

# LncRNA SOX2-OT Participates in Parkinson's Disease Through Regulating miRNA-942-5p/NAIF1 Axis

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## Research Article

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# Abstract

Parkinson's disease (PD) is a neurodegenerative disease. Studies have shown that lncRNA SOX2-OT was highly expressed in PD patients, but its specific functions and mechanisms still need further research. This study aimed to explore whether lncRNA SOX2-OT could regulate oxidative stress, inflammation and neuronal apoptosis in PD *in vitro* model and explored the underlying mechanism. An *in vitro* PD cell model was induced by 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>). The results of the biological software analysis and luciferase reporter assay indicated that miR-942-5p was a direct target of lncRNA SOX2-OT, and NAIF1 was a direct target of miR-942-5p. Experiments showed that the expression levels of lncRNA SOX2-OT and NAIF1 were increased, and miR-942-5p expression was decreased in SH-SY5Y cells following MPP<sup>+</sup> treatment. In addition, MPP<sup>+</sup> treatment reduced SH-SY5Y cell viability, induced apoptosis, increased cleaved-Caspase3 protein expression, and increased cleaved-Caspase3/Caspase3 ratio, increased LDH viability, and increased the levels of TNF- $\alpha$ , IL-1 $\beta$  and ROS in SH-SY5Y cells, reduced SOD activity, however, all these effects were inhibited by SOX2-OT-siRNA, and these inhibitions were reversed by miR-942-5p inhibitor. Moreover, the protective role of miR-942-5p mimic in MPP<sup>+</sup> induced SH-SY5Y cells was significantly eliminated by NAIF1-plasmid. In summary, this study confirmed that lncRNA SOX2-OT regulated oxidative stress, inflammation and neuronal apoptosis via directly regulating the miR-942-5p/NAIF1 signal axis, and then participated in the occurrence and development of PD. These data provide a new potential targets for PD diagnosis and treatment.

## Introduction

Parkinson's disease (PD) is a universal neurodegenerative disease, this disease results primarily from the death of dopaminergic neurons in the substantia nigra [1, 2]. There were many theories about the pathogenesis of PD. Patel *et al* pointed out that this disease was caused by a combination of age, genetic and environmental factors [3], These results showed that the pathogenesis was complicated. At present, more and more evidence indicated that deficits in mitochondrial function, oxidative stress, inflammation and apoptosis were the most important factors [4, 5], but the specific mechanism is not yet clear. Therefore, it is very necessary to study the mechanism and develop treatment methods of PD.

Long non-coding RNA (lncRNA) has a significant function in the development and disease of the central nervous system (CNS), a number of lncRNAs were highly expressed in the adult and developing brain [6]. Recent research demonstrated that lncRNAs interfere in transcriptional and translational processes, and there were 5 lncRNAs that were clearly differentially expressed in PD [7]. There were increasing evidences that lncRNA plays a crucial role in the development of PD [8, 9]. Long *et al* showed that miRNA and lncRNA co-regulate the pathogenesis of PD [9]. Lu *et al* found that lncRNA MALAT1 targeting miR-4 contributes to cell apoptosis in PD [10], and the research results of Xie *et al* showed that up-regulated lncRNA small nucleolar RNA host gene 1 promotes MPP<sup>+</sup>-induced reactive oxygen generation and cytotoxicity in human dopaminergic SH-SY5Y cells through the miR-15b-5p /GSK3 $\beta$  axis [11]. Moreover,

SOX2-OT as a lncRNA, and some microRNAs (miRNAs) have a targeting relationship in disease [12]. The role and specific mechanism of SOX2-OT in PD remain to be investigated.

As we all know, miRNAs regard as one kind of small non-coding RNAs that modulate gene expression at the post-transcriptional level [13]. In addition, miRNAs can participate in a variety of pathological processes in a variety of pathways [14]. miR-942-5p as a miRNA was related to the pathogenesis of many neurodegenerative diseases, such as attention deficit hyperactivity disorder [15], however, there were few reports on the role of miR-942-5p in PD. Past studies had shown that cell apoptosis is involved in the pathological mechanisms of PD [16, 17], however, the nuclear apoptosis-inducing factor 1 (NAIF1) was a very important substance in the process of cell apoptosis [18, 19]. Moreover, study has shown that the expression levels of miRNA and NAIF1 were related [20].

Therefore, this study aimed to explore whether lncRNA SOX2-OT participated in PD through regulating oxidative stress, inflammation and neuronal apoptosis via directly regulating miR-942-5p. Our research will provide new strategies for PD diagnosis and treatment.

## Materials And Methods

### Acquisition and culture of human neuroblastoma SH-SY5Y cells

Human neuroblastoma SH-SY5Y cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were stored in Dulbecco's modified Eagle medium (DMEM) medium (Gibco, Grand Island, NY, USA) supplemented with 1% penicillin/streptomycin, 10% fetal bovine serum (FBS, Gibco) and incubated in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C.

### Dual-luciferase reporter verification

The association between lncRNA SOX2-OT and miR-942-5p was identified by bioinformatics software (Starbase). In addition, TargetScan determined the relationship between miR-942-5p and NAIF1. We then used a dual luciferase reporter gene plasmid vector (Guangzhou RiboBio Co., Ltd., Guangzhou, China) and the QuikChange Site-Directed Mutagenesis Kit (Stratagene, San Diego, CA) to generate NAIF1-WT, NAIF1-MUT, SOX2-OT-WT and SOX2-OT-MUT according to the manufacturer's instructions. Finally, the luciferase activity was analyzed by the dual luciferase reporter gene analysis system (Promega, USA).

### Establishment of PD cell model *in vitro*

To explore the expression levels of miR-942-5p, lncRNA SOX2-OT and NAIF1 in the PD cell model. SH-SY5Y cells were treated with 0, 0.25, 0.5, 1 or 2 mM MPP<sup>+</sup> (Sigma, St. Louis, MO, USA) for 24 h, or exposed to 1 mM MPP<sup>+</sup> for 0, 6, 12, 24 or 48 h [21].

### Cell transfection assay

SH-SY5Y cells were inoculated at a concentration of  $5 \times 10^4$  cells/ml in 6-well plates and incubated overnight. The miR-942-5p inhibitors was used to down-regulate miR-942-5p expression in SH-SY5Y cells using an inhibitor-control as the negative control. SOX2-OT-siRNA was used for SOX2-OT down-regulation. The miR-942-5p mimic and NAIF1-plasmid were used to up-regulate miR-942-5p and NAIF1 expression in SH-SY5Y cells. Control-siRNA, SOX2-OT-siRNA, inhibitor control, miR-942-5p inhibitor, SOX2-OT-siRNA + inhibitor control or SOX2-OT-siRNA + miR-942-5p inhibitor, and mimic control, miR-942-5p mimic, control-plasmid, NAIF1-plasmid, miR-942-5p mimic + control-plasmid- or miR-942-5p mimic + NAIF1-plasmid were transfected into SH-SY5Y cells using Lipofectamine 2000 reagent (Invitrogen, USA) according to the manufacturer's instructions. After 48 h of cell culture at 37°C, the cells were collected to test the transfection efficiency using qRT-PCR, or further cultured in the presence of 1 mM MPP<sup>+</sup> for 24 h.

### **RNA extraction and quantitative real-time PCR (qRT-PCR)**

Total cellular RNA was isolated from SHSY5Y cells using TRIzol reagent (Invitrogen, USA), and reverse transcribed into first-strand cDNA using a cDNA Synthesis Kit (Invitrogen) according to the instructions provided by the manufacturer. The expression levels of miR-942-5p, lncRNA SOX2-OT and NAIF1 mRNA were quantified by the Prism 7000 real-time PCR system using Power SYBR Green Master mix (Vazyme, Piscataway, NJ, USA) according to the instructions provided by the manufacturer. The amplification conditions were as follows: denaturation at 94°C for 35 cycles of 60 seconds, anneal at 60°C for 60 seconds, then extend at 72°C for 1 minute, and then at 72°C for 10 minutes. U6 and GAPDH were used as inner control genes. Calculate the relative expression levels of miR-942-5p, lncRNA SOX2-OT and NAIF1 mRNA by the  $2^{-\Delta\Delta C_t}$  method [22].

### **3-(4,5-dimethylthiazol-2-yl)-2,5-dipheyltetrazolium bromide (MTT) assay**

SH-SY5Y cells were inoculated into 96-well plates in triplicate, incubating overnight. Subsequently, the medium was removed, and after transfection of the cells at 37°C, the cells were treated with 1 mM MPP<sup>+</sup>. The cells were incubated with 10  $\mu$ l MTT solution (Beyotime, Shanghai, China) for 4 hours. Subsequently, after removing the solution, adding 100  $\mu$ l dimethyl sulfoxide (DMSO) to each well to dissolve the formazan product. Detection was achieved by monitoring the absorbance at 570 nm with a microplate reader (Bio-Rad, Hercules, CA, USA). The optical density value was used to normalize the relative cell viability relative to the control group.

