRT1 could attenuate PD-associated neuropathology by improving mitochondrial homeostasis in MPTP-induced mice. TEM analysis revealed that mitochondria in the MPTP group exhibited pronounced ultrastructural abnormalities, including marked swelling, vacuolization, and disruption of cristae architecture. In contrast, mice treated with the SIRT1 agonist displayed substantially preserved mitochondrial morphology, characterized by reduced swelling and more intact cristae, indicating that pharmacological activation of SIRT1 markedly alleviated MPTP-induced mitochondrial structural damage (Fig. 10A).

At the molecular level, we investigated whether resveratrol treatment alleviated MPTP-induced disruption of mitochondrial homeostasis, encompassing both mitochondrial dynamics and mitophagy. WB analysis revealed that MPTP administration induced a pronounced shift toward excessive mitochondrial fission, as evidenced by increased expression of Drp1, Fis1, and Mff, along with decreased expression of the fusion-related proteins Mfn1, Mfn2, and Opa1 (Fig. 10B, E–J). In parallel, MPTP exposure caused marked dysregulation of mitophagy-related proteins, including Pink1, Parkin, p62, and LC3B, indicative of impaired mitochondrial clearance (Fig. 10B, L–O). Resveratrol treatment significantly reversed these pathological alterations. Specifically, the MPTP-induced imbalance in mitochondrial fission–fusion dynamics was markedly corrected, accompanied by partial normalization of mitophagy-related markers, suggesting an overall improvement in mitochondrial homeostasis under neurotoxic conditions (Fig. 10B, E–O).

Given that resveratrol is a well-established modulator of SIRT1 signaling, we further investigated its effects on SIRT1 expression and downstream pathways. WB analysis demonstrated that SIRT1 protein levels were significantly reduced in MPTP mice, whereas resveratrol treatment robustly restored SIRT1 expression in the SN. Consistent with these findings, the expression of the downstream transcription factor Foxo3a was also significantly upregulated following resveratrol treatment compared with the MPTP group (Fig. 10B–D, K). Collectively, these results indicate that resveratrol alleviates MPTP-induced mitochondrial dysfunction and neuronal pathology, at least in part, through modulation of the SIRT1–Foxo3a pathway.

To determine whether the structural and molecular improvements induced by resveratrol were accompanied by functional restoration of mitochondria, we next assessed mitochondria-related functional parameters. ATP levels in SN tissue were significantly reduced in MPTP mice, indicating impaired cellular energy metabolism. Resveratrol treatment markedly restored ATP levels compared with the MPTP group, suggesting improved mitochondrial bioenergetic capacity (Fig. S5C). In parallel, mitochondrial membrane potential was evaluated using JC-1 staining in mitochondria isolated from the SN. MPTP administration resulted in a pronounced decrease in the JC-1 red/green fluorescence ratio, reflecting mitochondrial membrane depolarization. In contrast, resveratrol treatment significantly restored mitochondrial membrane potential toward control levels (Fig. S5A–B).

Taken together, these findings demonstrate that pharmacological intervention with the SIRT1 agonist resveratrol can successfully mimic the protective effects of exercise on PD model mice at the behavioral, cellular, ultrastructural, and molecular levels. These results not only provide strong evidence for the sufficiency of SIRT1 in mediating neuroprotection, but also highlight SIRT1 as a highly promising pharmacological target for PD therapy. Through modulation of the SIRT1 to maintain mitochondrial homeostasis, may represent a novel therapeutic strategy for PD.

