

ISGylation of STING contributes to neuroinflammation and neurodegeneration via activating c-Fos in Parkinson's disease models

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Abstract

Type-I interferons (IFNs), as key mediators of innate immune response, have been implicated in neurodegenerative diseases, including Parkinson's disease (PD). IFN exerts various biological effects by activating hundreds of interferon-stimulated genes (ISGs), including ISG15. ISG15 mediate ISGylation by covalently linking modifying target proteins including STING. The present study aims to explore whether the IFN-ISG15 pathway contributes to PD pathogenesis by ISGylating its target STING. This study showed that ISG15 and ISGylation of STING (ISG-STING) were induced in *in vitro* PD models, including MPP⁺-treated and lentiviral synucleinA53T-infected SH-SY5Y cells. Transcriptomic analyses showed that c-Fos, an immediate early gene (IEG), is prominently induced by overexpression of STING.

Overexpression of STING-WT promoted ISGylation of STING, increased c-Fos and cell death in MPP⁺ treated SH-SY5Y, while non-ISGylation mutation STING K289A and shISG15 reduced the ISG-STING, c-Fos, and cell death. In *in vivo* PD models, including MPTP-injected and AAV-synucleinA53T mice, STING-K289A and shISG15 reduced the ISG15, ISG-STING, c-Fos, neuroinflammation, and dopaminergic neuronal death, and restored compromised behaviors. The present study supported that ISGylation of STING augmented its function and activity (gain-of-function), promoted PD pathogenesis by activating c-Fos and driving neuroinflammation. ISG-STING augments the induction of downstream IFN and ISG15, which further ISGylates STING, creating a positive feedback loop and potentiating the progression of neurodegenerative disease. Targeting ISG15-mediated ISGylation or ISG15-interacting proteins may provide a novel therapeutic approach for PD.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease, primarily characterized by the gradual degeneration of dopaminergic neurons (DN) in the substantia nigra compacta (SNc) and the formation of Lewy bodies. Clinical manifestations include motor symptoms such as resting tremor, muscle rigidity, bradykinesia, and postural instability, as well as non-motor symptoms such as constipation, sleep disturbances, and cognitive and psychiatric disorders[1, 2]. Studies suggest that PD is attributed to the aggregation of misfolded α -synuclein, neuroinflammation, and mitochondrial and endoplasmic reticulum-lysosome dysfunction[1, 2]. The main component of Lewy bodies, the pathological lesions, is extensively phosphorylated α -synuclein at Ser129 (pS129 α -synuclein), which serves as a decisive marker for PD and related synucleinopathies[3]. Relevant data indicate that the incidence rate of PD among individuals aged 65 and above in China is 1.6%, and it is projected to rise to 5 million by 2030, accounting for almost half of the global PD population [4]. There is an increasingly serious global public health burden for PD patients, caregivers, and socio-economic systems. Currently, there is a lack of effective treatments for PD, making it particularly urgent to understand the pathogenesis and explore treatment strategies for PD.

Neuroinflammation, reactive astrocytes, and microglia are prominent pathological features and crucial pathogenic mechanisms of patients with PD [5]. Within the neuroinflammatory cascade, type I interferons (IFNs) are key mediators of the innate immune response and are involved in the PD

pathogenesis [6]. Type I interferons are fundamental in protecting humans from external pathogens, such as neurotropic viruses. However, current studies indicated that chronic and prolonged interferon responses played a neurotoxic role and were key drivers for the pathogenesis of neurodegenerative diseases[7–9]. Type-I IFN pathway and its downstream interferon-stimulated gene (ISG) products are significantly elevated in the brains of clinically diagnosed AD patients[10]. Mice lacking the type-I IFN receptor (IFNAR1^{-/-}) displayed attenuated neuroinflammation and microglial activation in MPTP-injected mice [11], indicating that type-I IFNs played a detrimental role in the PD model. The IFN family is divided into three subfamilies: type I IFN (IFN-Is) (including 13 homologous human subtypes such as IFN- α /IFN- β); type II IFN (containing IFN- γ), and type III IFN (IFN- λ 1–3). IFN α / β binds to the IFNAR1/2 receptor. IFN-I activates and phosphorylates STAT1/2, binds to IRF9, initiates the transcription of interferon-stimulated genes (ISGs), antiviral genes, and IFN-I itself[12].

By activating hundreds of downstream interferon-stimulated genes (ISGs), Interferons exert various biological effects, such as antiviral and immune regulation [9]. ISG15 (interferon-stimulated gene 15) is an ISG and a ubiquitin-like protein (UBL) with a molecular weight of approximately 15 kD. Similar to ubiquitination, ISG15 shares homology with ubiquitin and requires a three-step enzymatic cascade involving E1, E2, and E3 conjugates to catalyze substrate modification[13–15]. ISG15 is covalently linked to lysine residues on target proteins via its C-terminal LRLRGG motif, leading to a post-translational modification known as ISGylation[13, 16]. Unlike ubiquitination, ISG15 modification does not necessarily lead to degradation of the target protein, and its impact on protein function is complex and diverse[14]. Multiple ISG15 substrates have been identified, including p53, NLRP3, and STING[15]. It is reported that activated STING underwent ISGylation at residue K289 by ISG15 [17]. The STING-K289A mutation blocked ISGylation and suppressed the interferon response [16]. Some studies have shown that ISG15 was abnormally elevated in patients with amyotrophic lateral sclerosis (ALS) and SOD1G93A-ALS mice [18]. In addition, ISG15 expression increased in the MCAO cerebral ischemia model and was mainly limited to neurons and astrocytes [19]. These suggest that ISG15-mediated protein ISGylation is involved in the pathogenesis of neurodegenerative diseases. However, The role of ISG15 and ISGylation in PD is not yet fully investigated. This study aims to determine whether the IFN-ISG15 pathway contributes to PD pathogenesis by ISGylating downstream targets such as STING.

Methods

Plasmid and Lentiviral vector

The full-length human STING cDNA sequence was purchased from Mailgene (NCBI: NM_198282.4; 1140 bp). The STING-WT and STING-K289A mutant sequences were obtained using PCR and site-directed mutagenesis. These sequences were inserted into the lentiviral plasmid pPLVX-CMV-AcGFP1-N1 (Clontech, product number: 632154) to generate the plasmids PPLV-STING-V5-P2A-AcGFP-WT and -K289A. Three pairs of shRNA sequences targeting both human and mouse ISG15 were designed by General Biol Company (<http://www.generalbiol.com/>; China). After gene sequencing verification, the lentiviral plasmids were used to generate lentiviral particles by General Biol Company. The lentiviral

packaging system consisted of three plasmids (PPLV, pAX2, pMD2G), and the viral titer was determined by qPCR, and the virus was aliquoted and stored at -80°C.

Cell culture

The human neuroblastoma cell line SH-SY5Y (Cat: CL-0208) and the mouse cerebellar astrocytes cell line C8-D1A (Cat: CL-0506) were obtained from Procella (China). Cells were maintained in Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS; PAN Biotech, Germany) and 1% penicillin/streptomycin (Solarbio, China). Cells were cultured in a humidified atmosphere containing 5% CO₂ at 37 °C. Upon reaching 50–60% confluence, the cells were infected with lentivirus (MOI: 5) and subjected to subsequent experimental procedures. To eliminate noninfected cells or establish a permanent infection, 10 µM puromycin was added to the culture medium. The concentration of C-176 treatment in SH-SY5Y was 10uM.

Western blotting

Cells were scraped off with a scraper and collected by centrifugation. For tissue, after anesthesia with 1.25% of Alphadolone in mice, the substantia nigra tissue was collected and cut into small pieces. The cell and tissue samples were added to 400 µL of RIPA lysis buffer containing protease inhibitors (K1007, APExBIO, China) and phosphatase inhibitors (K1015, APExBIO, China), and homogenized with a homogenizer. They were centrifuged at 12000 rpm for 5 minutes at 4°C, and the supernatant was aliquoted into 0.5 ml centrifuge tubes. Protein content was determined using the BCA method (Pierce™ Rapid Gold BCA Protein Assay Kit, Cat: A53225). After SDS-PAGE electrophoresis, the samples were transferred to PVDF membrane and were blocked with blocking buffer (5% skim milk powder in TBST) for 1 hour. The indicated primary antibodies were diluted with TBST to an appropriate concentration and incubated with the PVDF membrane at 4°C for 16-24 hours. After washing, the secondary antibodies were added, and PVDF was incubated at room temperature for 1 hour, followed by washing and chemiluminescence reaction. The antibodies are listed as following: Rabbit polyclonal anti-STING (Proteintech, 19851-1-AP,1:200); Rabbit polyclonal anti-ISG15 (Proteintech, 15981-1-AP,1:2000); Rabbit polyclonal anti-TNF-α (Proteintech, 17590-1-AP,1:2000); Rabbit polyclonal anti-NF-κB (Proteintech, 10745-1-AP,1:2000); Rabbit polyclonal anti-TH (Proteintech, 25859-1-AP,1:5000); Mouse monoclonal anti-c-Fos (Proteintech, 66590-1-Ig,1:5000); Rabbit polyclonal anti- Alpha Synuclein (Proteintech, 10842-1-AP,1:3000); Mouse polyclonal anti-Alpha Tubulin (Proteintech, 14555-1-AP, 1:100000); Mouse polyclonal anti-GAPDH (Proteintech, 60004-1-Ig, 1:100000).