### **Flow cytometry (FCM) to detect apoptosis**

SH-SY5Y cells were seeded into 6-well plates overnight, and collected by trypsinization following treatment. Washing the cells once with PBS buffer and subsequently re-suspended in  $1 \times$  binding buffer. A total of 100  $\mu$ l of cell suspension was transferred into a 5 ml test tube and mixed with 5  $\mu$ l fluorescein isothiocyanate (FITC)-Annexin V and 5  $\mu$ l propidium iodide (PI) (BD Biosciences, San Diego, CA), respectively, according to the manufacturer's specifications. We analyzed the induction of apoptosis with

a FACSCalibur flow cytometer (BD Biosciences, USA) within one hour, and analyzed the data with FlowJo software (version 7.6.1; FlowJo LLC).

### **Western blot analysis**

After 48 hours of transfection and cell culture, SH-SY5Y cells were treated with 1 mM MPP<sup>+</sup> for 24 hours, washed three times with cold PBS, and immediately lysed with RIPA lysis buffer (Beyotime, Shanghai, China). The lysate was centrifuged at 12,000 rpm at 4°C for 10 minutes, and the total protein level was measured by the BCA protein kit (Pierce, USA). Equal amounts of protein samples were separated using a sodium dodecyl sulfate (SDS) gel-polyacrylamide gel electrophoresis (PAGE), and then transfer it to the PVDF membranes. After sealing with 5% skim milk for 1 hour, the PVDF membranes were incubated with NAIF1 (1: 1,000; Cell Signaling Technology, Inc., Danvers, MA, USA), cleaved-Caspase3 (1: 1,000; Cell Signaling Technology, Inc.), Caspase3 (1: 1,000; Cell Signaling Technology, Inc.), GAPDH (1:1,000; Cell Signaling Technology, Inc.) antibodies, immediately overnight at 4°C. The next day, the membranes were washed 3 times with PBST buffer and incubated with horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G secondary antibody (1:2,000; Cell Signaling Technology, Inc.) at 37°C for 1 hour. We finally visualized the protein bands using ECL luminescent substrate (Pierce) according to the manufacturer's instructions. The experiments were repeated for 3 times at least.

### **Lactate dehydrogenase (LDH) activity assay**

SH-SY5Y cells were cultured with 1 mM MPP<sup>+</sup> for 24 hours, then the activity of LDH released into the culture medium was measured using a lactate dehydrogenase assay kit (Jiancheng Institute of Bioengineering, China) according to the manufacturer's instructions. A micro-plate reader (Bio-Rad, Hercules, CA, USA) was used to record the absorbance at 490 nm.

### **ELISA assay**

SH-SY5Y cells were treated with MPP<sup>+</sup> for 24 hours, gathered and centrifuged to detect the expression levels of TNF- $\alpha$  and IL-1 $\beta$  using an ELISA kit (BioLegend, Inc., CA, USA) according to the instructions provided by the manufacturer. A microplate reader (Bio-Rad, Hercules, CA, USA) was employed to measure the absorbance at 450 nm.

### **Reactive oxygen species (ROS) release and superoxide dismutase (SOD) activity test**

The treated cells were incubated with 10  $\mu$ M DCFH-DA (Sigma) at 37°C for 45 minutes in the dark. A fluorescence microplate reader (Labsystems Oy, Helsinki, Finland) was used to quantify the fluorescence intensity, using an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

SH-SY5Y cells were gathered and lysed using cell lysis buffer (Beyotime, Shanghai, China). According to the manufacturer's instructions, SOD activity assay kit (China Jiancheng Institute of Biological Engineering) was used to determine the SOD activity.

## Statistical analysis

The experimental data were indicated as the mean  $\pm$  standard deviation (SD) of at least three independent experiments. SPSS 13.0 software was performed for statistical analysis. The difference between the two groups were determined by Student's t-test, and the one-way analysis of variance followed by Bonferroni post-hoc test was applied to analyze the difference between multiple groups. The p-value below 0.05 ( $P < 0.05$ ) was identified as a significant difference.

## Results

### MiR-942-5p is a target of lncRNA SOX2-OT

In order to explore the molecular mechanism of lncRNA SOX2-OT and miR-942-5p in PD, the correlation between miR-942-5p and lncRNA SOX2-OT was examined. Starbase results demonstrated that the binding sites between lncRNA SOX2-OT and miR-942-5p (Figure 1A). Besides, a luciferase reporter assay was performed in 293T cells in order to confirm the predicted binding sites of lncRNA SOX2-OT and miR-942-5p. The experimental results confirmed the direct targeting relationship between lncRNA SOX2-OT and miR-942-5p (Figure 1B). Moreover, we confirmed that miR-942-5p mimic significantly enhanced miR-942-5p expression in 293T cells (Figure 1C).

### MPP<sup>+</sup> treatment causes an increase in the expression levels of lncRNA SOX2-OT and a decrease in the expression levels of miR-942-5p in SH-SY5Y cells

In order to research the function of lncRNA SOX2-OT and miR-942-5p in PD, the expression level of lncRNA SOX2-OT and miR-942-5p were examined by qRT-PCR in SH-SY5Y cells. And SH-SY5Y cells were treated with 0, 0.25, 0.5, 1 or 2 mM MPP<sup>+</sup> (Sigma, St. Louis, MO, USA) for 24 h, or exposed to 1 mM MPP<sup>+</sup> for 0, 6, 12, 24 or 48 h. The experimental results demonstrated that compared to the control group, MPP<sup>+</sup> increased the expression level of SOX2-OT (Figure 2A, 2B) in a dose-dependent and time-dependent manner, and reduced miR-942-5p (Figure 2C, 2D) expressed in SH-SY5Y cells.

### lncRNA SOX2-OT affects miR-942-5p expression in SH-SY5Y cells

To explore the contribution of lncRNA SOX2-OT and miR-942-5p to the development of PD, the SH-SY5Y cells were transfected with control-siRNA, SOX2-OT-siRNA, inhibitor, control, miR-942-5p inhibitor, SOX2-OT-siRNA + inhibitor control or SOX2-OT-siRNA + miR-942-5p inhibitor for 48 h. The transfection efficiency was measured by qRT-PCR. The experimental results showed that compared with the control-siRNA group, SOX2-OT-siRNA significantly reduced the expression of SOX2-OT in SH-SY5Y cells (Figure 3A). Compared with the inhibitor control group, miR-942-5p inhibitor observably decreased the expression of miR-942-5p in SH-SY5Y cells (Figure 3B). Compared to the control-siRNA group, SOX2-OT-siRNA observably improved the expression of miR-942-5p in SH-SY5Y cells, and the effect was eliminated by miR-942-5p inhibitor (Figure 3C).

## **LncRNA SOX2-OT influences neuronal apoptosis, inflammatory response and the induction of oxidative stress in MPP<sup>+</sup>-induced SHSY5Y cells**

To explore the mechanism of lncRNA SOX2-OT affecting PD, the SH-SY5Y cells were transfected with control-siRNA, SOX2-OT-siRNA, SOX2-OT-siRNA + inhibitor control or SOX2-OT-siRNA + miR-942-5p inhibitor for 48 h, and then treated with 1 mM MPP<sup>+</sup> for 24 h. These cells were divided into the following groups: control group; MPP<sup>+</sup> group; MPP<sup>+</sup> + control-siRNA group; MPP<sup>+</sup> + SOX2-OT-siRNA group; MPP<sup>+</sup> + SOX2-OT-siRNA + inhibitor control group; MPP<sup>+</sup> + SOX2-OT-siRNA + miR-942-5p inhibitor group. Subsequently, MTT detected the cell viability; LDH assay detected LDH activity; flow cytometry detected apoptosis; western blotting detected the protein expression of cleaved-Caspase3 and calculated the ratio of cleaved-Caspase3/Caspase3; ELISA was used to examine the secretion of TNF- $\alpha$  and IL-1 $\beta$ ; ROS release and SOD activity were also tested. The results revealed that compared to the control group, MPP<sup>+</sup> treatment observably decreased SH-SY5Y cell viability (Figure 4A), increased the activity of LDH (Figure 4B), induced apoptosis (Figure 4C and D), increased cleaved-Caspase3 protein expression (Figure 4E), and enhanced the ratio of cleaved-Caspase3/Caspase3 (Figure 4F); compared with the MPP<sup>+</sup> + control-siRNA treatment group, SOX2-OT-siRNA increased the viability of SH-SY5Y cells, decreased the activity of LDH, reduced cell apoptosis, and decreased cleaved-Caspase3 protein expression, and reduced the ratio of cleaved-Caspase3/Caspase3, and these effects were significantly eliminated by miR-942-5p inhibitor. At the same time, MPP<sup>+</sup> treatment significantly increased the levels of TNF- $\alpha$  (Figure 5A), IL-1 $\beta$  (Figure 5B) and ROS (Figure 5C) in SH-SY5Y cells, and decreased SOD activity (Figure 5D); and compared to the MPP<sup>+</sup> + control-siRNA group, SOX2-OT-siRNA significantly reduced the levels of TNF- $\alpha$  (Figure 5A), IL-1 $\beta$  (Figure 5B) and ROS (Figure 5C) in SH-SY5Y cells, and increased SOD activity (Figure 5D). These effects were significantly eliminated by miR-942-5p inhibitor.