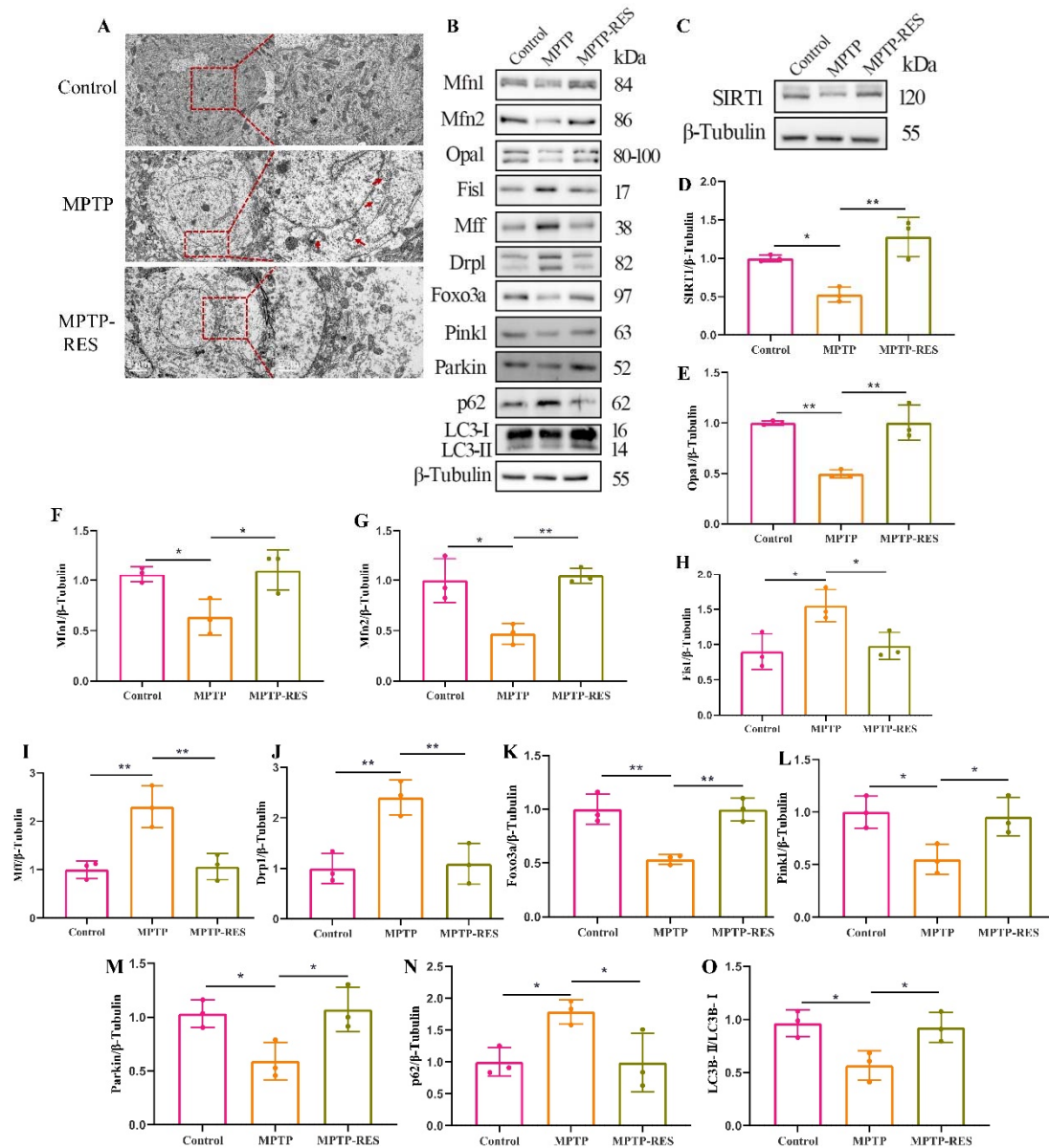


Figure 10. The SIRT1 agonist resveratrol ameliorates mitochondrial homeostasis in MPTP-induced PD mice. (A) TEM images of mitochondrial ultrastructure in the SN. Scale bars: 5 μ m, and 2 μ m. Red arrows indicate damaged mitochondria with cristae disruption and vacuolization. (B) Representative WB images showing the expression of mitochondrial dynamics-related proteins, including fission proteins (Drp1, Fis1, and Mff) and fusion proteins (Mfn1, Mfn2, and Opa1), as well as mitophagy-related proteins (Pink1, Parkin, p62, and LC3B), and Foxo3a. (C) Representative WB images of SIRT1. (D) Quantitative analysis of SIRT1 protein expression among groups. (E–O) Quantification of protein levels of Mfn1 (E), Mfn2 (F), Opa1 (G), Fis1 (H), Mff (I), Drp1 (J), Foxo3a (K), Pink1 (L), Parkin (M), p62 (N), and LC3B (O), normalized to β -Tubulin. $n = 3$, * $P < 0.05$, ** $P < 0.01$.

To obtain a comprehensive and unbiased characterization of the molecular landscape underlying the neuroprotective effects of resveratrol, we performed quantitative proteomic analysis of SN tissues from the Control, MPTP, and MPTP-RES groups. Compared with the Control group, the MPTP group exhibited substantial alterations in protein expression (Fig. 11A). Importantly, resveratrol treatment significantly reversed the expression of many of these dysregulated proteins (Fig. 11D). To further elucidate the functional implications of resveratrol-regulated DEPs, GO enrichment analysis demonstrated that these proteins were predominantly associated with biological processes related to synaptic structure and function—including synapse organization, neurotransmitter transport, and regulation of synaptic plasticity—as well as processes

involved in cellular responses to oxidative stress (Fig. 11E). KEGG pathway analysis demonstrated that resveratrol-modulated proteins were significantly enriched in several neuroactive pathways, including neuroactive ligand–receptor interaction, GABAergic synapse, cAMP signaling, and the FoxO signaling pathway (Fig. 11C, F). Notably, the FoxO signaling pathway, a key downstream effector of SIRT1, is critically involved in the regulation of oxidative stress responses and mitochondrial homeostasis, highlighting its potential contribution to the neuroprotective actions of resveratrol.

Collectively, these proteomic findings indicate that the neuroprotective effects of resveratrol are not mediated by a single molecular target but rather involve a coordinated, multi-pathway network. This network includes the preservation of synaptic integrity, attenuation of oxidative stress, and modulation of key neuronal signaling pathways, with SIRT1-associated signaling emerging as an integral component of this broader regulatory framework.