Co-Immunoprecipitation (Co-IP)

SH-SY5Y cells were collected and lysed with a cold RIPA buffer with protease (Cat: K1007, APExBIO, China) and phosphatase (Cat: K1015, APExBIO, China) inhibitor cocktail. After centrifuging at 12,000 rpm at 4°C for 15 min, the supernatants were collected and incubated with the indicated primary antibodies and 20 µL protein A/G MagBeads (Cat: 36417ES03, Yeasen, China) at 4°C overnight. The beads were

washed three times. Then, 80 μ L of loading buffer was added, and the mixture was heated at 100 °C for 10 min, followed by Western blot analysis.

RNA-sequencing analysis

SH-SY5Y were infected with Lentivirus PLV-Con, PLV-STING-WT, and PLV-STING-K289A for 48 hours and collected for transcriptomic analysis. The cDNA library was constructed and sequenced using Illumina HiSeq™ X Ten by Novogene, China. The sequencing reads were mapped to the mouse reference genome (mm9) using HISAT2. Feature counts (v1.5.0-p3) were used to count the reads mapped to each gene. Differential expressed genes (DEG) analysis of the two groups was performed using the edgeR R package (3.22.5). The p-values were adjusted using the Benjamini & Hochberg method. $\text{padj} \leq 0.05$ and $|\log_2(\text{foldchange})| \geq 1$ were set as the threshold for significantly differential expression. Gene Ontology (GO) enrichment analysis and KEGG (Kyoto Encyclopedia of Genes and Genomes) database pathways of differentially expressed genes were performed using the clusterProfiler R package (3.8.1).

CCK-8 assay

The Cell Counting Kit-8 (CCK-8) was employed to assess cell viability in SH-SY5Y cells treated with MPP⁺. SH-SY5Y was subcultured in 96-well plates, followed by lentiviral infection for 2 days and subsequent exposure to 1 mM MPP⁺ for 24 hours. Cell viability was then determined using the CCK-8 assay kit (C0048S, Beyotime, China) according to the manufacturer's instructions.

Animal

C57BL/6J mice were obtained from Beijing Huafukang Biotechnology Co., Ltd. (license number: SCXK (Jing) 2024-0003). All animals were housed in a barrier facility under controlled environmental conditions: 22–25 °C, 50–60% relative humidity, and a 12-hour light/12-hour dark cycle, with 4–5 animals per cage. All experimental protocols and procedures were approved by the Animal Protection and Use Committee of Fujian Medical University (Approval No. IACUC FJMU 2024-Y-1554). Efforts were made to minimize both the number of animals used and their suffering throughout the experiments.

Stereotaxic lentivirus injection

Mice (10 per group) were anesthetized with a 2.5% isoflurane-nitrogen -oxygen mixture ($\text{N}_2:\text{O}_2 = 70:30$, flow rate 400 mL/min) and placed in a stereotaxic frame (RWD Life Science Co., Ltd., China). A total of 5 μ L of lentivirus (2×10^8 U/mL) was bilaterally injected into the substantia nigra at the following coordinates: anteroposterior (AP) –3.1 mm, mediolateral (ML) +1.4 mm, and dorsoventral (DV) –4.4 mm relative to bregma. A 10 μ L Hamilton syringe connected to an infusion pump (RWD Life Science, China) was used, and the virus was delivered at a rate of 0.5 μ L/min. Following the injection, the needle was left in place for an additional 5 minutes to allow for diffusion.

MPTP injection PD model

8-10W male C57BL/6 mice were subjected to one week of adaptive feeding prior to the experiment. Each mouse then received intraperitoneal injections of freshly prepared MPTP (MCE; Cat. No. HY-15608) at a dose of 20 mg/kg body weight. The first injection was followed by a second injection 2 hours later, and a total of four injections were administered on the same day at 2-hour intervals. For administration of the STING antagonist C-176 (HY-112906, MedChemExpress, China), the compound was dissolved in 200 μ L of corn oil and injected intraperitoneally at a dose of 10 mg/kg per mouse. Treatment commenced 1 day prior to the first MPTP injection and continued daily for the next 4 days (a total of 5 injections).

AAV-Synuclein-A53T PD model

The adeno-associated virus vector encoding α -synuclein A53T (AAV-SynA53T) was purchased from OBio Technology (Shanghai, China). Mice were anesthetized and secured in a stereotaxic frame. A total volume of 2 μ L of AAV2/1-SynA53T (1×10^{12} viral units/mL) was bilaterally injected into the substantia nigra at the following coordinates relative to bregma: anteroposterior (AP) -3.1 mm, mediolateral (ML) ± 1.4 mm, and dorsoventral (DV) -4.4 mm. The mice were then housed for an additional two months prior to subsequent experiments.

Open field test

The open field test was used to assess exploratory behavior and general locomotor activity in mice. The test was conducted on day 3 following MPTP injection; for AAV-SynA53T mice, it was performed 2 months post-AAV injection and on the third day of the rotarod testing regimen. Prior to the examination, each mouse was placed in the center of a square opaque box ($40 \times 40 \times 40$ cm) and allowed to acclimate for 30 minutes. Following a 10-minute period of free exploration, the total distance traveled and movement speed were automatically recorded. The acquired data were analyzed using ANY-Maze video-tracking software (Stoelting, CO, USA).

Rotarod test

Motor function in mice was evaluated using an accelerating rotarod system (Product No. SA102, SANS Bio Instrument, China). Prior to MPTP injection, all animals underwent two days of pre-test training. During training, mice were placed on the rotating rod at speeds ranging from 5 to 25 rpm for three adaptation trials, each lasting no more than 180 seconds. A minimum inter-trial interval of 5 minutes was observed to minimize fatigue and stress. The rotarod test was conducted from day 1 to day 4 following MPTP injection. On each test day, mice were placed on the accelerating rotarod (5–25 rpm), and the latency to fall was automatically recorded. Each animal performed three consecutive trials, and the mean latency across the three trials was used as the retention time for subsequent statistical analysis. For AAV-SynA53T mice, the test was performed 2 months after viral injection and lasted 4 days.

Pole Test

The pole test is commonly employed to assess coordination ability and muscle function in rodents. The test was conducted from day 1 to 4 following MPTP injection; for AAV-SynA53T mice, it was performed 2

months post-AAV injection and lasted 4 days. Prior to formal testing, the animals underwent a training period. During the formal testing phase, each mouse was placed at the top of the pole, with its head facing upward. The animal naturally turned downward and descended along the pole. Three trials were conducted per mouse. The time taken for the mouse to orient downward (T-turn) and the time required to climb down to the base of the pole (T-total) were recorded as pole climbing time for subsequent statistical analysis.

Immunohistochemistry and Immunofluorescence

Mice were deeply anesthetized with 1.25% isoflurane and transcardially perfused with saline (NS) followed by 4% paraformaldehyde (PFA). The brains were post-fixed in 4% PFA for 24 hours, cryoprotected in 30% sucrose for two days, and embedded in optimal cutting temperature (OCT) compound. Free-floating continuous coronal frozen sections (30 μ m thick) of the ventral midbrain were collected for immunostaining. For immunohistochemistry (IHC), sections were blocked with serum for 1 hour and then incubated with primary antibodies overnight at 4 °C. Immunoreactivity was detected using the avidin-biotin peroxidase complex (ABC) method with 3,3'-diaminobenzidine (DAB; P0203, Beyotime, China). For immunofluorescence (IF), after primary antibody incubation, sections were treated with Alexa-488-conjugated anti-rabbit IgG (1:200) or Alexa-594-conjugated anti-mouse IgG (1:200) secondary antibodies. The following primary and secondary antibodies were used: Rabbit polyclonal anti-STING (Proteintech, 19851-1-AP, 1:200); Mouse monoclonal anti-c-Fos (Proteintech, 66590-1-Ig, 1:5000); goat polyclonal anti-Iba1 (Abcam, ab5076, 1:1000); mouse monoclonal anti-glial fibrillary acidic protein (GFAP; Sigma, G3893, 1:1000); mouse monoclonal anti-NeuN [1B7] (Abcam, ab104224, 1:1000); goat anti-rabbit IgG H&L (biotin) (Abcam, AB207995, 1:200); goat anti-mouse IgG H&L (biotin) (Abcam, AB6788, 1:200); Multi-rAb® CoraLite® Plus 594-goat anti-rabbit recombinant secondary antibody (H+L) (Proteintech, RGAR003, 1:200); and Alexa Fluor® 555 AffiniPure® donkey anti-goat IgG (H+L) (Jackson ImmunoResearch, 705-545-147, 1:200).