### **NAIF1 is a target of miR-942-5p**

In order to study the molecular mechanism of miR-942-5p and NAIF1 in PD, the relationship between miR-942-5p and NAIF1 was examined. TargetScan results indicated the binding sites between miR-942-5p and NAIF1 (Figure 6A). Moreover, a luciferase reporter assay was performed in 293T cells in order to ascertain the predicted binding sites of NAIF1 and miR-942-5p. The experimental results confirmed the direct targeting relationship between miR-942-5p and NAIF1 (Figure 6B).

Besides, the expression level of NAIF1 were detected by qRT-PCR in SH-SY5Y cells treated with MPP<sup>+</sup>. And SH-SY5Y cells were treated with 0, 0.25, 0.5, 1 or 2 mM MPP<sup>+</sup> (Sigma, St. Louis, MO, USA) for 24 h, or exposed to 1 mM MPP<sup>+</sup> for 0, 6, 12, 24 or 48 h. The experimental results revealed that compared to the control group, MPP<sup>+</sup> increased the mRNA expression level of NAIF1 (Figure 6C, 6D) in a dose-dependent and time-dependent manner in SH-SY5Y cells.

### **MiR-942-5p negatively regulate NAIF1 expression in SH-SY5Y cells**

To explore the contribution of miR-942-5p and NAIF1 to the development of PD, the SH-SY5Y cells were transfected with mimic control, miR-942-5p mimic, control-plasmid, NAIF1-plasmid, miR-942-5p mimic + control-plasmid or miR-942-5p mimic + NAIF1-plasmid for 48 h. The transfection efficiency was determined by qRT-PCR and western blotting. The experimental results revealed that compared to the mimic control group, miR-942-5p mimic observably increased the expression of miR-942-5p in SH-SY5Y cells (Figure 7A); Compared to the control-plasmid group, NAIF1-plasmid observably increased the expression of NAIF1 mRNA in SH-SY5Y cells (Figure 7B). Compared with the mimic control group, miR-942-5p mimic significantly reduced the expression of NAIF1 mRNA and protein in SH-SY5Y cells, and these reductions were significantly eliminated by NAIF1-plasmid (Figure 7C and D).

### **MiR-942-5p influences the neuronal apoptosis, inflammatory response and the induction of oxidative stress in MPP<sup>+</sup>-induced SHSY5Y cells**

To explore the mechanism of miR-942-5p affecting PD, the SH-SY5Y cells were transfected with mimic control, miR-942-5p mimic, miR-942-5p mimic + control-plasmid or miR-942-5p mimic + NAIF1-plasmid for 48 h, and then treated with 1mM MPP<sup>+</sup> for 24 h. These cells were divided into the following groups: Control, MPP<sup>+</sup>, MPP<sup>+</sup> + mimic control group; MPP<sup>+</sup> + miR-942-5p mimic group; MPP<sup>+</sup> + miR-942-5p mimic + control-plasmid group; MPP<sup>+</sup> + miR-942-5p mimic + NAIF1-plasmid group. The results showed that compared with the MPP<sup>+</sup> + mimic control treatment group, miR-942-5p mimic significantly increased SH-SY5Y cell viability (Figure 8A), reduced the activity of LDH (Figure 8B), reduced cell apoptosis (Figure 8C, 8D), reduced cleaved-Caspase3 protein expression (Figure 8E), and reduce the ratio of cleaved-Caspase3/Caspase3 (Figure 8F), and all these effects were significantly eliminated by NAIF1-plasmid.

Compared to the MPP<sup>+</sup> + mimic control group, miR-942-5p mimic significantly reduced the levels of TNF- $\alpha$  (Figure 9A), IL-1 $\beta$  (Figure 9B) and ROS (Figure 9C) in SH-SY5Y cells, and increased SOD activity (Figure 9D). These effects were significantly eliminated by NAIF1-plasmid.

## **Discussion**

LncRNAs had appeared as a novel regulator of neurogenesis [23]. SOX2OT as a lncRNAs which harbors one of the primary regulator of pluripotency [24], at present, studies had proved that lncRNA was significantly highly expressed in PD [25–27]. In addition, a mass of studies had proved that miRNAs can participate in the development of various diseases by regulating the expression levels of key factors for cell growth and apoptosis [28–30], there were reports in the literature that there was a targeting relationship between NAIF1 and certain miRNAs [31]. However, inflammation, oxidative stress and neuronal apoptosis were also related to the pathogenesis of PD. These evidences indicated that lncRNA may have a certain relationship with miRNAs and NAIF1 to participate in the development of PD, but the specific mechanism is still unclear.

In this study, the prediction analysis confirmed that lncRNA SOX2-OT directly targeted miR-942-5p and NAIF1. This research revealed that the expression level of lncRNA SOX2-OT and NAIF1 were increased

significantly, while the expression level of miR-942-5p was decreased following an increase in the concentration levels of MPP<sup>+</sup> and in the treatment period, which was similar to the results of previous studies [1, 21]. In addition, the effects of lncRNA SOX2-OT, miR-942-5p and NAIF1 were studied on the *in vitro* PD cell model, MPP<sup>+</sup> was a commonly used substance for establishing PD *in vitro* models [32]. The results demonstrated that the downregulation of the expression level of lncRNA SOX2-OT significantly increased SH-SY5Y cell viability, reduced cell apoptosis, and decreased LDH activity in MPP<sup>+</sup>-treated SH-SY5Y cells, and these effects were significantly eliminated by miR-942-5p inhibitor. Apoptosis or necrosis can cause cell membrane rupture to release LDH, the released LDH activity can represent the amount of cell necrosis and was widely used in cytotoxicity testing [33]. Furthermore, preceding studies had shown that proinflammatory factors were related to the pathogenesis of PD [34]. In our current research, ELISA was employed to examine IL-1 $\beta$  and TNF- $\alpha$  and other pro-inflammatory factors, and western blotting was employed to examine the expression level of cleaved-Caspase3 protein. The results manifested that the down-regulated expression of lncRNA SOX2-OT leads to decrease of the expression levels of TNF- $\alpha$ , IL-1 $\beta$  and cleaved-Caspase3 protein in SH-SY5Y cells treated with MPP<sup>+</sup>, moreover, reduces the cleaved-Caspase3/Caspase3 ratio. In addition, we further noticed that the down-regulated expression of lncRNA SOX2-OT significantly reduced the level of ROS and enhanced the SOD activity in MPP<sup>+</sup>-treated SH-SY5Y cells in the *in vitro* PD cell model. These results showed that the lncRNA SOX2-OT inhibition suppresses MPP<sup>+</sup>-induced oxidative stress, however, miR-942-5p inhibitor significantly reversed all the above effects in SH SY5Y cells treated with MPP<sup>+</sup>. Further studies had shown that miR-942-5p mimic significantly improves SH-SY5Y cell viability, reduces cell apoptosis, the expression of cleaved-Caspase3 protein, the ratio of cleaved-Caspase3/Caspase3, the LDH activity, the levels of TNF- $\alpha$ , IL-1 $\beta$  and ROS in SH-SY5Y cells, and increase the activity of SOD, but these effects were significantly eliminated by NAIF1-plasmid; these results indicated that lncRNA SOX2-OT modulated the inflammatory response and the induction of oxidative stress and neuronal apoptosis by targeting miR-942-5p and NAIF1 in an *in vitro* PD cell model.

In summary, our experiments have proved that lncRNA SOX2-OT regulates inflammation, oxidative stress and neuronal apoptosis by directly regulating the miR-942-5p/NAIF1 signal axis, and then participates in the occurrence and development of Parkinson's. The present study provides a novel potential target for PD diagnosis and therapy.

## Declarations

### Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

### Competing interests

The authors declare that they have no competing interests.