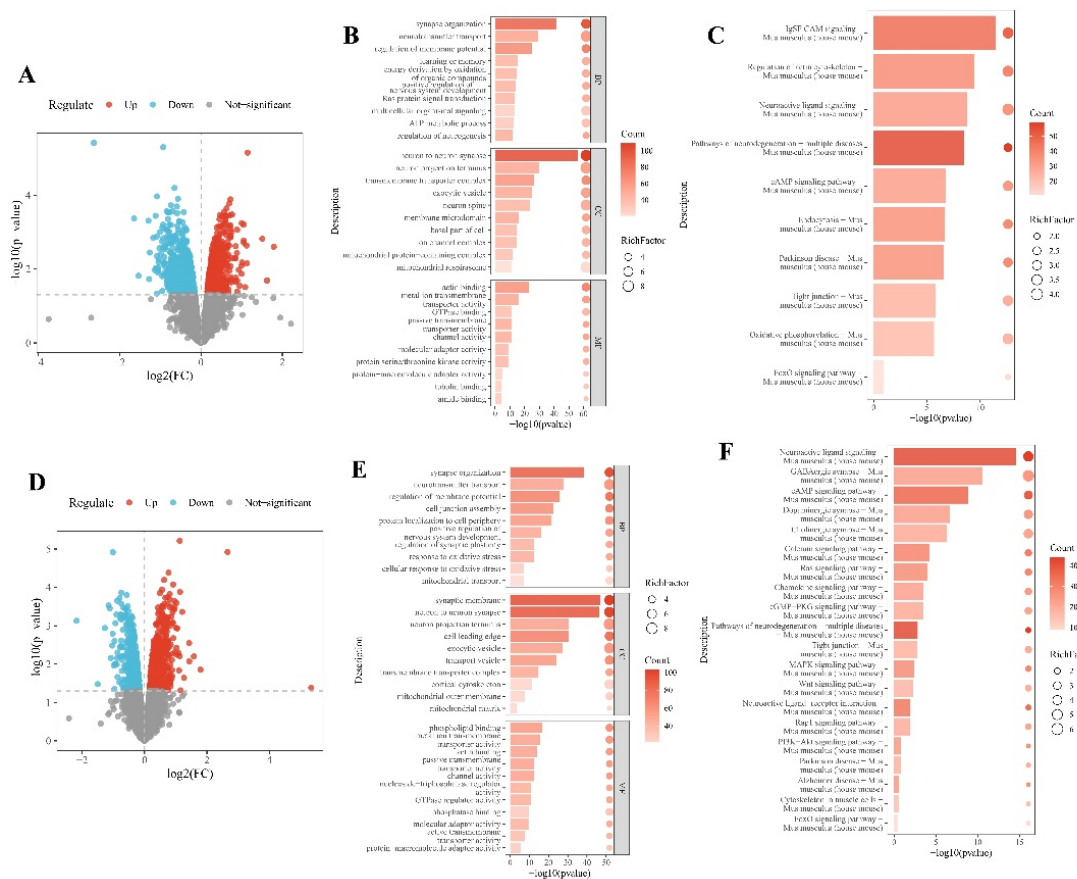


Figure 11. Functional enrichment analysis of DEPs. (A) Volcano plot showing DEPs between the Control and MPTP groups. The x-axis represents the $\log_2$ fold change ($\log_2\text{FC}$), and the y-axis represents $-\log_{10}(P \text{ value})$. Red dots indicate significantly upregulated proteins, blue dots indicate significantly downregulated proteins, and gray dots indicate proteins with no significant difference. Dashed lines denote the thresholds for differential expression. (B) GO enrichment analysis of DEPs between the Control and MPTP groups, encompassing biological process (BP), cellular component (CC), and molecular function (MF) categories. The x-axis represents $-\log_{10}(P \text{ value})$, and bar length reflects the significance of enrichment. Bubble size indicates the enrichment factor (RichFactor), and color intensity represents the number of enriched proteins (Count). (C) KEGG pathway enrichment analysis of DEPs between the Control and MPTP groups. The x-axis represents $-\log_{10}(P \text{ value})$, and the y-axis lists the enriched signaling pathways. Bubble size indicates the enrichment factor (RichFactor), and color intensity represents the number of DEPs in each pathway (Count). (D) Volcano plot showing DEPs between the MPTP and MPTP-RES groups. (E) GO enrichment analysis of DEPs between the MPTP and MPTP-RES groups. (F) KEGG pathway enrichment analysis of DEPs between the MPTP and MPTP-RES groups. $n = 3$.

3.6 The SIRT1–Foxo3a–Drp1 signaling pathway contributes to mitochondrial protection in MPP⁺-induced neurotoxicity

Given the proteomic evidence indicating modulation of FoxO signaling, and considering the well-established role of the SIRT1–Foxo3a pathway in regulating mitochondrial dynamics and quality control, we hypothesized that this pathway represents a key mechanism underlying the mitochondrial benefits conferred by resveratrol. Accordingly, we focused on validating the involvement of the SIRT1–Foxo3a–Drp1 signaling cascade. To this end, we conducted mechanistic intervention experiments in MN9D cells using resveratrol as a pharmacological activator of SIRT1, in combination with pharmacological inhibition of Foxo3a and downstream Drp1.

WB analysis demonstrated that MPP⁺ exposure caused a marked reduction in dopaminergic markers, including TH and DAT, and induced abnormal α -syn accumulation. Resveratrol treatment significantly reversed these pathological changes in the MPP⁺-RES group, indicating protection against MPP⁺-induced dopaminergic injury. However, pharmacological inhibition of Foxo3a with AS1842856 largely abolished the protective effects of resveratrol (MPP⁺-RES-AS group), as evidenced by reduced TH and DAT expression and increased α -syn accumulation. Notably, inhibition of Drp1 with Mdivi-1 in the presence of Foxo3a blockade (MPP⁺-RES-AS-Mdivi-1 group) substantially restored TH and DAT levels and reduced α -syn accumulation (Fig. 12A–D). Together, these findings suggest that Foxo3a is required for resveratrol-mediated neuroprotection and that Drp1-dependent mitochondrial fission represents a key downstream effector process that can be targeted to partially circumvent Foxo3a inhibition.

At the level of mitochondrial homeostasis, resveratrol normalized MPP⁺-induced alterations in mitochondrial dynamics-related proteins and concurrently improved the expression profile of mitophagy-related markers. Inhibition of Foxo3a largely antagonized these resveratrol-mediated effects, resulting in the re-emergence of dysregulated mitochondrial dynamics and aberrant mitophagy-related protein expression (Fig. 12H–S). Importantly, inhibition of Drp1 partially counteracted the disruptive effects of Foxo3a blockade, indicating that Drp1-dependent excessive mitochondrial fission constitutes a critical downstream regulatory node in this model.

Furthermore, resveratrol modulated SIRT1 expression in MPP⁺-induced cells. Treatment with the Foxo3a inhibitor AS1842856 did not alter SIRT1 protein levels but markedly reduced Foxo3a expression, supporting the notion that Foxo3a is positioned downstream of SIRT1 in this experimental context (Fig. 12F–G, J). By contrast, inhibition of Drp1 with Mdivi-1 did not affect Foxo3a protein expression, yet substantially ameliorated MPP⁺-induced pathological phenotypes and mitochondrial disruption. This pattern suggests that Drp1 functions downstream of Foxo3a as an effector node. Consistently, TOM20 IF analysis revealed that resveratrol markedly alleviated MPP⁺-induced mitochondrial fragmentation, whereas Foxo3a inhibition abolished this protective effect on mitochondrial morphology. Notably, Drp1 inhibition substantially improved mitochondrial network integrity even in the presence of Foxo3a inhibition, providing

morphological evidence supporting the downstream positioning of Drp1 relative to Foxo3a in the regulation of pathological mitochondrial fragmentation (Fig. 12E).

In addition to mitigating mitochondrial structural abnormalities, resveratrol also alleviated MPP⁺-induced mitochondrial functional impairment, as evidenced by restoration of the JC-1 red/green fluorescence ratio (Fig. S6D–E), increased ATP production (Fig. S6C), and reduced ROS levels (Fig. S6A–B). These functional improvements were markedly attenuated by Foxo3a inhibition, whereas downstream blockade of Drp1 partially restored the resveratrol-mediated benefits, indicating that the Foxo3a–Drp1 pathway contributes to the maintenance of mitochondrial function under MPP⁺-induced stress.

This study provides a detailed characterization of the downstream molecular events underlying the neuroprotective effects of SIRT1. Within this signaling framework, SIRT1 is positioned upstream and is associated with alterations in the expression of the transcription factor Foxo3a under MPP⁺-induced stress. Foxo3a appears to function as a critical intermediate, as pharmacological inhibition of Foxo3a largely diminished the protective phenotype elicited by SIRT1-targeted intervention. Notably, modulation of Foxo3a was accompanied by corresponding changes in the expression of Drp1, a central regulator of mitochondrial fission. Importantly, downstream inhibition of Drp1 partially restored mitochondrial network integrity and attenuated PD-related pathological alterations even in the presence of Foxo3a blockade, supporting Drp1 as a key downstream effector contributing to mitochondrial disruption. Collectively, these findings support a functional SIRT1–Foxo3a–Drp1 signaling pathway, whereby SIRT1-targeted intervention mitigates dopaminergic neurotoxicity, at least in part, through Foxo3a-dependent regulation of Drp1 to suppress excessive mitochondrial fission, thereby preserving mitochondrial homeostasis and neuronal integrity. Together, these results demonstrate that the SIRT1–Foxo3a–Drp1 pathway represents a critical mechanism through which resveratrol attenuates MPP⁺-induced mitochondrial dysfunction and neuronal damage.