Quantification of Dopaminergic Neuronal Loss

According to a previously established protocol in our laboratory[20], the number of tyrosine hydroxylase (TH)-positive dopaminergic neurons was quantified in sections encompassing the medial terminal nucleus (MTN), an anatomical landmark used to assess substantia nigra integrity. The sum number of TH-positive cells in three consecutive coronal sections corresponding to bregma levels -3.07 mm, -3.16 mm, and -3.25 mm was calculated per animal.

Statistical Analysis

Statistical analyses were performed using Prism 8 (GraphPad Software). Prior to analysis, data were assessed for normality of distribution and homogeneity of variances. All results are expressed as mean $\pm$ standard error of the mean (SEM). Comparisons between two groups were conducted using two-tailed Student's t-tests, while comparisons among multiple groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test. p-value of less than 0.05 was

considered statistically significant. The level of significance is denoted as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Results

ISG15 and ISGylation of STING were induced in *in vitro* PD models

WB was initially applied to investigate ISG15 expression and STING ISGylation in an *in vitro* PD model of MPP⁺-treated SH-SY5Y cells. Results indicated that ISG15, STING, and ISGylated STING (ISG-STING) were induced in MPP⁺ treated SH-SY5Y compared to NS treatment. ISG-STING was both recognized with anti-ISG15 antibody (ISG15-ISG-STING) and anti-STING antibody (STING-ISG-STING) at around 52K in WB, which is close to the sum of ISG (15K) and STING (37K) molecular weight (Fig. 1A). Similar results were obtained in another *in vitro* PD model, lentiviral PLV-SynulceinA53T infected SH-SY5Y (SYN, Fig. 1B). Next, the Co-IP was applied to clarify the status of ISG-STING. Anti-ISG15 antibody pulled down the ISG15 and ISG-STING but not STING (Fig. 1C); on the other hand, anti-STING antibody pulled down the STING and ISG-STING but not ISG (Fig. 1D). Full length of WB blot indicated that the similar molecular weight of the IPed ISG-STING recognized by both anti-ISG15 and anti-STING antibodies was around 52K (Fig. 1C-D). Three set of shISG15 a-c treatments all knocked down the ISG15 in C8-D1A cells, and ISG15 and ISG-STING were decreased, but the STING was not affected, verifying the status ISGylated STING (supplemental Fig. 1). ShISG15-a was selected for further experiments. These data indicated that ISG15 and ISG-STING were induced in *in vitro* PD models.

ISG-STING induced c-Fos in *in vitro* PD models

To investigate the molecular mechanism underlying ISGylation of STING, PLV-Con, PLV-STING-WT, and non-ISGylation mutation PLV-STING-K289A were infected in SH-SY5Y for 1 day and processed for transcriptomic analyses. When the screening criterion was set to $FC \geq 2$ and $p < 0.01$, the transcriptomic profile showed that only 8 genes were upregulated in the PLV-STING-WT vs PLV-Con group (Fig. 2A). The five of eight genes were immediate early genes (IEGs) such as Egr1, c-Fos, Junb, Ler2 and Arc in STING-WT vs Con groups. All the five IEGs were decreased in STING-K289A vs WT groups and c-Fos was selected for further investigation (Fig. 2B). WB results showed that overexpression of STING-WT increased ISG15, ISG-STING, c-Fos in SH-SY5Y (Fig. 2C) compared to control group while overexpression of non-ISGylation mutation STING-K289A reduced the ISG15, ISG-STING and c-Fos compared to STING-K289A group (Fig. 2C), supporting that K289 is the ISGylated site in STING. shISG15 treatment reduced the ISG15, ISG-STING, and c-Fos (Fig. 2D, Supplemental Fig. 1). STING inhibitor C-176 treatment also reduced the ISG15, ISG-STING, and c-Fos (Fig. 2E). All these data indicated that overexpression of STING-WT induced ISG-STING and c-Fos, while non-ISGylation mutation STING-K289A, shISG15, and STING inhibitor C-176 all block ISG-STING and subsequently reduced c-Fos. Both ISGylation and ubiquitination can target the same lysine (K) site on a protein, and they are potentially competitive. The ubiquitination status of STING was investigated to examine the association between ISGylation and ubiquitination of STING, and the results showed that overexpression of STING-

WT increased ISG-STING, while STING-K289A blocked ISG-STING. However, overexpression of STING-WT also increased the ubiquitination of whole proteins (Supplemental Fig. 2). shISG15 treatment decreased the ISG-STING but also decreased the ubiquitination of whole proteins (Supplemental Fig. 3). STING was pulled down to check the status of ubiquitination of STING, and it showed that shISG15 decreased ISG-STING and also decreased ubiquitination of STING (Fig. 2F). Evidence showed that neurons express STING and other associated immune mediators such as NF- κ B and tumor necrosis factor- α (TNF- α) [21]. Therefore, we further investigated whether ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α were induced in MPP⁺-treated SH-SY5Y cells. WB results showed that ISG15, ISG-STING, c-Fos, NF- κ B and TNF- α were increased in MPP⁺ treated SH-SY5Y (Fig. 3A). In MPP⁺ treated SH-SY5Y, overexpression of PLV-STING-WT further increased the ISG15, ISG-STING, c-Fos, NF- κ B and TNF- α compared to control group while PLV-STING-K289A overexpression reduced the ISG15, ISG-STING, c-Fos, NF- κ B and TNF- α compared to STING-WT group (Fig. 3A). Overexpression of STING promoted cell death analyzed by CCK8 assay and STING-K289A rescued the survival compared to STING-WT group in MPP⁺ treated SH-SY5Y (Fig. 3B). shISG15 knockdown treatment reduced ISG15, ISG-STING, c-Fos, NF- κ B and TNF- α (Fig. 3C), and protect cell death in MPP⁺ treated SH-SY5Y (Fig. 3D). STING inhibitor C-176 treatment also reduced the induced ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α (Fig. 3E), and protected against cell death in MPP⁺ treated SH-SY5Y (Fig. 3F). The STING-c-Fos axis was also investigated in another *in vitro* model, PLV-synucleinA53T infected SH-SY5Y. In this model, ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α were also induced (Fig. 4). overexpression of STING-K289A (Fig. 4A-B), shISG15 (Fig. 4C-D), C-176 (Fig. 4E-F) all blocked the induced ISG15, ISG-STING, c-Fos, NF- κ B and TNF- α (Fig. 4A, C, E) and protected cell death (Fig. 4B, D,F). Summary, these data indicated that all the non-ISGylation mutation STING K289A, shISG15, and STING inhibitor C-176 reduced the ISG15, ISG-STING, c-Fos, and inflammatory mediators NF- κ B and TNF- α , and protected against cell death in *in vitro* PD models. ISGylated-STING at K289A by ISG15 promoted cell death via c-Fos induction and related inflammatory mediators.

STING-K289A ameliorated motor behaviors, reduced neuroinflammation, and dopaminergic neuronal death in MPTP-injected mice