## References

1. Wang M, Sun H, Yao Y, Tang X, Wu B. MicroRNA-217/138-5p downregulation inhibits inflammatory response, oxidative stress and the induction of neuronal apoptosis in MPP<sup>+</sup>-induced SH-SY5Y cells. *Am J Transl Res* 2019;11(10):6619-6631.
2. Dauer W, Przedborski S. Parkinson's disease: mechanism and models. *Neuron* 2003;39(6):889-909.
3. Patel S, Singh V, Kumar A, Gupta YK, Singh MP. Status of antioxidant defense system and expression of toxicant responsive genes in striatum of maneb- and paraquat-induced Parkinson's disease phenotype in mouse: mechanism of neurodegeneration. *Brain Res* 2006;1081(1):9-18.
4. Skaper SD. The Brain as a Target for Inflammatory Processes and Neuroprotective Strategies. *Ann N Y Acad Sci* 2007;1122:23-34.
5. Moore DJ, West AB, Dawson VL, Dawson TM. Molecular pathophysiology of Parkinson's disease. *Annu Rev Neurosci* 2005;28:57-87.
6. Ng SY, Lin L, Soh BS, Stanton LW. Long noncoding RNAs in development and disease of the central nervous system. *Trends Genet* 2013;29(8):461-468.
7. Kraus T, Haider M, Spanner J, Steinmaurer M, Dietinger V, Kretschmar HA. Altered Long Noncoding RNA Expression Precedes the Course of Parkinson's Disease—a Preliminary Report. *Mol Neurobiol* 2017;54(4):2869-2877.
8. Wang Y, Chen ZY, Chen JQ, Chen HM. LncRNA NEAT1 promotes autophagy in MPTP-induced Parkinson's disease through stabilizing PINK1 protein. *Biochem Biophys Res Commun* 2017;496(4):1019-1024.
9. Long L, Cai X, Liao J, Ma X, Chen X. H<sub>2</sub>S alleviates Parkinson-like phenotypes by modulating lncRNA-CasC7/miR-30c/BNIP3L signaling pathway. *Archives of Medical Science* 2021.
10. Lu Y, Gong Z, Jin X, Zhao P, Wang Z. LncRNA MALAT1 targeting miR4 regulates DAPK1 expression contributes to cell apoptosis in Parkinson's Disease. *J Cell Biochem* 2020;121(12):4838-4848.
11. Xie N, J Qi, Li S, Deng J, Chen Y, Lian Y. Upregulated lncRNA small nucleolar RNA host gene 1 promotes 1-methyl-4-phenylpyridiniumion-induced cytotoxicity and reactive oxygen species production through miR-15b-5p/GSK3- $\beta$ axis in human dopaminergic SH-SY5Y cells. *J Cell Biochem* 2019;120(4):5790-5801.
12. Wo Q, Zhang D, Hu L, Jia L, Qi X. Long noncoding RNA SOX2-OT facilitates prostate cancer cell proliferation and migration via miR-369-3p/CFL2 axis. *Biochem Biophys Res Commun* 2019;520(3):586-593.
13. Lu TX, Rothenberg ME. MicroRNA. *J Allergy Clin Immunol* 2018;141(4):1202-1207.
14. Li M, Marin-Muller C, Bharadwaj U, Chow KH, Yao QZ, Chen CY. MicroRNAs: control and loss of control in human physiology and disease. *World J Surg* 2009;33(4):667-684.
15. Nuzziello N, Craig F, Simone M, Consiglio A, Liguori M. Integrated Analysis of microRNA and mRNA Expression Profiles: An Attempt to Disentangle the Complex Interaction Network in Attention Deficit Hyperactivity Disorder. *Brain sci* 2019;9(10):288.

16. Flower TR, Chesnokova LS, Froelich CA. Heat Shock Prevents Alpha-synuclein-induced Apoptosis in a Yeast Model of Parkinsons Disease. *J Mol Biol* 2005;351(5):1081-1100.
17. Banati RB, Daniel SE, Blunt SB. Glial pathology but absence of apoptotic nigral neurons in long-standing Parkinson's disease. *Mov Disord* 2010;13(2):221-227.
18. Luo Q, Zhao M, Zhong J, Ma Y, Deng G, Liu J, Wang J, Yuan X, Huang C. NAIF1 is down-regulated in gastric cancer and promotes apoptosis through the caspase-9 pathway in human MKN45 cells. *Oncol Rep* 2011;25(4):1117-1123.
19. Lv B, Shi T, Wang X, Song Q, Zhang Y, Shen Y, Ma D, Lou Y. Overexpression of the novel human gene, nuclear apoptosis-inducing factor 1, induces apoptosis. *Int J Biochem Cell Biol* 2006;38(4):671-683.
20. Kong D, Zhang Z. NAIF1 suppresses osteosarcoma progression and is regulated by miR-128. *Cell Biochem Funct* 2018;36(8):443-449.
21. Geng L, Zhang T, Liu W, Chen Y. miR-494-3p modulates the progression of *in vitro* and *in vivo* Parkinson's disease models by targeting SIRT3. *Neurosci Lett* 2018;675:23-30.
22. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2- $\Delta\Delta$ CT method. *Methods* 2001;25:402-408.
23. Shahryari A R, Malakootian M, Shafiee. Identification A Novel Regulatory Mechanism Governing One of The Key Stem Cell Pluripotency Genes, SOX2, Mediated by The Non-Coding RNA SOX2OT. *Cell Journal* 2013;15:77-78.
24. Alireza S, Saghaeian JM, Samaei NM, Mowla SJ. Long non-coding RNA SOX2OT: expression signature, splicing patterns, and emerging roles in pluripotency and tumorigenesis. *Front Genet* 2015;6:196.
25. Wu P, Zuo X, Deng H, Liu X, Ji A. Roles of long noncoding RNAs in brain development, functional diversification and neurodegenerative diseases. *Brain Res Bull* 2013;97:69-80.
26. Ying L, Bai L, Qin C. Long noncoding RNAs in neurodevelopment and Parkinson's disease. *Animal models exp med* 2019;002(004):239-251.
27. Zhou Y, Gu C, Li J, Zhu L, Huang G, Dai J. Aberrantly expressed long noncoding RNAs and genes in Parkinson's disease. *Neuropsychiatr dis treat* 2018;14:3219-3229.
28. Lu X, Cui Z, Liu S, Yin F. MiRNAs participate in the diagnosis, pathogenesis and therapy of Parkinson's disease. *Histol Histopathol* 2017;33(5):447-453.
29. Afjeh S, Ghaderian S. The role of microRNAs in cardiovascular disease. *Int J Mol Cell Med* 2013;2(2):50-57.
30. Ventura A, Young AG, Winslow MM, Lintault L, Meissner A, Erkland SJ. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miRNA clusters. *Cell* 2008;132(5):875-886.
31. Wu X, Hu C, Long C, Zhai X, Yu Z. MicroRNA-351 Promotes the Proliferation and Invasion of Glioma Cells through Downregulation of NAIF1. *J Mol Neurosci* 2020;70(10):1493-1499.

32. Wang S, He H, Chen L, Zhang W, Zhang X, Chen J. Protective Effects of Salidroside in the MPTP/MPP<sup>+</sup>-Induced Model of Parkinson's Disease through ROS-NO-Related Mitochondrion Pathway. *Mol Neurobiol* 2015;51(2):718-728.
33. Guo H, Ning-Ning LI, Wang GX, Cai XL. Roles of reproductive hormone,LDH and PARP in apoptosis in spermatogenic cells of rats exposed to manganese. *Anatomy Res* 2016;2:24-27.
34. Depino AM, Earl C, Kaczmarczyk E, Ferrari C, Oertel WH. Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease. *Eur J Neurosci* 2015;18(10):2731-2742.