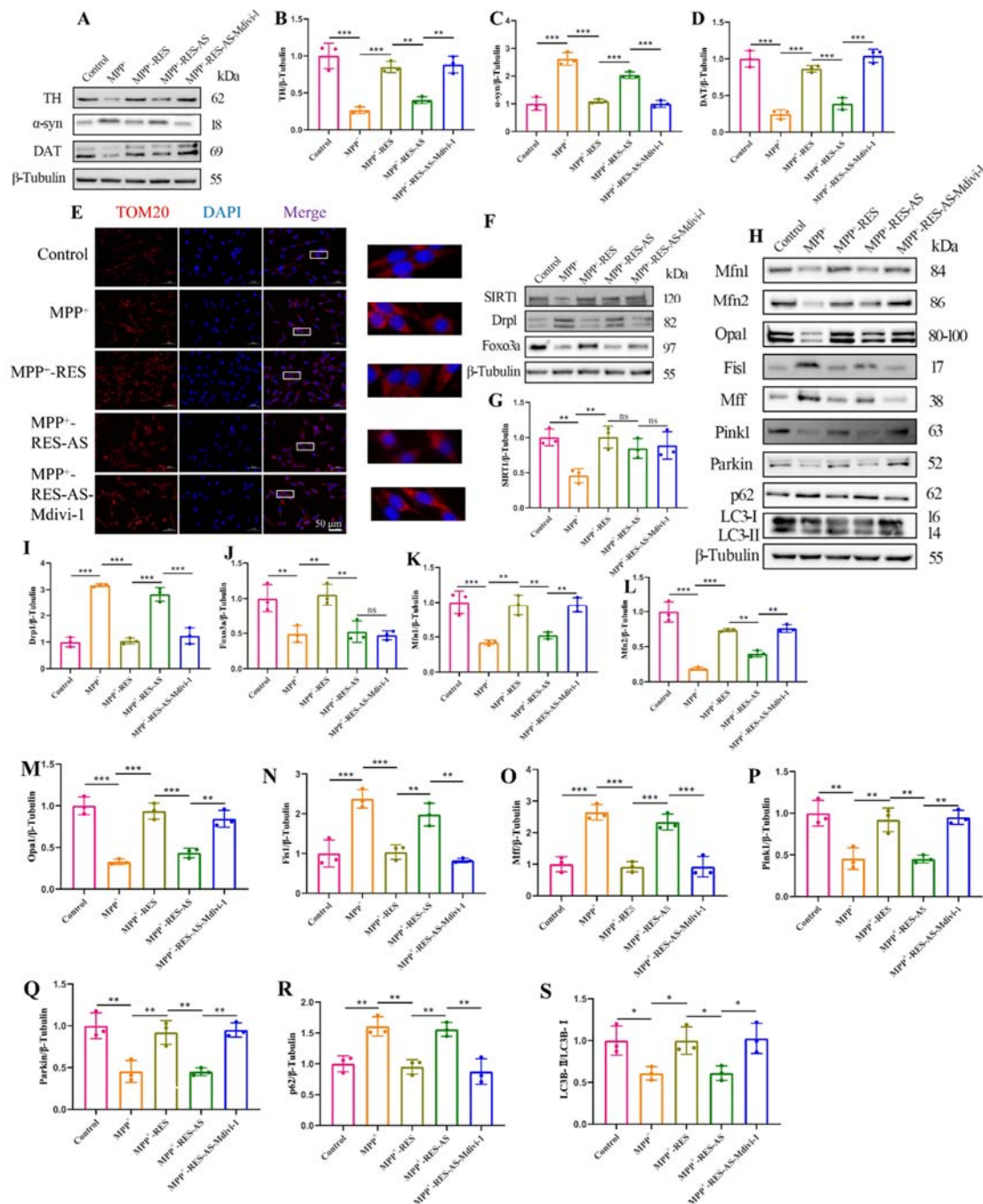


Figure 12. The SIRT1–Foxo3a–Drp1 signaling pathway promotes mitochondrial homeostasis and neuronal survival in MPP⁺-injured neurons. Cells were exposed to MPP⁺ to induce dopaminergic neuronal injury and were subsequently treated with the SIRT1 agonist resveratrol, with or without the Foxo3a inhibitor AS1842856 (AS) and/or the Drp1 inhibitor Mdivi-1. (A) Representative WB images showing protein expression levels of TH, α -syn, and DAT in cells. (B–D) Quantification of protein levels of TH (B), α -syn (C), and DAT (D), normalized to β -Tubulin. (E) Representative TOM20 IF staining images showing mitochondrial network morphology in each group. Scale bar: 50 μ m. (F) Representative WB images showing protein expression levels of SIRT1, Foxo3a, and Drp1 in cells. (G) Quantification of protein levels of SIRT1, normalized to β -Tubulin. (H) Representative WB images showing the expression of mitochondrial dynamics-related proteins, including fission proteins (Fis1, and Mff) and fusion proteins (Mfn1, Mfn2, and Opa1), as well as mitophagy-related proteins (Pink1, Parkin, p62, and LC3B). (I–J) Quantification of protein levels of Drp1 (I), and Foxo3a (J), normalized to β -Tubulin. (K–S) Quantification of protein levels of Mfn1 (K), Mfn2 (L), Opa1 (M), Fis1 (N), Mff (O), Pink1 (P), Parkin (Q), p62 (R), and LC3B (S), normalized to β -Tubulin. $n = 3$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Discussion

Our study seeks to integrate previous findings on the role of SIRT1 in PD by establishing a comprehensive mechanistic framework. Beyond confirming that both exercise and the SIRT1 agonist

resveratrol upregulate SIRT1 and enhance mitochondrial function, we demonstrate the necessity of this pathway using complementary gain- and loss-of-function approaches involving EX527. Furthermore, through the use of exercise serum, we provide direct evidence that the neuroprotective effects of exercise are, at least in part, humorally mediated and dependent on SIRT1. This triangulation of evidence—from whole-body behavioral outcomes to circulating factors and intracellular signaling—positions SIRT1 as a central integrative hub that translates exercise-mediated signals into enhanced mitochondrial resilience in neurons. Collectively, our findings establish a multi-level, causally validated framework that integrates behavioral, humoral, and pharmacological approaches to delineate this pathway.