To investigate the role of ISG-STING in an *in vivo* model MPTP-injected mice, PLV-Con, PLV-STING-WT, and PLV-STING-K289A were injected into the SNc of mice for 2 weeks before MPTP injection. The behavior test and subsequent experiments were then followed (Fig. 5A). In saline (NS) injected control groups, there were no differences in distance traveled, center time, and mean speed of mice tested with the open field test (OFT) between PLV-Con, STING-WT, and STING-K289A injected groups. Compared to control, MPTP injection resulted in the reduced distance traveled, center time and mean speed of mice (Fig. 5B). Overexpression of STING-WT further worsened the motor behavior while STING-K289A ameliorated the distance, center time and mean speed of mice compared to STING-WT group (Fig. 5B). The Rotarod test indicated that PLV-STING-WT and STING-K289A injection did not affect the retention time spent on the rod in NS injected groups compared to PLV-Con group. MPTP injection resulted in

reduced retention time in the PLV-Con group compared to the NS group (Fig. 5C). PLV-STING-WT further shortened the retention time compared to the PLV-Con group, while PLV-STING-K289A extended the retention time compared to PLV-STING-WT (Fig. 5C). For the pole test, in the NS-injected groups, PLV-Con, PLV-STING-WT, and STING-K289A injection was not different in the time on pole climbing time. MPTP injection resulted in a longer pole climbing time in PLV-Con group. PLV-STING-WT further elongated the pole climbing time compared to the PLV-Con group, while PLV-STING-K289A shortened the pole climbing time compared to the PLV-STING-WT group (Fig. 5D). TH immunostaining indicated that there was no difference in dopaminergic neuronal death between PLV-Con, STING-WT, and STING-K289A-injected groups in NS-injected mice (Fig. 5E). MPTP injection significantly reduced the number of TH-positive cells in the PLV-Con group. PLV-STING-WT further reduced the number of TH-positive cells in the SNc of MPTP-injected mice (Fig. 5E), while PLV-STING-K289A rescued the dopaminergic neuronal death compared to PLV-STING-WT group. In NS-injected groups, there were no differences in the numbers of GFAP-positive (astrocyte marker) among PLV-Con, STING-WT, and STING-K289A groups. MPTP injection significantly elevated the intensity of GFAP and Iba1-positive cells in the PLV-Con group. PLV-STING-WT further increased the intensity of GFAP and Iba1-positive cells in the SNc of MPTP-injected mice compared to the PLV-Con group (Fig. 5F), while PLV-STING-K289A reduced the intensity of GFAP and Iba1-positive cells compared to PLV-STING-WT (Fig. 5F). WB results showed that ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α were increased in the SNc of MPTP-injected mice (Fig. 5G). Overexpression of PLV-STING-WT further increased the ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α compared to the control group, while PLV-STING-K289A overexpression reduced the ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α compared to the STING-WT group in MPTP-injected mice (Fig. 5G). These results indicated that STING-WT promoted ISGylation of STING, c-Fos induction, neuroinflammation, dopaminergic neuronal death, and compromised behavior in MPTP-injected mice, whereas the non-ISGylation mutation STING-K289A reduced these phenotypes compared with STING-WT treatment.

STING induced c-Fos in neurons in MPTP-injected mice

Immunostaining showed that both STING and c-Fos were induced in SNc of MPTP-injected mice. PLV-STING-WT further increased the intensity of STING and c-Fos in the SNc of MPTP-injected mice compared to PLV-Con group (Fig. 6A) while PLV-STING-K289A reduced intensity of STING and c-Fos compared to PLV-STING-WT (Fig. 6A). The double-labeling immunofluorescence indicated that STING was mainly co-localized with the neuronal marker NeuN (~ 60%) and less with GFAP or Iba1 (Fig. 6B) and the induced c-Fos was also mainly expressed in neurons in SNc of MPTP-injected mice (Fig. 6C). In MPTP-injected mice, it showed that STING and c-Fos are mainly co-localized (Fig. 6D). The data indicated that STING and c-Fos mainly were induced in neurons in MPTP injected mice.

ISG15 knockdown reduced ISG-STING and protected against neuroinflammation and dopaminergic neuronal death in MPTP-injected mice

In MPTP-injected mice, PLV-shISG15 treatment enhanced the distance, center time and mean speed of mice in the open field test compared to the PLV-shCon group (Fig. 7A). PLV-shISG15 extended the retention time in the Rotarod test compared to the shCon group (Fig. 7B). Pole test showed that PLV-shISG15 shortened the pole climbing time compared to shCon group (Fig. 7C). PLV-shISG15 restored the number of TH-positive cells (Fig. 7D) and reduced the intensity of GFAP and Iba1-positive cells compared to shCon group (Fig. 7E). Immunostaining indicated that PLV-shISG15 reduced the intensity of STING and c-Fos (Fig. 7F) in MPTP-injected mice. WB indicated that PLV-shISG15 reduced ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α compared to shCon (Fig. 7G). These data supported that ISG15 knockdown reduced the induced ISG15, ISG-STING, c-Fos, neuroinflammation, and dopaminergic neuronal death, and restored compromised behaviors in MPTP-injected mice.

STING inhibitor C-176 reduced c-Fos and protected against neuroinflammation and dopaminergic neuronal death in MPTP-injected mice

STING inhibitor C-176 was applied to verify the effect of STING and ISG-STING on c-Fos. In MPTP-injected mice, C-176 treatment increased the distance, center time, and mean speed in the open field test compared with the control group (Fig. 8A). C-176 extended the retention time in the Rotarod test compared to the control group (Fig. 8B). The pole test showed that C-176 shortened the pole climbing time compared to the control group (Fig. 8C). C-176 restored the number of TH-positive cells (Fig. 8D) and reduced the intensity of GFAP and Iba1-positive cells in the SNc of MPTP-injected mice compared to the control group (Fig. 8E). Immunostaining also indicated that C-176 reduced STING and c-Fos in the SNc of MPTP-injected mice (Fig. 8F). WB indicated that C-176 reduced ISG15, ISG-STING, c-Fos, NF- κ B, and TNF- α compared with the Con group (Fig. 8G). These data indicated that STING inhibitor C-176 reduced ISG15, ISG-STING, c-Fos, neuroinflammation, and dopaminergic neuronal death, and restored compromised behavior.

STING-K289A reduced c-Fos, and protected against neuroinflammation, dopaminergic neuronal death and behavior deficiency in AAV-SynA53T mice

We further investigate the role of the STING-c-Fos axis in another *in vivo* model AAV-SynA53T injected mice. Compared to control, AAV-SynA53T resulted in the reduced the distance, center time and mean speed of mice (Fig. 9A). Overexpression of STING-WT further worsened the motor behavior while PLV-STING-K289A increased the distance, center time and mean speed of mice compared to STING-WT group (Fig. 9A). In the Rotarod test, PLV-STING-WT shortened the retention time compared to PLV-Con group while PLV-STING-K289A elongated the retention time compared to PLV-STING-WT in AAV-SynA53T mice (Fig. 9B). For Pole test, PLV-STING-WT elongated the pole climbing time compared to PLV-Con group while PLV-STING-K289A reduced the time compared to PLV-STING-WT group in AAV-SynA53T mice (Fig. 9C). TH immunostaining indicated that PLV-STING-WT further reduced the number of TH-positive cells in the SNc of AAV-SynA53T mice (Fig. 9D), while PLV-STING-K289A restored the dopaminergic neuronal death compared to PLV-STING-WT group. PLV-STING-WT further increased the intensity of GFAP and Iba1-positive cells in the SNc of AAV-SynA53T mice compared to PLV-Con group

(Fig. 9E) while PLV-STING-K289A reduced the intensity of GFAP and Iba1-positive cells compared to PLV-STING-WT (Fig. 9E). PLV-STING-WT increased the density of c-Fos compared to control group while PLV-STING-K289A decreased c-Fos compared to PLV-STING-WT group (Fig. 9F). WB indicated that PLV-STING-WT further increased the ISG15, STING, ISG-STING, c-Fos, NF- κ B and TNF- α in AAV-SynA53T mice compared to PLV-Con group and PLV-STING-K289A blocked the induced ISG15, STING, ISG-STING, c-Fos, NF- κ B and TNF- α in PLV-STING-WT groups (Fig. 9G). These results indicated that overexpression of STING-WT promoted c-Fos, neuroinflammation, dopaminergic neuronal death, and compromised behavior in AAV-SynA53T mice, while STING-K289A reduced these phenotypes compared to STING-WT treatment.

Discussion

This study showed that ISG15 and ISGylation of STING (ISG-STING) were induced in *in vitro* PD models, including MPP⁺-treated and PLV-synuclein A53T-infected SH-SY5Y cells. ShISG15 treatment reduced ISG-STING levels, supporting that ISGylation of STING was mediated by ISG15. Transcriptomic analyses showed that c-Fos was prominently induced by STING in SH-SY5Y. Overexpression of STING-WT promoted ISG-STING, increased c-Fos, and cell death, while non-ISGylation mutation STING K289A and shISG15 reduced the ISG-STING, c-Fos, and cell death in *in vitro* PD models. In *in vivo* PD models, including MPTP-injected and AAV-synucleinA53T mice, STING-K289A, shISG15, and STING inhibitor C-176 reduced ISG15, ISG-STING, c-Fos, neuroinflammation, and dopaminergic neuronal death, and restored compromised behavior.

This study indicated that ISG15 and ISG-STING were induced in PD models. Both anti-ISG15 and STING antibodies pulled down and recognized ISGylation of STING (ISG-STING, MW ~ 52K), whose molecular weight was close to the sum of ISG15 (MW ~ 15K) and STING (MW ~ 37K). ShISG15 and STING-K289A both reduced ISG-STING levels, supporting that K289 was the ISGylation site of STING by ISG15. Both ISGylation and ubiquitination can target the same lysine (K) site on protein, and they are potential competitors. However, this study showed that shISG15 treatment decreased ISG-STING and also decreased STING ubiquitination. These indicated that ISGylation did not compete with ubiquitination of STING, which was linked to proteasomal degradation. Recent study also showed that ISGylation of STING stabilized STING by blocking autophagic degradation but was not dependent on ubiquitination and proteasomal turnover in lung cancer models [22].