## Figures

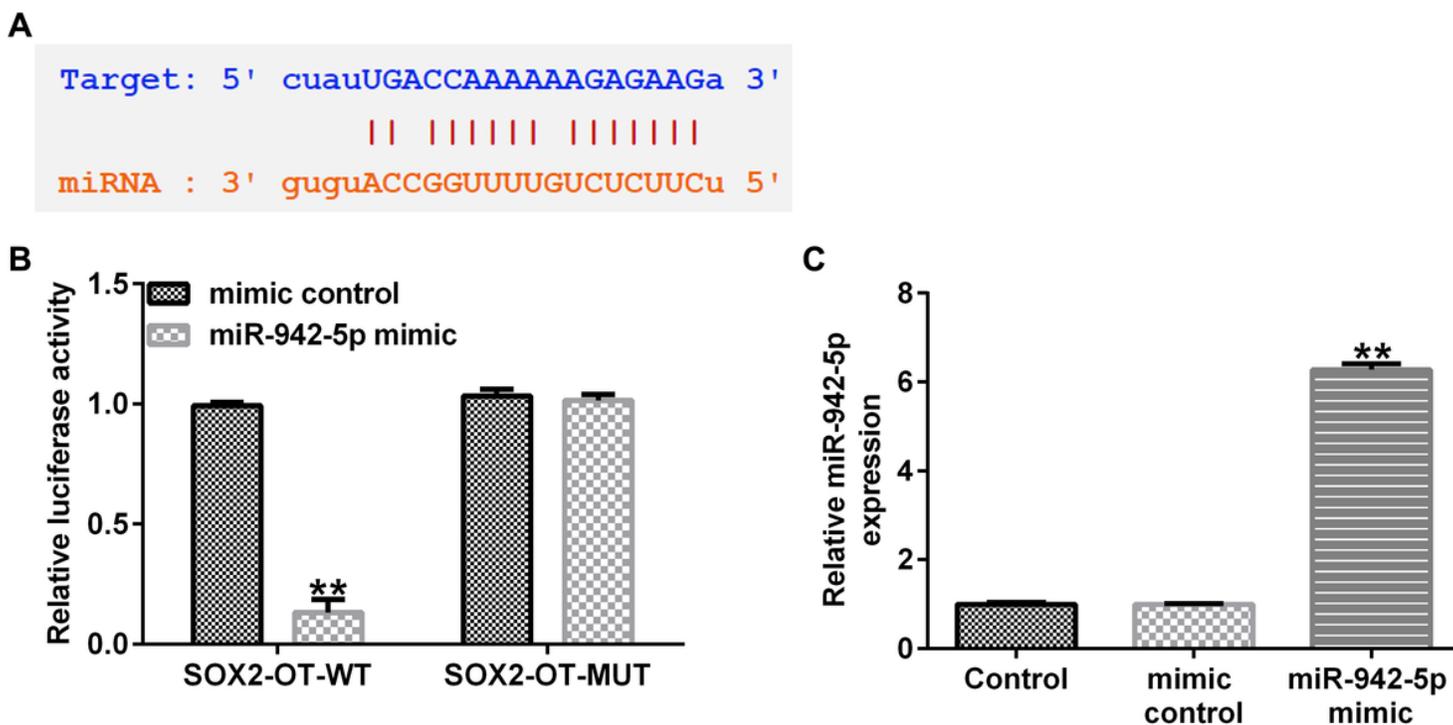
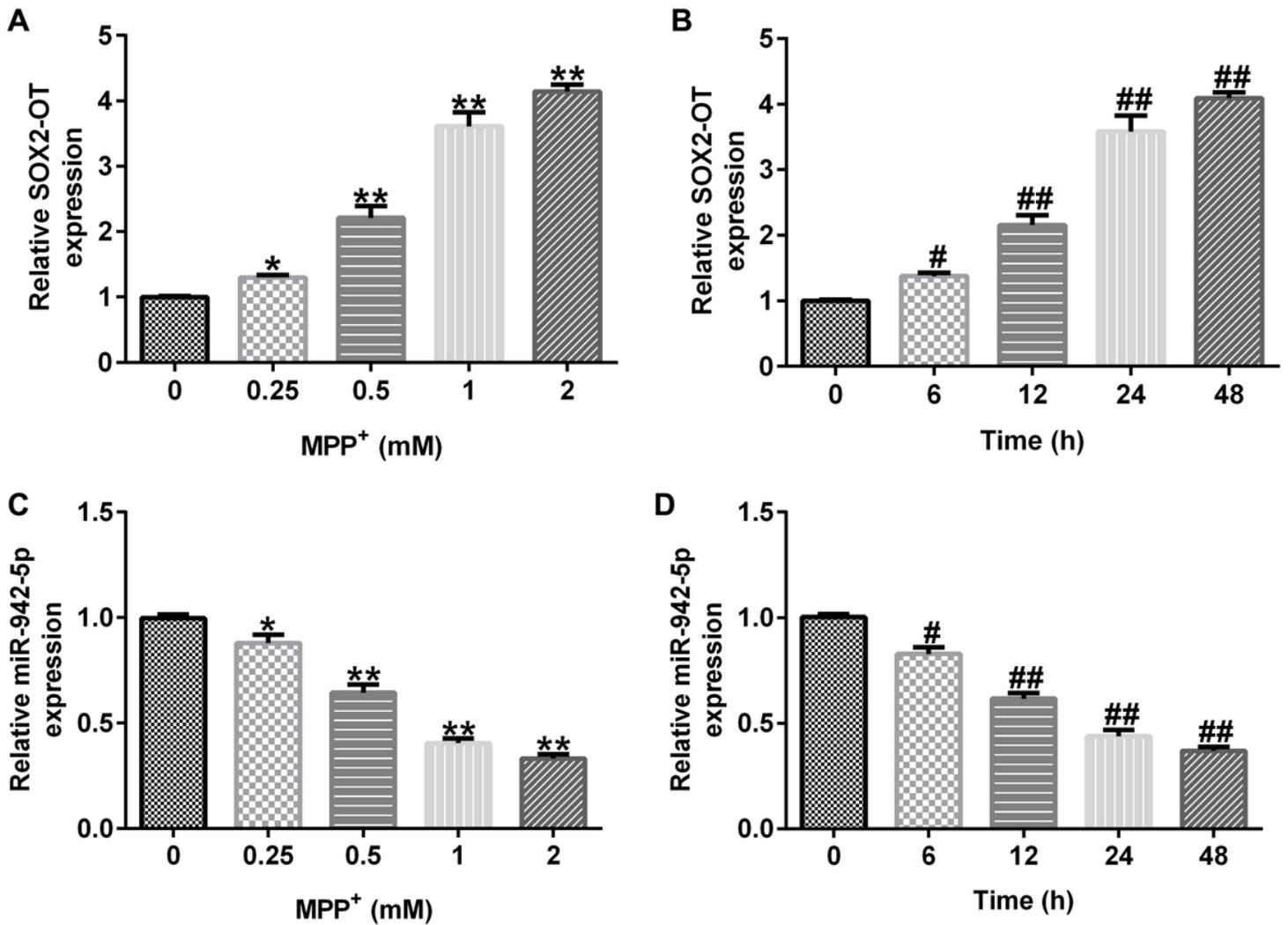


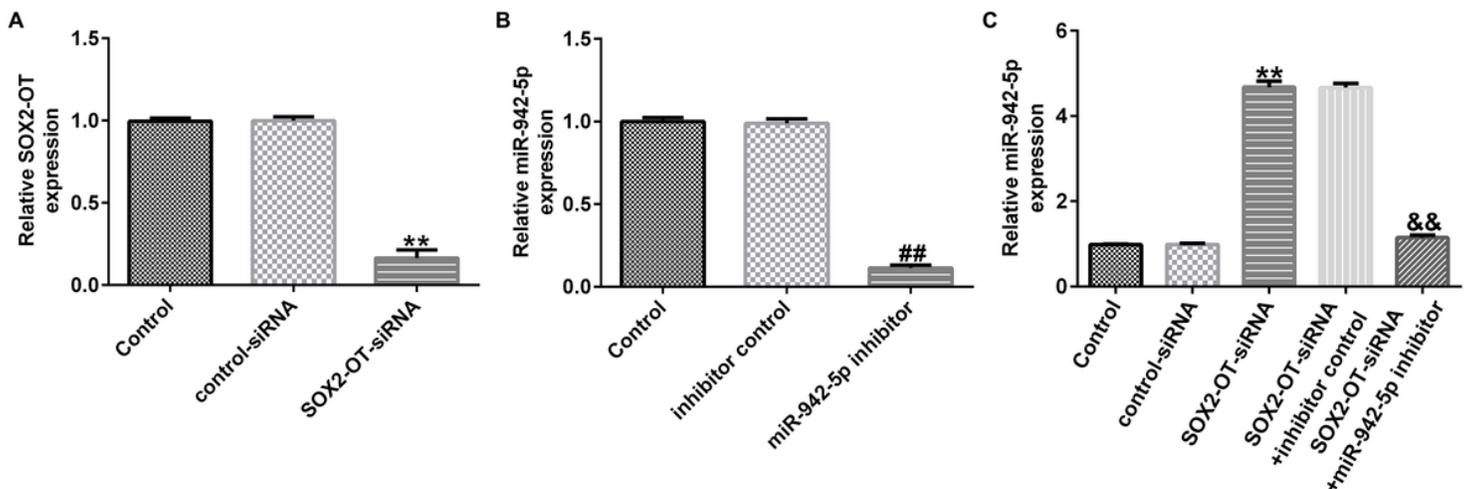
Figure 1

LncRNA SOX2-OT directly targeted miR-942-5p. A. The Starbase discovered the binding site between lncRNA SOX2-OT and miR-942-5p. B. Dual-luciferase reporter assay was used to reveal the relationship between lncRNA SOX2-OT and miR-942-5p. C. The level of miR-942-5p in 293T cells transfected with mimic control or miR-942-5p mimic was determined using qRT-PCR.