A central translational implication of our work is the “exercise mimetic” hypothesis. Although we did not identify the specific exerkins within exercise serum responsible for SIRT1 upregulation, our serum transfer experiments provide critical proof-of-concept evidence that circulating factors released by exercise are sufficient to recapitulate exercise-like neuroprotection in a SIRT1-dependent manner. This finding directly demonstrates the existence of functional humoral mediators and establishes a robust bioassay for their future identification. Accordingly, our study shifts the focus from whether humoral factors are involved to how they signal, identifying SIRT1 as an indispensable central signaling hub mediating the beneficial effects of exercise. SIRT1 may function as a downstream integrative node for multiple exerkins, translating peripherally generated humoral signals into intracellular adaptive responses. Future proteomic and metabolomic analyses of exercise serum, guided by functional readouts such as mitochondrial network integrity and SIRT1/Foxo3a expression, represent a logical and high-yield next step.

Our study elucidates the dual significance of SIRT1 in the context of PD. Its necessity as a mediator of exercise-induced benefits was substantiated through multi-level validation: at the animal level, inhibition of SIRT1 completely abolished all improvements in behavior, dopaminergic neuron survival, and mitochondrial integrity conferred by exercise. At the cellular level, SIRT1 inhibition similarly negated the protective effects of exercise serum. More notably, we uncovered a second role of SIRT1 as an endogenous neuroprotective factor^[35]. This study found that pathological injury induced by SIRT1 inhibition alone was even more severe than that in the MPTP-only model group, suggesting that endogenous SIRT1 constitutes an important self-protective mechanism against neurotoxicity during the pathogenesis and progression of PD. Exercise intervention may further enhance this endogenous protective system, thereby maximizing its neuroprotective benefits. Resveratrol treatment may also potentiate this endogenous defense system, amplifying neuroprotection through mitochondrial restoration and transcriptional reprogramming. This discovery significantly enriches the understanding of the pathophysiological significance of SIRT1 in neurodegenerative disorders.

Furthermore, this study sought to delineate the specific downstream mechanisms by which SIRT1 confers mitochondrial protection. We recognize that SIRT1 regulates a broad and complex network of downstream effectors. To obtain an unbiased assessment of this complexity, we performed proteomic analyses. Although these analyses revealed modest enrichment of proteins associated with the FoxO signaling

pathway among resveratrol-induced changes, they more broadly identified concurrent alterations in additional processes relevant to neuroprotection. This global proteomic profile underscores the multifaceted nature of SIRT1-mediated adaptive responses. In light of this complexity, we subsequently employed targeted functional experiments to interrogate the specific contribution of Foxo3a. Our results demonstrate that Foxo3a acts as a necessary downstream mediator of SIRT1-dependent protection in our experimental models. Notably, pharmacological inhibition of Foxo3a using AS1842856 completely abolished the neuroprotective and mitochondrial-stabilizing effects of both resveratrol and exercise serum, confirming its essential role. We further identified Drp1 as a critical downstream effector of Foxo3a, as evidenced by the partial restoration of mitochondrial morphology and neuronal survival following Drp1 inhibition with Mdivi-1, even under conditions of Foxo3a blockade. Collectively, this integrated approach—combining proteomic profiling to contextualize the signaling landscape with functional pharmacology to establish causality—defines the SIRT1–Foxo3a–Drp1 pathway as a necessary and contributory component of the broader adaptive program engaged by SIRT1 activation to restore mitochondrial homeostasis in PD models. Notably, our mechanistic model identifies Foxo3a as a critical downstream effector of SIRT1 signaling. However, an important limitation of this conclusion is that it is derived from protein expression analyses and pharmacological inhibition studies, without direct assessment of the proposed molecular event—namely, Foxo3a deacetylation. Accordingly, we characterize this relationship as a functionally required signaling pathway rather than a definitively established molecular mechanism. Future studies directly examining Foxo3a acetylation dynamics will be essential to determine whether this specific regulatory process operates within our model of exercise- and resveratrol-induced protection.

Our findings are consistent with and extend existing evidence supporting SIRT1 as a neuroprotective target. The efficacy of resveratrol observed in this study aligns with the reported benefits of other SIRT1-activating compounds, such as SRT1720, in models of neurodegeneration, thereby reinforcing the therapeutic potential of this pathway^[36]. The novelty of the present work resides in the direct and comparative demonstration that both a pharmacological intervention (resveratrol) and exercise-derived humoral factors converge on SIRT1 to activate a specific downstream signaling pathway—Foxo3a–Drp1—that promotes mitochondrial repair. This integrated approach establishes a more unified mechanistic framework linking the well-documented benefits of exercise with those of SIRT1-targeted pharmacological strategies in PD.

The transition from behavioral to pharmacological intervention represents a critical step in translating basic research into clinical application. In this study, administration of the natural SIRT1 agonist resveratrol was found to closely replicate multiple protective effects of exercise in animal models, including amelioration of deficits, preservation of dopaminergic neurons, suppression of α -syn pathology, and improvement of mitochondrial structure and function. These findings suggest that pharmacologically targeting SIRT1 is a viable therapeutic strategy for mimicking exercise-induced benefits. For patients with advanced PD or individuals unable to perform effective physical activity due to comorbid conditions, the development of SIRT1-targeting drugs carries significant clinical translational implications. Resveratrol, which has attracted

considerable interest due to its natural origin and favorable safety profile^[37], is supported by robust preclinical evidence in this study for its potential application in PD.

Although this study systematically elucidates a comprehensive signaling pathway from behavioral intervention to the preservation of intracellular mitochondrial homeostasis—providing a theoretical framework for understanding the neuroprotective mechanisms of exercise—it is inevitably subject to inherent limitations, which also point to promising avenues for future investigation. First, our study employed a 14-day MPTP regimen that induces a subacute parkinsonian phenotype. Although this model is well suited for investigating mitochondrial dysfunction and represents a more prolonged exposure than acute bolus paradigms^[38], it does not fully recapitulate the chronic, progressive course or Lewy body pathology characteristic of human PD. Validation of our findings in chronic toxin-based or genetic models would therefore enhance their translational relevance. Second, all experiments were conducted exclusively in male mice. Given well-established sex differences in PD epidemiology, clinical manifestations, and underlying pathophysiology^[39], evaluating the efficacy of the SIRT1-mediated pathway in females represents an important and necessary direction for future research.