The STING pathway has been implicated in many neurological disorders, including neurodegeneration, neuroinflammation, brain injury, and pain. The main role of cGAS-STING was in the immune response, and the TBK1-IRF3-interferon pathway was the canonical immune pathway response[23]. Type I IFNs are fundamental for protecting humans against external pathogens, such as viral infections[24]. However, current studies indicate that chronic long-term IFN responses exert neurotoxic effects[25]. In neurodegenerative diseases, age-related immune abnormalities, protein aggregation, and tissue injury can all trigger IFN responses[25]. The main interferon (IFN)-producing cells in the brain are glial cells, astrocytes and microglia (especially for Type I IFNs like IFN- β during viral infections), followed by

neurons[10]. Nucleic acids derived either from invading pathogens or damaged organelles induce the production of IFN-Is and ISGs for innate immune response. Studies demonstrated that the target substrate of ISG15-mediated ISGylation included cytoplasmic dsRNA sensors, melanoma differentiation-associated protein 5 (MDA5), and the DNA sensor cGAS-STING. The ISGylation positively regulates their oligomerization and activation [26]. ISGylation modulates protein stability and activity either positively or negatively[16]. This study showed that ShISG15 and STING K289A overexpression reduced ISG-STING and blocked the induction of c-Jun and PD pathogenesis, supporting the idea that ISGylation of STING augments STING function and activity (gain of function) to promote neuroinflammation and neurodegeneration. STING induces downstream IFN and ISG15, which ISGylates and positively regulates STING, creating a positive feedback loop that may potentiate the progression of PD pathogenesis [26]. These elements implicated in ISGylation modulation were helpful in the novel design of therapeutic strategies. Targeting ISG15-mediated ISGylation or ISG15-interacting proteins may provide a novel therapeutic approach for PD.

It was also reported that STING was mainly expressed in microglia and in astrocytes, neurons, and other cells in the brain[23]. This study indicated that STING-WT, K289A, and shISG15 treatments were associated with NF- κ B, TNF- α , glial activation, and neuroinflammation. On the other hand, recent reports indicated that STING was prominently induced in neurons in the context of neurological pathology, such as experimental autoimmune encephalomyelitis (EAE) and inflammation-induced neuronal death[27]. Neuronal STING activation induces a non-canonical signaling pathway and is associated with neurological pathogenesis[28]. In Huntington's disease, STING activation in neurons triggered apoptosis and autophagic responses[29]. Through transcriptomic analyses, our study showed that c-Fos, an immediate early gene (IEG), was induced in STING-infected SH-SY5Y cells. Our data indicated that STING-K289A, shISG15, and the STING inhibitor C-176 reduced c-Fos activation in *in vitro* and *in vivo* PD models, supporting the notion that c-Fos is downstream of STING. Our data demonstrated that both STING and c-Fos were mainly induced and co-localized in neurons in *in vivo* PD models. In physiological conditions, transient c-Fos induction is implicated in cellular homeostasis and serves as a marker of neuronal activity[30]. However, c-Fos, as a component of AP-1, executed the pro-death role under some pathological stress with prolonged activation. In animal models of various neurodegenerative diseases, protein misfolding, ROS, and neuroinflammation consistently result in aberrant AP-1 activation, which promotes apoptosis, synaptic dysfunction, and inflammatory signaling [31]. It was reported that AP-1 contributed to neuronal apoptosis by inducing pro-death genes, such as mitogen-activated protein kinase phosphatase 1 (Mkp1), jun D proto-oncogene (Jund), and BCL-2-interacting mediator of cell death (Bim) [32]. Inhibiting the JNK-c-Jun/AP-1 pathway has been proven to effectively reduce neuronal death in neurological disorders [32, 33]. The present study showed that five Immediate Early Genes (IEGs), including c-Fos, were upregulated in SH-SY5Y cells overexpressing STING using transcriptomic analyses, indicating that the IEGs were strongly associated with STING activation. IEGs are triggered by neuronal activation within minutes and are essential for neuronal functions such as synaptic plasticity and stress responses[34]. Studies also implicated that aberrant IEG has been observed in neurodegenerative disorders such as Alzheimer's disease and other neurological disorders. Studies

suggest that ISGs may upregulate gene transcription, constituting a detrimental pathway for neuronal function and death [33]. How STING activates c-Fos and IEGs is not well known yet. It is reported that the STING-TNF- α pathway was involved in AP-1/c-Fos activation[35]. Recent evidence showed that neurons released multiple cytokines, including NF-KB and TNF- α , which induced chronic long-term c-Fos expression in neurons [21, 36]. Neuronal-sourced cytokines could pass signal to and cross-talk with glial cells, endothelial cells, etc. There is an abundance of neurons in the periphery, including sensory, motor, and enteric neurons, which exert effects on cells of other systems outside the central nervous system[21]. For example, the nervous system and the peripheral immune system interact profoundly. The nervous system precisely orchestrates other system development and homeostasis and shapes tumor initiation and progression [37, 38]. Neural-body interaction was involved in complex signaling. The neuron-expressed cytokines may play an essential role in this communication. These indicate that neurons may act as systemic immunomodulators by expressing cell-autonomous cytokines. c-Fos may serve as an indicator of neuronal cytokines and immunomodulation.

Conclusion

This study presented that ISG15 and ISGylation of STING (ISG-STING) were induced in *in vitro* and *in vivo* PD models. Transcriptomic analyses indicated that c-Fos is prominently induced by STING. In *in vivo* PD models, including MPTP-injected and AAV-synucleinA53T mice, STING and c-Fos were induced and mainly co-localized in neurons. Non-ISGylation mutation STING-K289A, shISG15, and STING inhibitor C-176 reduced ISG15, ISG-STING, c-Fos, neuroinflammation, and dopaminergic neuronal death, and restored compromised behavior. The present study supported that ISGylation of STING contributes to pathogenesis by activating c-Fos and inflammatory mediators. ISGylation of STING augments its function and activation (gain-of-function), inducing downstream IFN and ISG15, which further ISGylated STING, creating a feedback loop and potentiating the progression of PD (Fig. 10). Targeting ISG15-mediated ISGylation or ISG15-interacting proteins may provide a novel therapeutic avenue for controlling the Pathogenesis of PD.

Declarations

Acknowledgments

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Authors' contributions

X. L., T. H., Q.Y., and E. H. designed experiments and data analyses; X.L. performed main experiments; P. H. and X. J. provided technical support; X. L. and E. H. wrote the manuscript; Q.Y. and E.H. edited the manuscript and provided the funding; E.H. supervised the project; All authors have reviewed the manuscript.

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Availability of data and materials

The data in this study are available upon reasonable request from the corresponding authors.

Ethics approval

All experimental procedures were approved by the Animal Subjects Committees at Fujian Medical University, in accordance with the guidelines (No. IACUC FJMU 2024-Y-1554).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