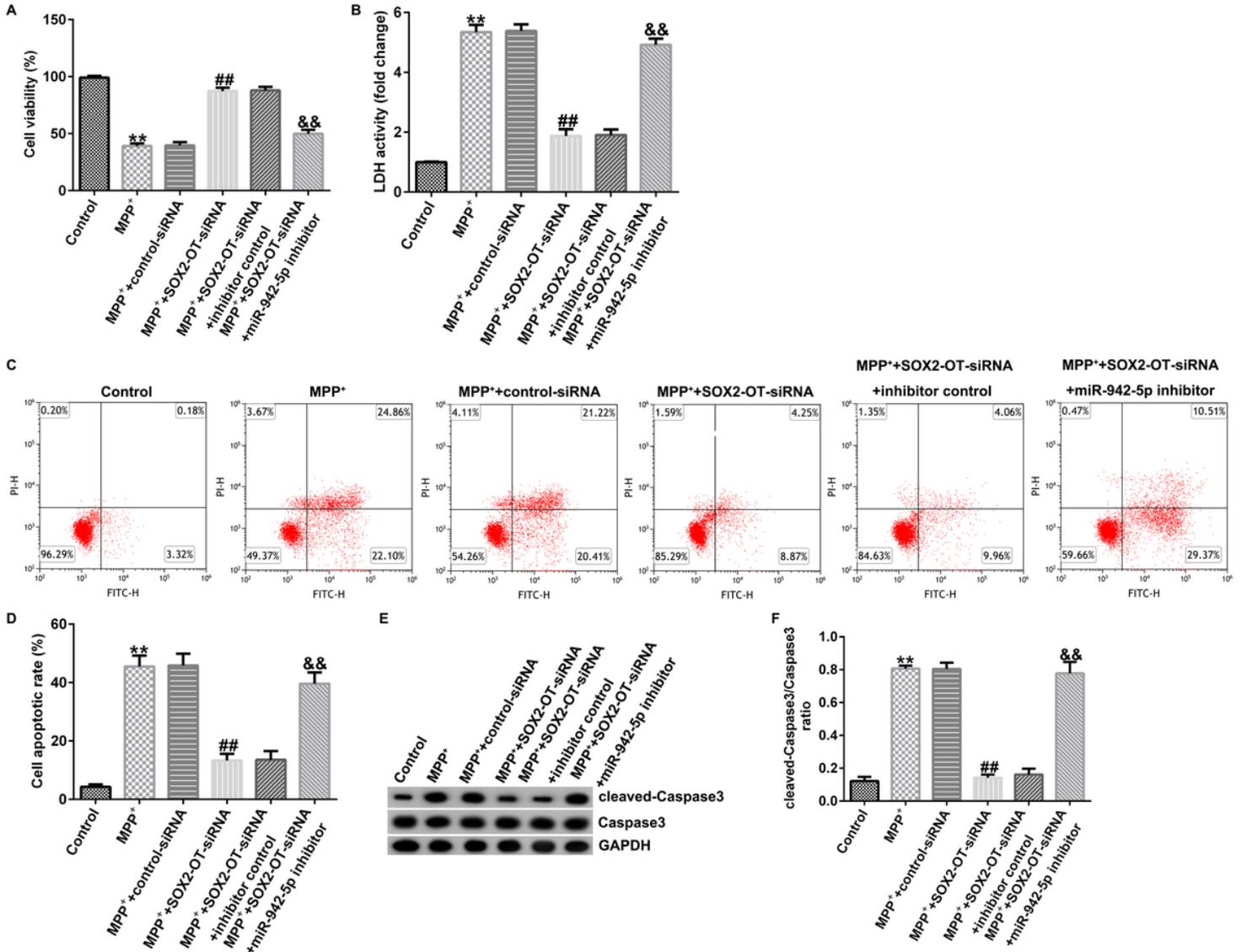
**Figure 2**

Expression of lncRNA SOX2-OT and miR-942-5p in MPP<sup>+</sup>-induced SH-SY5Y cells. The expression of SOX2-OT (A) and miR-942-5p (C) levels were analyzed by qRT-PCR in SH-SY5Y cells treated with different doses of MPP<sup>+</sup> for 24 h. The expression of SOX2-OT (B) and miR-942-5p (D) levels were also determined by qRT-PCR in SH-SY5Y cells cultured with 1 mM MPP<sup>+</sup> for different times.



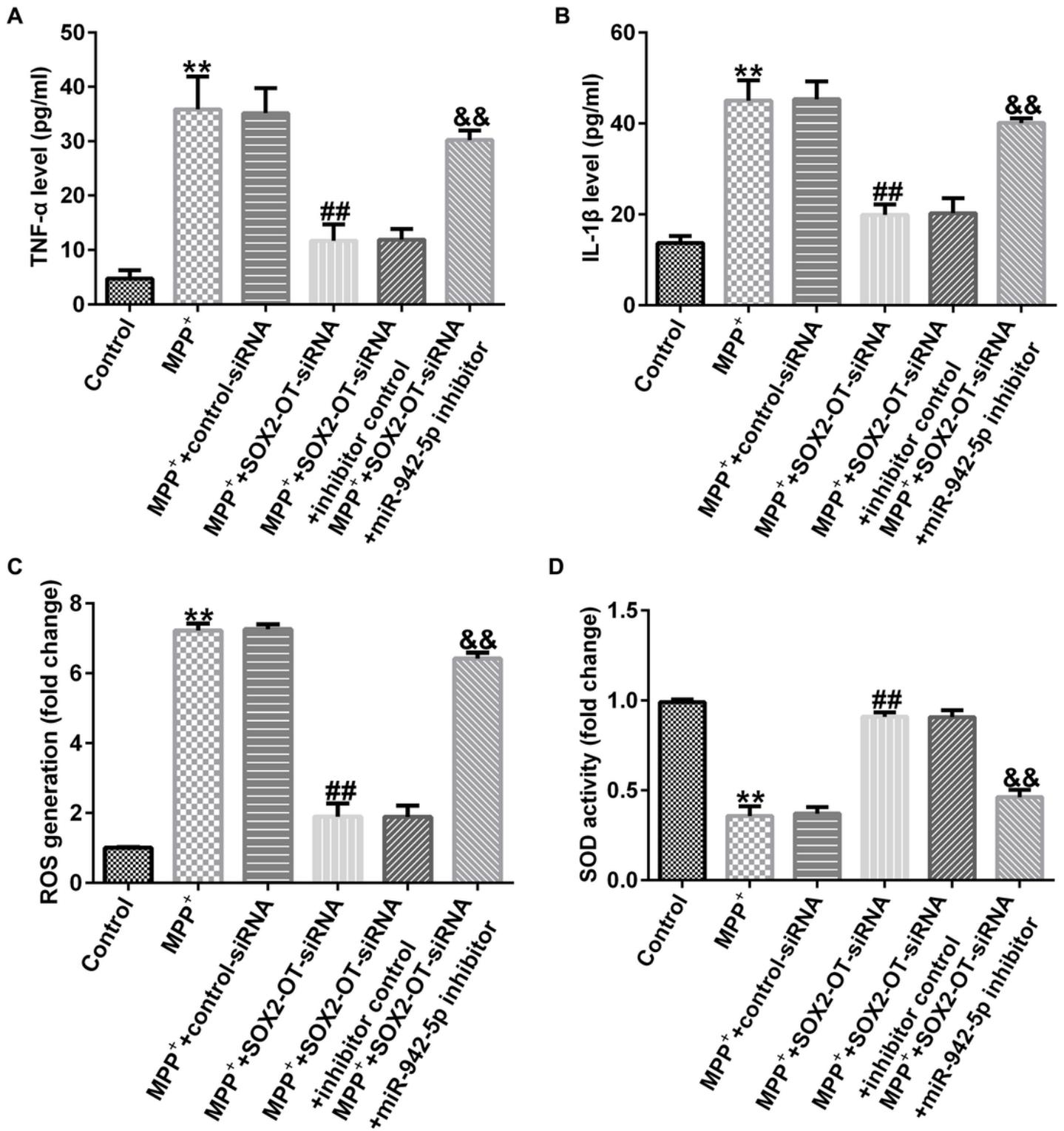
**Figure 3**

Effects of lncRNA SOX2-OT down-regulation on expression of miR-942-5p in SH-SY5Y cells. A. The level of SOX2-OT in SH-SY5Y cells was detected by qRT-PCR. B. The level of miR-942-5p in treated SH-SY5Y cells was examined by qRT-PCR assay. C. The levels of miR-942-5p in SH-SY5Y cells transfected with control-siRNA, SOX2-OT-siRNA, SOX2-OT-siRNA+inhibitor control, or SOX2-OT-siRNA+miR-942-5p inhibitor were detected by qRT-PCR assay.