Moreover, several technical limitations in the current study should be addressed in future investigations. Although this work primarily relied on pharmacological tools (e.g., EX527, AS1842856) to manipulate target molecules, and while these inhibitors are relatively selective, the potential for off-target effects cannot be entirely excluded. To more specifically validate the cell-autonomous role of this pathway in dopaminergic neurons, future studies should utilize conditional knockout mice with dopaminergic neuron-specific deletion of SIRT1 or Foxo3a. In addition, the gait test was based on manual measurement of paw prints rather than automated gait analysis systems. Future studies incorporating automated approaches may provide more reliable and quantitative assessments of motor parameters. Furthermore, regarding the assessment of mitochondrial morphology, although TOM20 staining in this study accurately and consistently visualizes mitochondrial network architecture independent of changes in mitochondrial membrane potential, this method does not concurrently provide functional insights into mitochondrial status. Subsequent research should integrate the use of fluorescent dyes, such as MitoTracker Red (dependent on membrane potential) and MitoTracker Green (independent of membrane potential), co-stained with structural markers to simultaneously evaluate both mitochondrial morphology and quality. This approach would enable a more comprehensive assessment of mitochondrial health.

Lastly, from a translational perspective, although resveratrol successfully served as a proof-of-concept agent in this study, its unfavorable pharmacokinetic properties—such as low oral bioavailability and rapid metabolism—limit its clinical translational potential^[40]. Future studies should aim to identify or develop more druggable SIRT1 agonists and systematically evaluate their long-term efficacy and safety in large animal models (e.g., non-human primate models of PD, ultimately facilitating the clinical translation of novel PD treatment strategies based on the concept of "exercise mimetic" therapeutics. In summary, these limitations not only delineate the boundaries of the current study but also provide clear directions for future investigation.

By identifying key exerkinases, providing direct molecular evidence, developing more specific genetic models, employing multi-dimensional assessments of mitochondrial function, and validating findings in models closer to human disease, subsequent research can further strengthen the conclusions of this work and ultimately offer new targets and strategies for the treatment of PD.

In summary, this study integrates behavioral interventions with molecular analyses to establish a coherent mechanistic framework. We delineate a pathway by which exercise mitigates PD pathology through the preservation of mitochondrial homeostasis mediated by the SIRT1–Foxo3a–Drp1 pathway, and we further propose a complementary humoral mechanism whereby exercise-induced circulating factors activate SIRT1 signaling. Moreover, by demonstrating that the natural compound resveratrol pharmacologically recapitulates these effects, we validate SIRT1 as a convergent target for both behavioral and pharmacological interventions. Collectively, these findings provide mechanistic insight into how exercise-derived signals intersect with pharmacological actions at the level of mitochondrial regulation, thereby establishing a strong scientific foundation for the development of SIRT1-targeted “exercise mimetic” therapeutics for the prevention and treatment of PD.

5. Conclusion

This study demonstrates that exercise alleviates PD pathology by upregulating SIRT1, thereby restoring mitochondrial homeostasis through the Foxo3a–Drp1 pathway. Notably, the natural SIRT1 agonist resveratrol recapitulates these exercise-induced benefits. Collectively, our findings identify SIRT1 as a convergent therapeutic target for both behavioral and dietary interventions, supporting the development of “exercise mimetic” strategies for PD (Fig. 13).

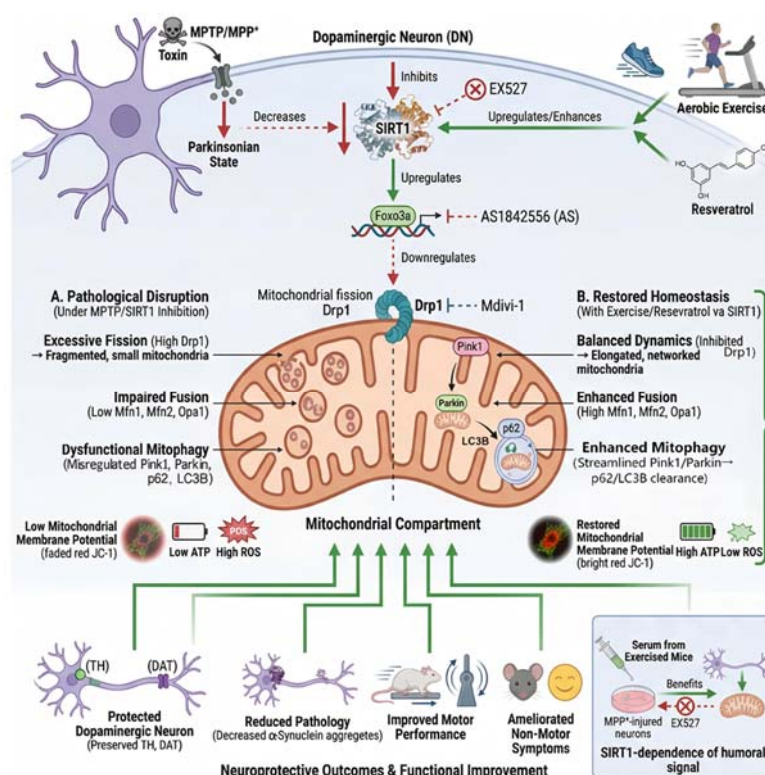


Figure 13. Schematic representation contrasting PD pathology with exercise- and resveratrol-mediated neuroprotection via the SIRT1 signaling pathway.

Left (Pathology): MPTP/MPP⁺-induced toxicity disrupts mitochondrial homeostasis, resulting in an imbalance between mitochondrial fission and fusion characterized by excessive fission, impaired mitophagy, and subsequent mitochondrial dysfunction, including reduced membrane potential, decreased ATP production, and elevated ROS. These alterations ultimately lead to dopaminergic neuron degeneration, as evidenced by reduced TH and DAT expression, along with increased α -syn aggregation.