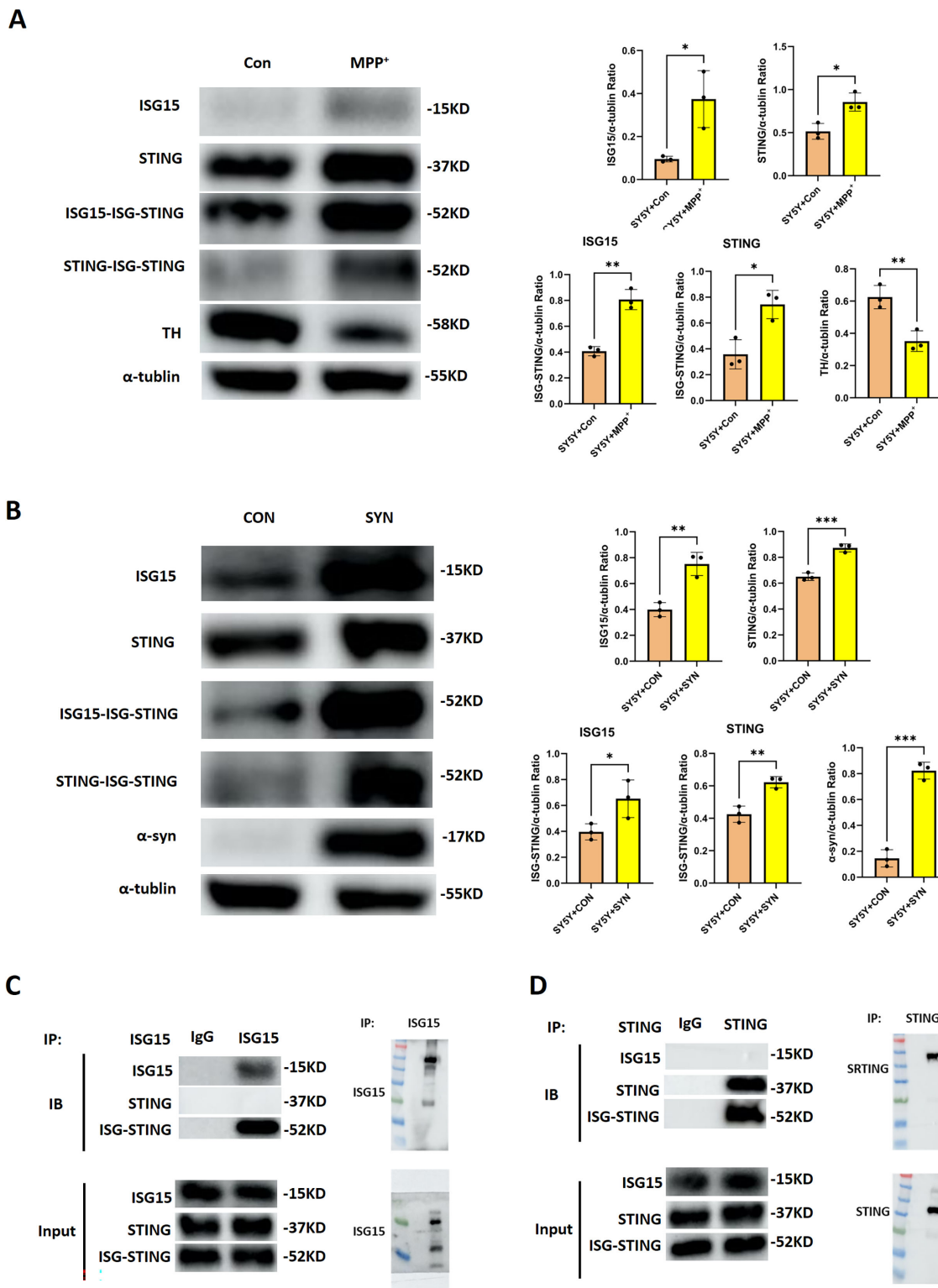


Figure 1

ISG15 and ISGylation of STING were induced in *in vitro* PD models. (A) SH-SY5Y cells were treated with 1mM MPP⁺ for 24 hr, then processed to WB with the indicated antibodies and quantified with ImageJ. (B) PLV-CON and PLV-SYNA53T(SYN) permanently infected SH-SY5Y cells were processed to WB with indicated antibodies and quantified with ImageJ. (C-D) The SH-SY5Y cell lysate was co-

immunoprecipitated (Co-IP) with control IgG and ISG15(C) or STING (D) antibodies, and the precipitated proteins were probed with the indicated antibodies by WB and quantified with ImageJ. All n=4.

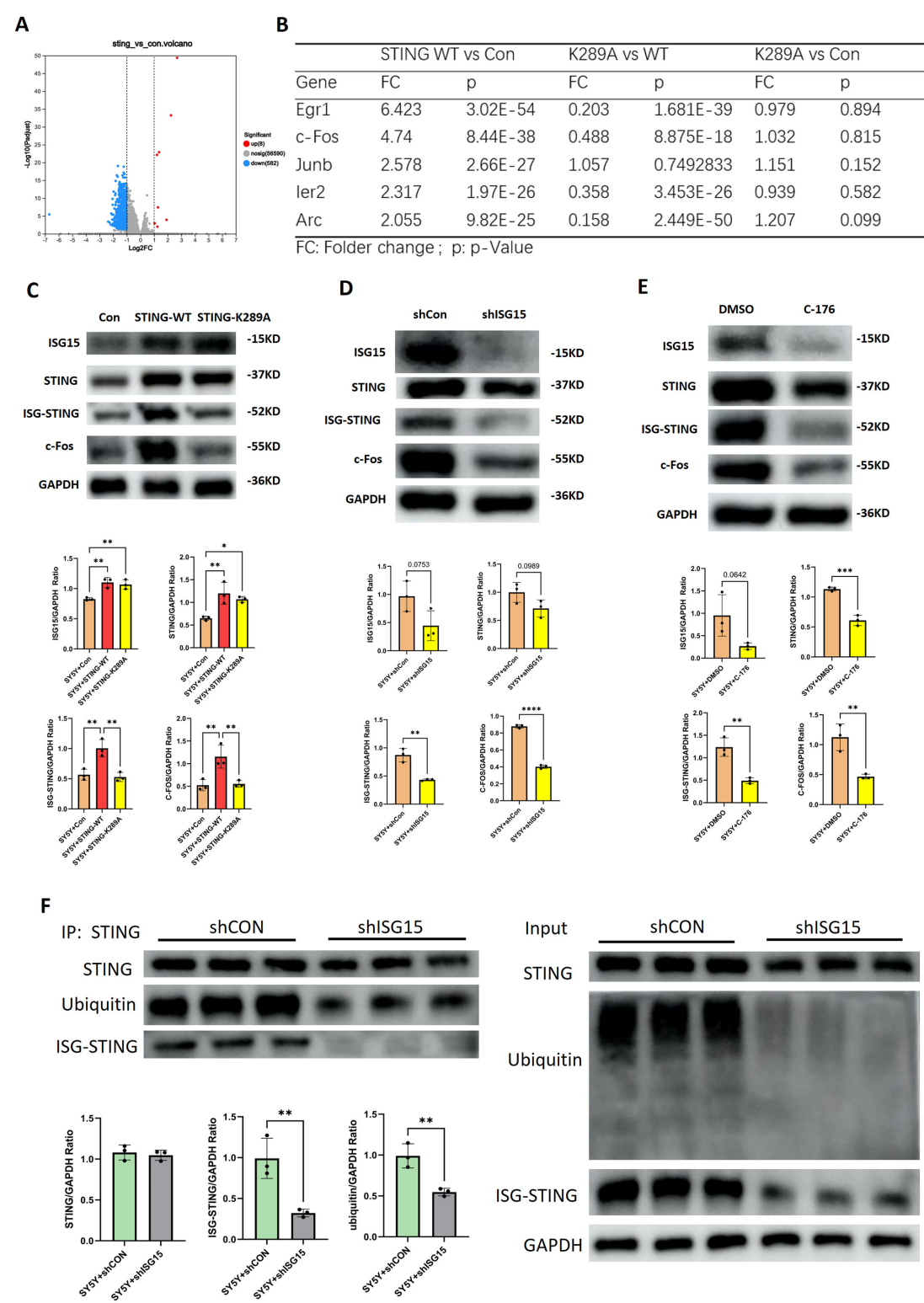


Figure 2

ISG-STING induced c-Fos in *in vitro* PD models. (A) Volcano Plot of DEG (Differential Expressed Gene) in LV-STING-WT vs LV-CON infected SH-SY5Y with transcriptomic analysis. (B) The list of top DEGs related

to immediate early genes (IEGs) among PLV-CON, PLV-STING-WT, and PLV-STING-K289A infected SH-SY5Y cells with transcriptomic analysis. (C) SH-SY5Y cells were infected with PLV-CON, PLV-STING-WT, or PLV-STING-K289A for 2 days, then processed for WB with the indicated antibodies and quantified with ImageJ. (D) SH-SY5Y cells were infected with PLV-shCon and PLV-shISG15 for 3 days, then processed for WB with the indicated antibodies and quantified with ImageJ. (E) SH-SY5Y cells were treated with DMSO and C-176 for 24h, then processed for WB with the indicated antibodies and quantified with ImageJ. (F) shISG15 blocked ISGylation of STING but also decreased ubiquitination of STING. SH-SY5Y were infected with PLV-shCON and PLV-shISG15 for 3 days and processed to IP with anti-STING and processed to WB with indicated antibodies and quantified with ImageJ. All n=4.