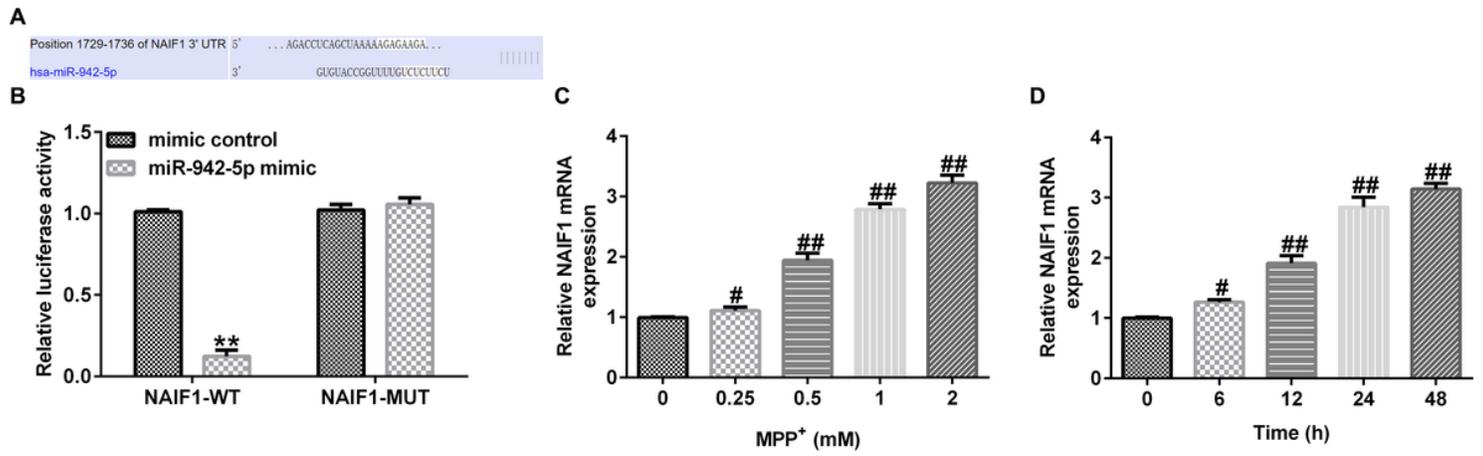
**Figure 4**

Effects of lncRNA SOX2-OT inhibition on MPP<sup>+</sup>-induced SH-SY5Y cells viability and apoptosis. A. The cell viability in SH-SY5Y cells following treatments was measured by MTT assay. B. LDH release assay was applied to detect LDH activity of treated SH-SY5Y cells. C and D. Flow cytometry analysis was employed to detect apoptotic rate of treated SH-SY5Y cells. E and F. Western blotting was employed to examine the expression of cleaved-Caspase3 protein and calculated the ratio of cleaved-Caspase3/Caspase3 of treated SH-SY5Y cells.



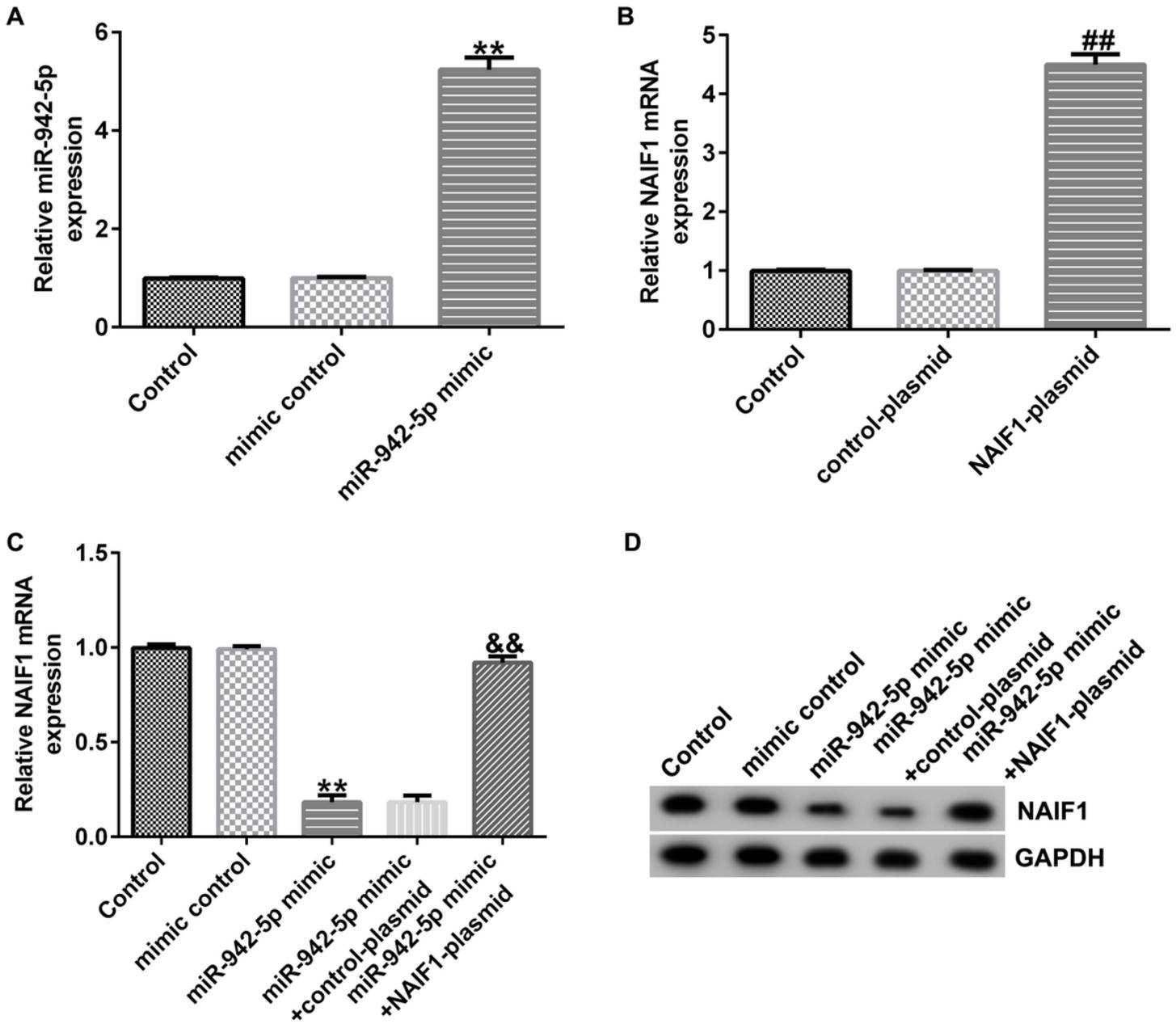
**Figure 5**

Effects of lncRNA SOX2-OT inhibition on inflammatory response and oxidative stress in MPP<sup>+</sup>-induced SH-SY5Y cells. A and B. The levels of TNF- $\alpha$  and IL-1 $\beta$  in treated SH-SY5Y cells were measured by ELISA. C and D. The intracellular level of ROS release and SOD activity.