Right (Intervention and Protection): Both physical exercise and the SIRT1-activating polyphenol resveratrol converge to upregulate SIRT1 expression. Activated SIRT1 engages the downstream Foxo3a–Drp1 signaling pathway, thereby restoring the balance between mitochondrial fission and fusion, enhancing mitophagic clearance, and improving mitochondrial function, as indicated by restored membrane potential, increased ATP levels, and reduced ROS production. As a result, dopaminergic neurons are preserved, with maintained TH and DAT expression and attenuated α -syn pathology, culminating in overall neuroprotection and functional improvement.

Funding

This project was partially supported by the National Natural Science Foundation of China (81271410, 32161143021), Henan Provincial Science and Technology Key Projects Program (252102311160), Henan Provincial Medical Science and Technology Key Projects Program (SBGJ202402075) and Henan Natural Science Foundation of China (182300410313).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The working model of this study was drawn using BioRender (<https://BioRender.com>).

References

- [1] Wu KM, Xu QH, Liu YQ, et al., Neuronal FAM171A2 mediates α -synuclein fibril uptake and drives Parkinson's disease, *Science*. 387 (2025) 892-900. <https://doi.org/10.1126/science.adp3645>.
- [2] Tolosa E, Garrido A, Scholz SW, et al., Challenges in the diagnosis of Parkinson's disease, *Lancet Neurol*. 20 (2021) 385-397. [https://doi.org/10.1016/S1474-4422\(21\)00030-2](https://doi.org/10.1016/S1474-4422(21)00030-2).
- [3] Su D, Cui Y, He C, et al., Projections for prevalence of Parkinson's disease and its driving factors in 195 countries and territories to 2050: modelling study of Global Burden of Disease Study 2021, *BMJ*. 388 (2025) e080952. <https://doi.org/10.1136/bmj-2024-080952>.
- [4] Moreau C, Odou P, Labreuche J, et al., Intracerebroventricular anaerobic dopamine in Parkinson's disease with L-dopa-related complications: a phase 1/2 randomized-controlled trial, *Nat Med*. 31 (2025) 819-828. <https://doi.org/10.1038/s41591-024-03428-2>.
- [5] Chen C, McDonald D, Blain A, et al., Parkinson's disease neurons exhibit alterations in mitochondrial quality control proteins, *NPJ Parkinsons Dis*. 9 (2023) 120. <https://doi.org/10.1038/s41531-023-00564-3>.
- [6] Geibl FF, Henrich MT, Xie Z, et al., α -Synuclein pathology disrupts mitochondrial function in dopaminergic and cholinergic neurons at-risk in Parkinson's disease, *Mol Neurodegener*. 19 (2024) 69. <https://doi.org/10.1186/s13024-024-00756-2>.

-
- [7] Henrich MT, Oertel WH, Surmeier DJ, et al., Mitochondrial dysfunction in Parkinson's disease - a key disease hallmark with therapeutic potential, *Mol Neurodegener.* 18 (2023) 83. <https://doi.org/10.1186/s13024-023-00676-7>.
- [8] Ahn EH, Lei K, Kang SS, et al., Mitochondrial dysfunction triggers the pathogenesis of Parkinson's disease in neuronal C/EBP β transgenic mice, *Mol Psychiatry.* 26 (2021) 7838-7850. <https://doi.org/10.1038/s41380-021-01284-x>.
- [9] Chemla A, Arena G, Sacripanti G, et al., Parkinson's disease mutant Miro1 causes mitochondrial dysfunction and dopaminergic neuron loss, *Brain.* 148 (2025) 3607-3622. <https://doi.org/10.1093/brain/awaf051>.
- [10] Picca A, Faltg J, Auwerx J, et al., Mitophagy in human health, ageing and disease, *Nat Metab.* 5 (2023) 2047-2061. <https://doi.org/10.1038/s42255-023-00930-8>.
- [11] Court AC, Vega-Letter AM, Parra-Crisóstomo E, et al., Mitochondrial transfer balances cell redox, energy and metabolic homeostasis in the osteoarthritic chondrocyte preserving cartilage integrity, *Theranostics.* 14 (2024) 6471-6486. <https://doi.org/10.7150/thno.96723>.
- [12] Brown HJ, Fan RZ, Bell R, et al., Imbalanced mitochondrial dynamics in human PD and α -synuclein mouse brains, *Neurobiol Dis.* 212 (2025) 106976. <https://doi.org/10.1016/j.nbd.2025.106976>.
- [13] Narendra DP, Youle RJ, The role of PINK1-Parkin in mitochondrial quality control, *Nat Cell Biol.* 26 (2024) 1639-1651. <https://doi.org/10.1038/s41556-024-01513-9>.
- [14] Peng K, Xiao J, Yang L, et al., Mutual Antagonism of PINK1/Parkin and PGC-1 α Contributes to Maintenance of Mitochondrial Homeostasis in Rotenone-Induced Neurotoxicity, *Neurotox Res.* 35 (2019) 331-343. <https://doi.org/10.1007/s12640-018-9957-4>.
- [15] Königstein K, Dipla K, Zafeiridis A, Training the Vessels: Molecular and Clinical Effects of Exercise on Vascular Health-A Narrative Review, *Cells.* 12 (2023) 2544. <https://doi.org/10.3390/cells12212544>.
- [16] Li J, Liu T, Xian M, et al., The Power of Exercise: Unlocking the Biological Mysteries of Peripheral-Central Crosstalk in Parkinson's Disease, *J Adv Res.* 78 (2025) 717-732. <https://doi.org/10.1016/j.jare.2025.03.001>.
- [17] van der Kolk NM, de Vries NM, Kessels RPC, et al., Effectiveness of home-based and remotely supervised aerobic exercise in Parkinson's disease: a double-blind, randomised controlled trial, *Lancet Neurol.* 18 (2019) 998-1008. [https://doi.org/10.1016/S1474-4422\(19\)30285-6](https://doi.org/10.1016/S1474-4422(19)30285-6).
- [18] Johansson ME, Cameron IGM, Van der Kolk NM, et al., Aerobic Exercise Alters Brain Function and Structure in Parkinson's Disease: A Randomized Controlled Trial, *Ann Neurol.* 91 (2022) 203-216. <https://doi.org/10.1002/ana.26291>.
- [19] Li J, Xu Y, Liu T, et al., The Role of Exercise in Maintaining Mitochondrial Proteostasis in Parkinson's Disease, *Int J Mol Sci.* 24 (2023) 7994. <https://doi.org/10.3390/ijms24097994>.
- [20] Zhang X, Xu S, Hu Y, et al., Irisin exhibits neuroprotection by preventing mitochondrial damage in Parkinson's disease, *NPJ Parkinsons Dis.* 9 (2023) 13. <https://doi.org/10.1038/s41531-023-00453-9>.
- [21] Yan P, Li Z, Xiong J, et al., LARP7 ameliorates cellular senescence and aging by allosterically enhancing SIRT1 deacetylase activity, *Cell Rep.* 37 (2021) 110038. <https://doi.org/10.1016/j.celrep.2021.110038>.
- [22] Li J, Liu T, Xian M, et al., Therapeutic applications of exercise in neurodegenerative diseases: focusing on the mechanism of SIRT1, *Mol Cell Biochem.* 480 (2025) 4921-4939. <https://doi.org/10.1007/s11010-025-05299-8>.
- [23] Hu P, Wang Y, Qi XH, et al., SIRT1 in the BNST modulates chronic stress-induced anxiety of male mice via FKBP5 and corticotropin-releasing factor signaling, *Mol Psychiatry.* 28 (2023) 5101-5117. <https://doi.org/10.1038/s41380-023-02144-6>.
- [24] Lei Y, Wang J, Wang D, et al., SIRT1 in forebrain excitatory neurons produces sexually dimorphic effects on depression-related behaviors and modulates neuronal excitability and synaptic transmission in the medial prefrontal cortex, *Mol Psychiatry.* 25 (2020) 1094-1111. <https://doi.org/10.1038/s41380-019-0352-1>.
- [25] Lian B, Zhang J, Yin X, et al., SIRT1 improves lactate homeostasis in the brain to alleviate parkinsonism via deacetylation and inhibition of PKM2, *Cell Rep Med.* 5 (2024) 101684. <https://doi.org/10.1016/j.xcrm.2024.101684>.
- [26] Xu Y, An Z, Hou K, et al., ACE2 Alleviates Microglia Neuroinflammation by RANK-RANKL-OPG Axis in Parkinson's Disease, *Inflammation.* 48 (2025) 4364-4378. <https://doi.org/10.1007/s10753-025-02331-3>.
- [27] Guo YJ, Dong SY, Cui XX, et al., Resveratrol alleviates MPTP-induced motor impairments and pathological changes by autophagic degradation of α -synuclein via SIRT1-deacetylated LC3, *Mol Nutr Food Res.* 60 (2016) 2161-2175. <https://doi.org/10.1002/mnfr.201600111>.