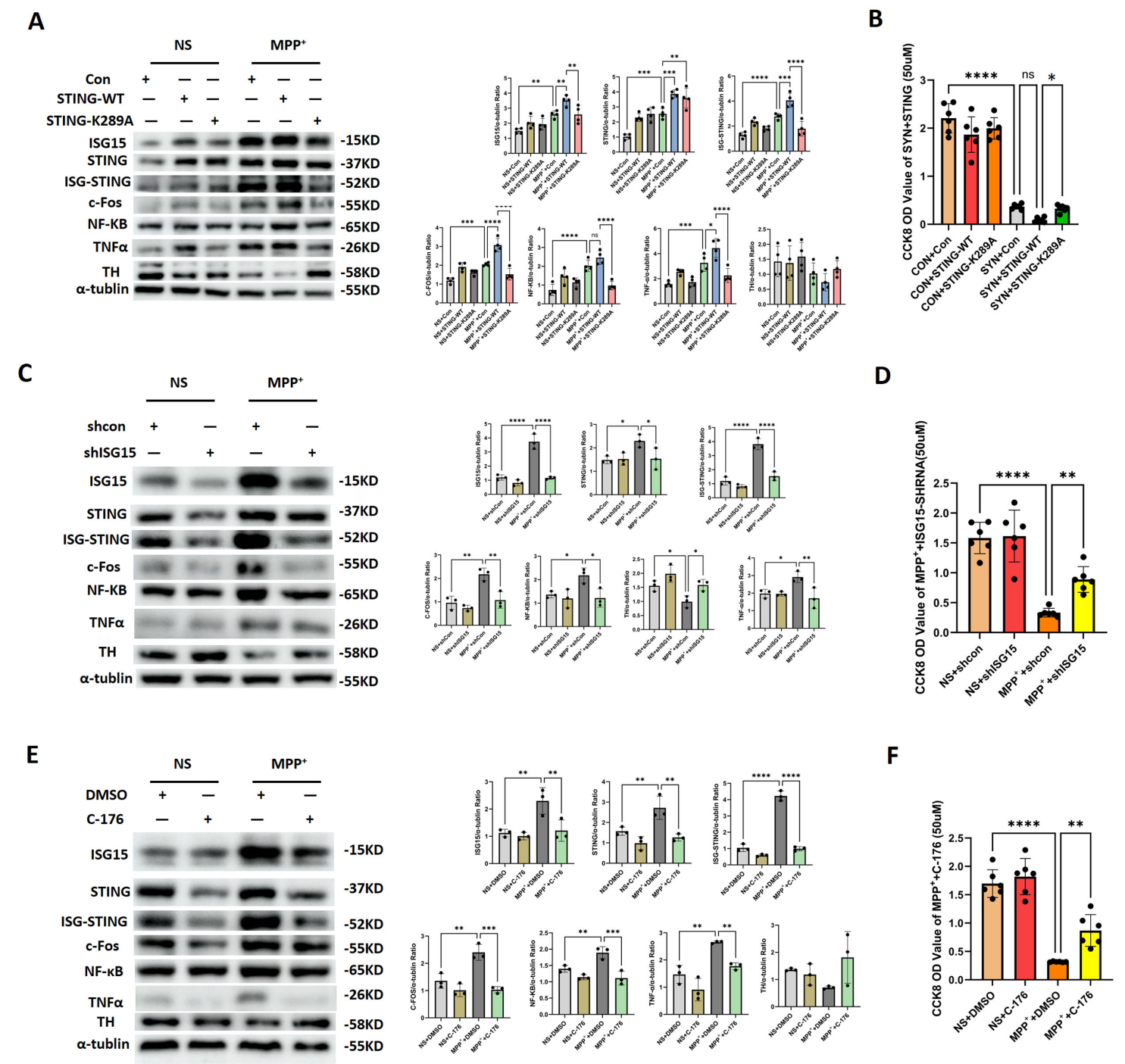


Figure 3

ISG-STING and c-Fos were induced in MPP⁺ treated SH-HY5Y. (A-B) SH-SY5Y was infected with PLV-CON, PLV-STING-WT, and PLV-STING-K289A for 2 days; (C-D) SH-SY5Y was infected with PLV-shCon and PLV-shISG15 for 3 days; (E-F) SH-SY5Y was treated with DMSO and 10uM C-176 for 2h, then SH-SY5Y was treated with 10 μ M MPP⁺ and 10uM C-176 for 24h and processed to WB with the indicated antibodies and quantified with ImageJ (A, C, E) and CCK8 assay (B, D, E). all n=4.

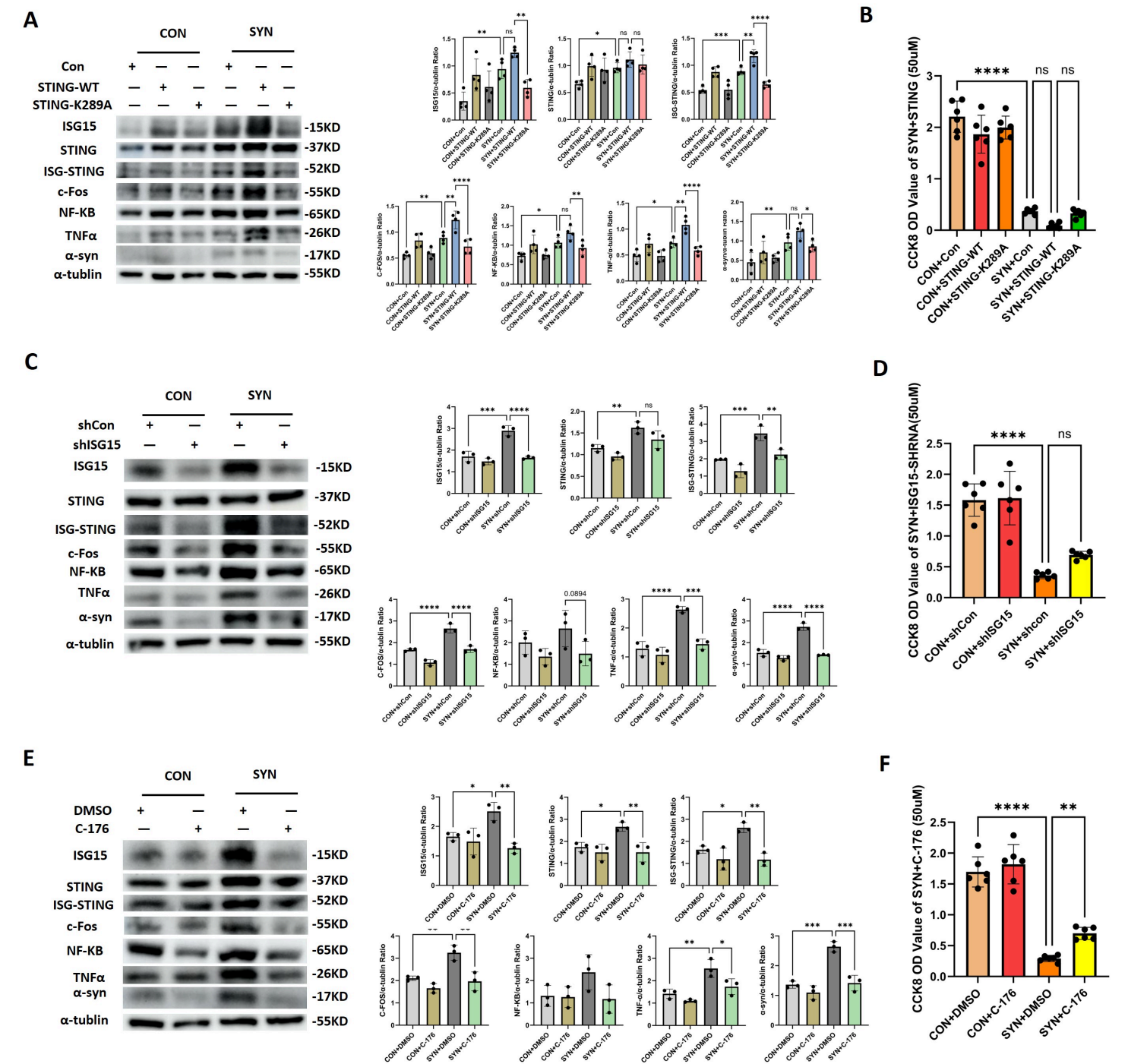


Figure 4

ISG-STING and c-Fos were induced in PLV-SYNA53T-infected SH-SY5Y. PLV-SYNA53T-infected SH-SY5Y was (A-B) infected with PLV-CON, PLV-STING-WT, and PLV-STING-K289A for 2 days; (C-D) infected with PLV-shCon and PLV-shISG15 for 4 days; (E-F) treated with DMSO and 10uM C-176 for 24h; and processed to WB with indicated antibodies and quantified with ImageJ (A, C, E) and CCK-8 assay (B, D, E). all n=4.