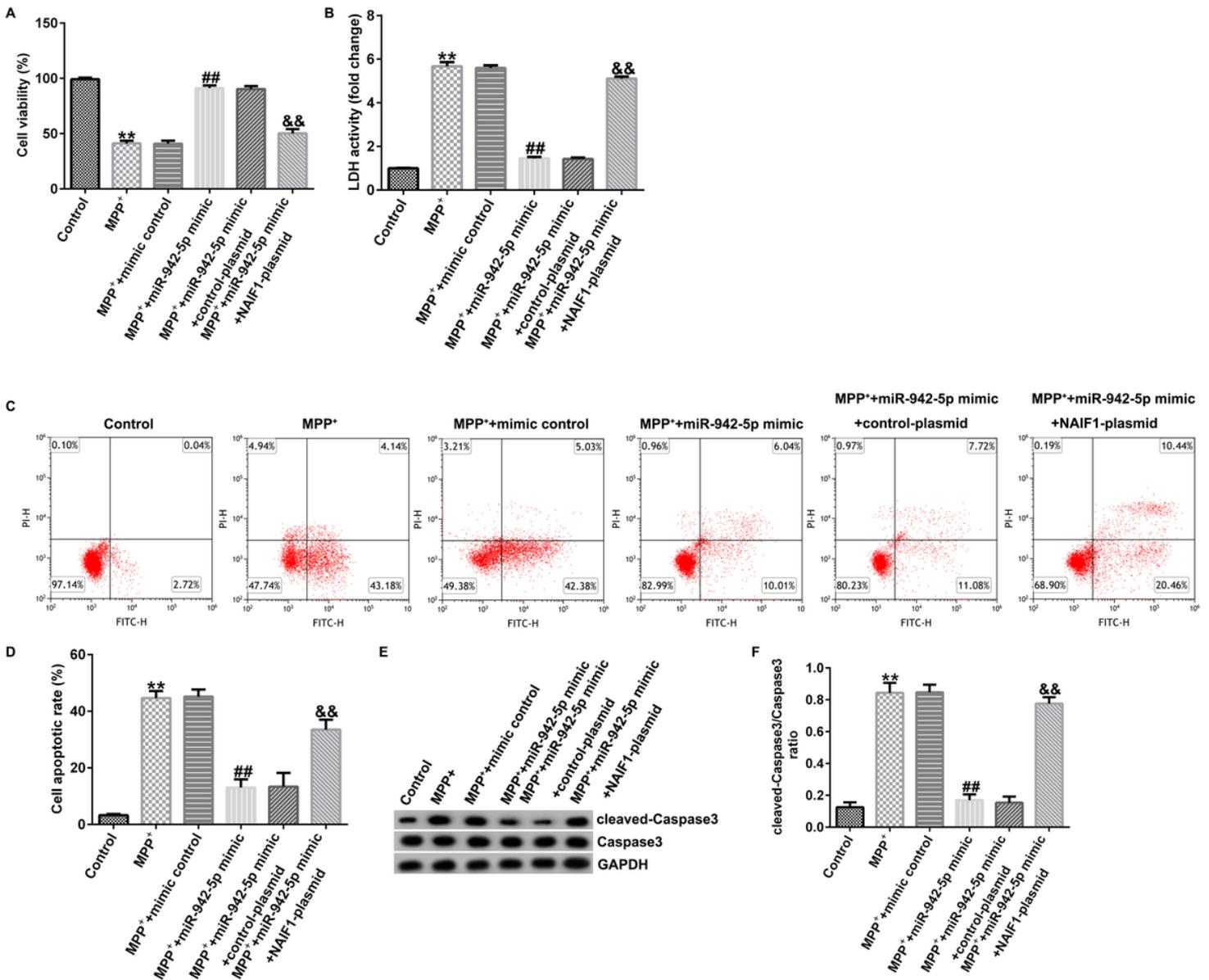
**Figure 6**

MiR-942-5p directly targeted NAIF1 and the expression of NAIF1 in MPP<sup>+</sup>-induced SH-SY5Y cells. A. The TargetScan discovered the binding site between miR-942-5p and NAIF1. B. Dual-luciferase reporter assay was used to reveal the relationship between miR-942-5p and NAIF1. C. The expression of NAIF1 levels were analyzed by qRT-PCR in SH-SY5Y cells treated with different doses of MPP<sup>+</sup> for 24 h. D. The expression of NAIF1 levels were analyzed by qRT-PCR in SH-SY5Y cells treated with 1 mM MPP<sup>+</sup> for different times.



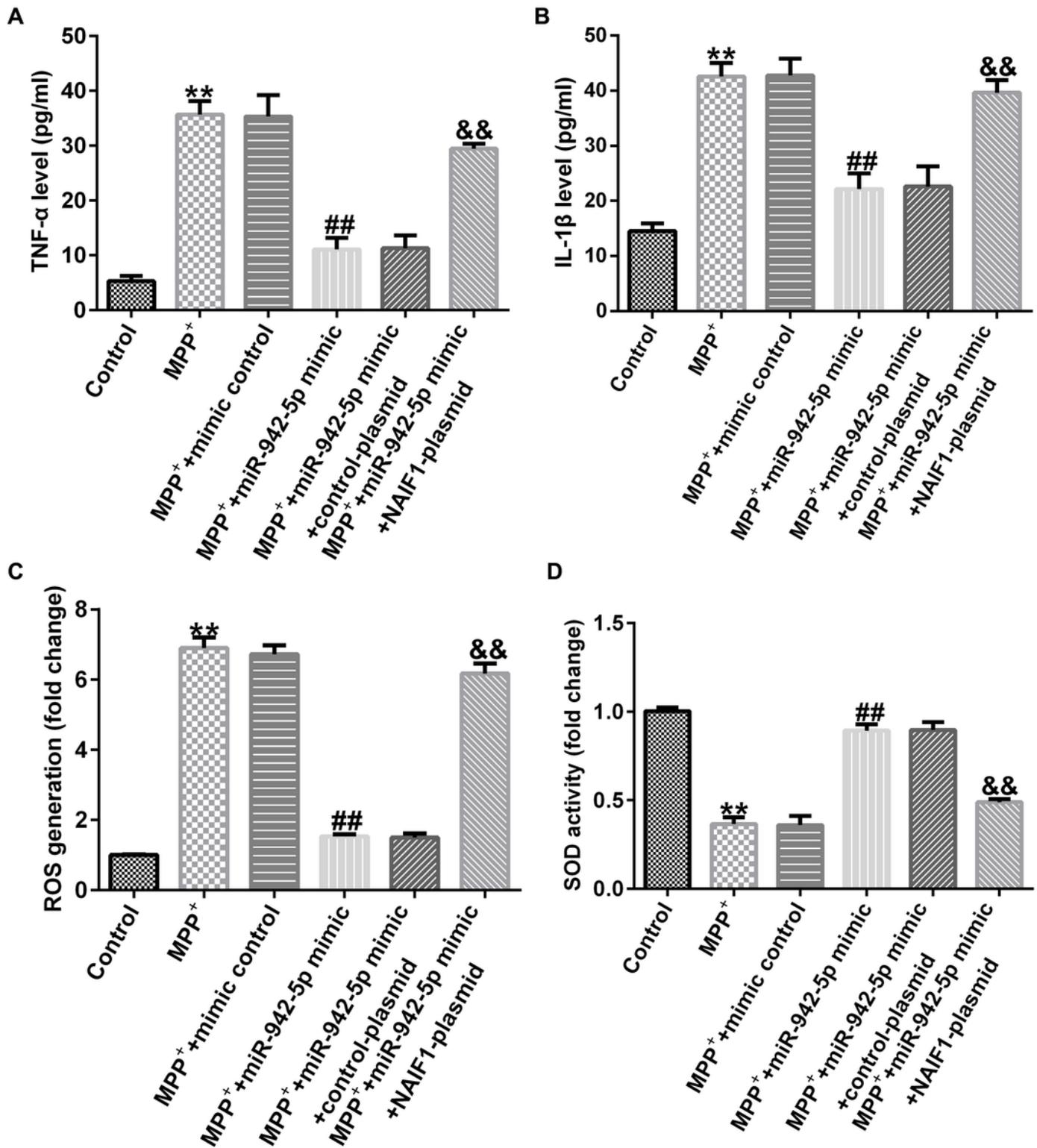
**Figure 7**

Effects of miR-942-5p up-regulation on expression of NAIF1 in SH-SY5Y cells. A. The level of miR-942-5p in SH-SY5Y cells was examined by qRT-PCR. B. The level of NAIF1 mRNA in treated SH-SY5Y cells was detected by qRT-PCR assay. C. The levels of NAIF1 mRNA and protein expression in SH-SY5Y cells transfected with mimic control, miR-942-5p mimic, miR-942-5p mimic+control-plasmid, or miR-942-5p mimic+NAIF1-plasmid were detected by qRT-PCR and western blotting assay.



**Figure 8**

Effects of miR-942-5p on MPP<sup>+</sup>-induced SH-SY5Y cells viability and apoptosis. A. The cell viability in SH-SY5Y cells following treatments was measured by MTT assay. B. LDH release assay was applied to detect LDH activity of treated SH-SY5Y cells. C and D. Flow cytometry analysis was employed to detect apoptotic rate of treated SH-SY5Y cells. E and F. Western blotting was employed to examine the expression of cleaved-Caspase3 protein and calculated the ratio of cleaved-Caspase3/Caspase3 of treated SH-SY5Y cells.



**Figure 9**

Effects of miR-942-5p on inflammatory response and oxidative stress in MPP<sup>+</sup>-induced SH-SY5Y cells. A and B. The levels of TNF- $\alpha$  and IL-1 $\beta$  in treated SH-SY5Y cells were measured by ELISA. C and D. The intracellular level of ROS release and SOD activity.