-
- [28] Wang ZH, Zhang JL, Duan YL, et al., MicroRNA-214 participates in the neuroprotective effect of Resveratrol via inhibiting α -synuclein expression in MPTP-induced Parkinson's disease mouse, *Biomed Pharmacother.* 74 (2015) 252-256. <https://doi.org/10.1016/j.biopha.2015.08.025>.
- [29] Schefer V, Talan MI, Oxygen consumption in adult and AGED C57BL/6J mice during acute treadmill exercise of different intensity, *Exp Gerontol.* 31 (1996) 387-392. [https://doi.org/10.1016/0531-5565\(95\)02032-2](https://doi.org/10.1016/0531-5565(95)02032-2).
- [30] Zhao R, Tian X, Xu H, et al., Aerobic Exercise Restores Hippocampal Neurogenesis and Cognitive Function by Decreasing Microglia Inflammasome Formation Through Irisin/NLRP3 Pathway, *Aging Cell.* 24 (2025) e70061. <https://doi.org/10.1111/accel.70061>.
- [31] Gao Y, Li S, Zhang S, et al., Atractylenolide-I Attenuates MPTP/MPP⁺-Mediated Oxidative Stress in Parkinson's Disease Through SIRT1/PGC-1 α /Nrf2 Axis, *Neurochem Res.* 50 (2024) 18. <https://doi.org/10.1007/s11064-024-04258-x>.
- [32] Kang RR, Sun Q, Chen KG, et al., Resveratrol prevents benzo(a)pyrene-induced disruption of mitochondrial homeostasis via the AMPK signaling pathway in primary cultured neurons, *Environ Pollut.* 261 (2020) 114207. <https://doi.org/10.1016/j.envpol.2020.114207>.
- [33] Zhang M, Sui W, Xing Y, et al., Angiotensin IV attenuates diabetic cardiomyopathy *via* suppressing FoxO1-induced excessive autophagy, apoptosis and fibrosis, *Theranostics.* 11 (2021) 8624-8639. <https://doi.org/10.7150/thno.48561>.
- [34] Yan XD, Fan RH, Wang Y, et al., α -asarone activates mitophagy to relieve diabetic encephalopathy via inhibiting apoptosis and oxidative stress, *Metab Brain Dis.* 40 (2025) 126. <https://doi.org/10.1007/s11011-025-01556-3>.
- [35] Villeda SA, Plambeck KE, Middeldorp J, et al., Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice, *Nat Med.* 20 (2014) 659-663. <https://doi.org/10.1038/nm.3569>.
- [36] Chao CC, Huang CL, Cheng JJ, et al., SRT1720 as an SIRT1 activator for alleviating paraquat-induced models of Parkinson's disease, *Redox Biol.* 58 (2022) 102534. <https://doi.org/10.1016/j.redox.2022.102534>.
- [37] Wu SX, Xiong RG, Huang SY, et al., Effects and mechanisms of resveratrol for prevention and management of cancers: An updated review, *Crit Rev Food Sci Nutr.* 63 (2023) 12422-12440. <https://doi.org/10.1080/10408398.2022.2101428>.
- [38] Santoro M, Fadda P, Klephan KJ, et al., Neurochemical, histological, and behavioral profiling of the acute, sub-acute, and chronic MPTP mouse model of Parkinson's disease, *J Neurochem.* 164 (2023) 121-142. <https://doi.org/10.1111/jnc.15699>.
- [39] Vaidya B, Dhamija K, Guru P, et al., Parkinson's disease in women: Mechanisms underlying sex differences, *Eur J Pharmacol.* 895 (2021) 173862. <https://doi.org/10.1016/j.ejphar.2021.173862>.
- [40] Ren B, Kwah MX, Liu C, et al., Resveratrol for cancer therapy: Challenges and future perspectives, *Cancer Lett.* 515 (2021) 63-72. <https://doi.org/10.1016/j.canlet.2021.05.001>.