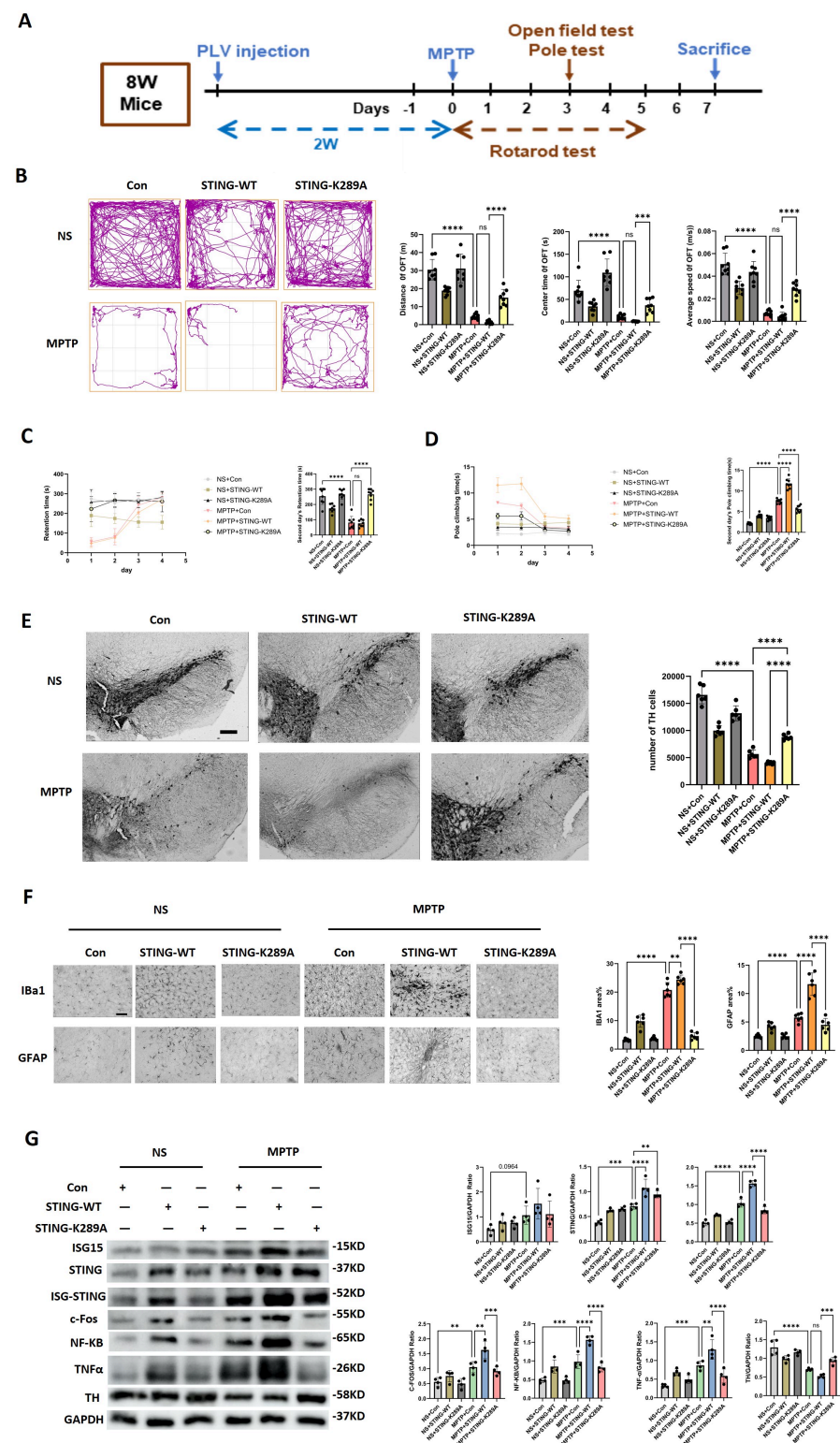


Figure 5

STING-K289A improved motor behaviors, reduced neuroinflammation, and dopaminergic neuronal death in MPTP-injected mice. (A) Schematic diagram of the protocol for the MPTP-injected mice PD model. PLV-Con, PLV-STING-WT, and PLV-STING-K289A were injected into the SNc of mice (each group n=10) for 2 weeks prior to MPTP injection and processed to: (B-D) behavior test (n=8), including (B) the trajectory map, distance traveled, center time, and mean speed of mice in the open field test. (C) The retention time of mice at days 1-4 in the Rotarod test and the statistical analysis on day 2. (D) Pole climbing time of mice at days 1-4 in the Rotarod test and the statistical analysis on day 2. (E-G) After behavior test at day 7, mice were sacrificed and processed to: (E) Immunohistochemistry for TH. n=6. Scale bar =250um. (F) Immunohistochemistry for GFAP and IBA1. n=6. Scale bar = 20 μ m. (G) WB using the indicated antibodies. n=4.

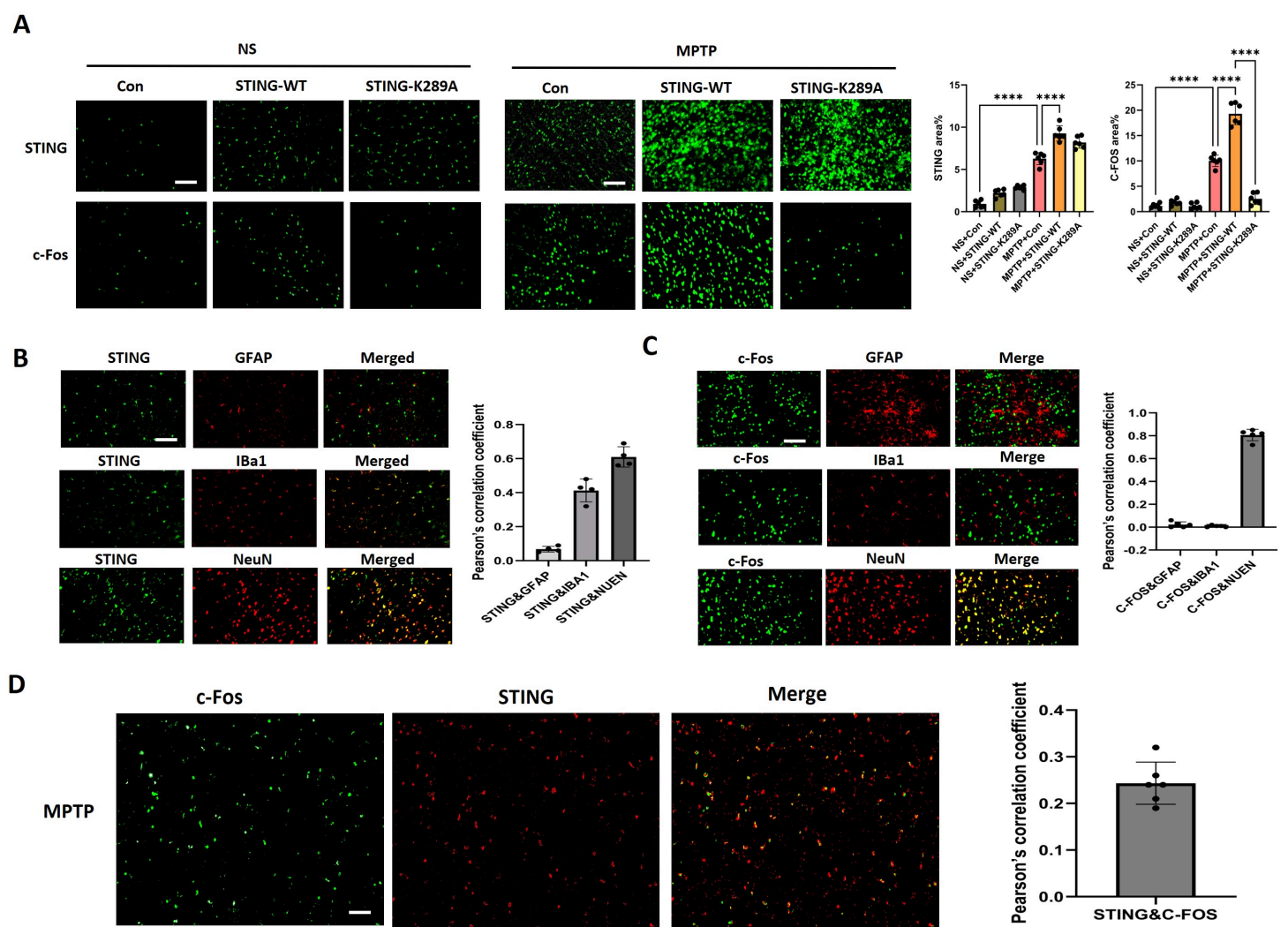


Figure 6

STING induced c-Fos in MPTP-injected mice. PLV-Con, PLV-STING-WT, and PLV-STING-K289A were injected into the SNc of mice (n=10) for 2 weeks prior to MPTP injection, and the SNc sections were processed to: (A) immunofluorescence for STING and c-Fos. (B) Double immunolabeling of STING with GFAP, IBA1, and NeuN. Co-localization was quantified with Manders' co-localization coefficients. (C)

Double immunolabeling of c-Fos with GFAP, IBA1, and NeuN. (D) Double immunofluorescence for STING and c-Fos. All n=6. Scale bar = 50 μ m.

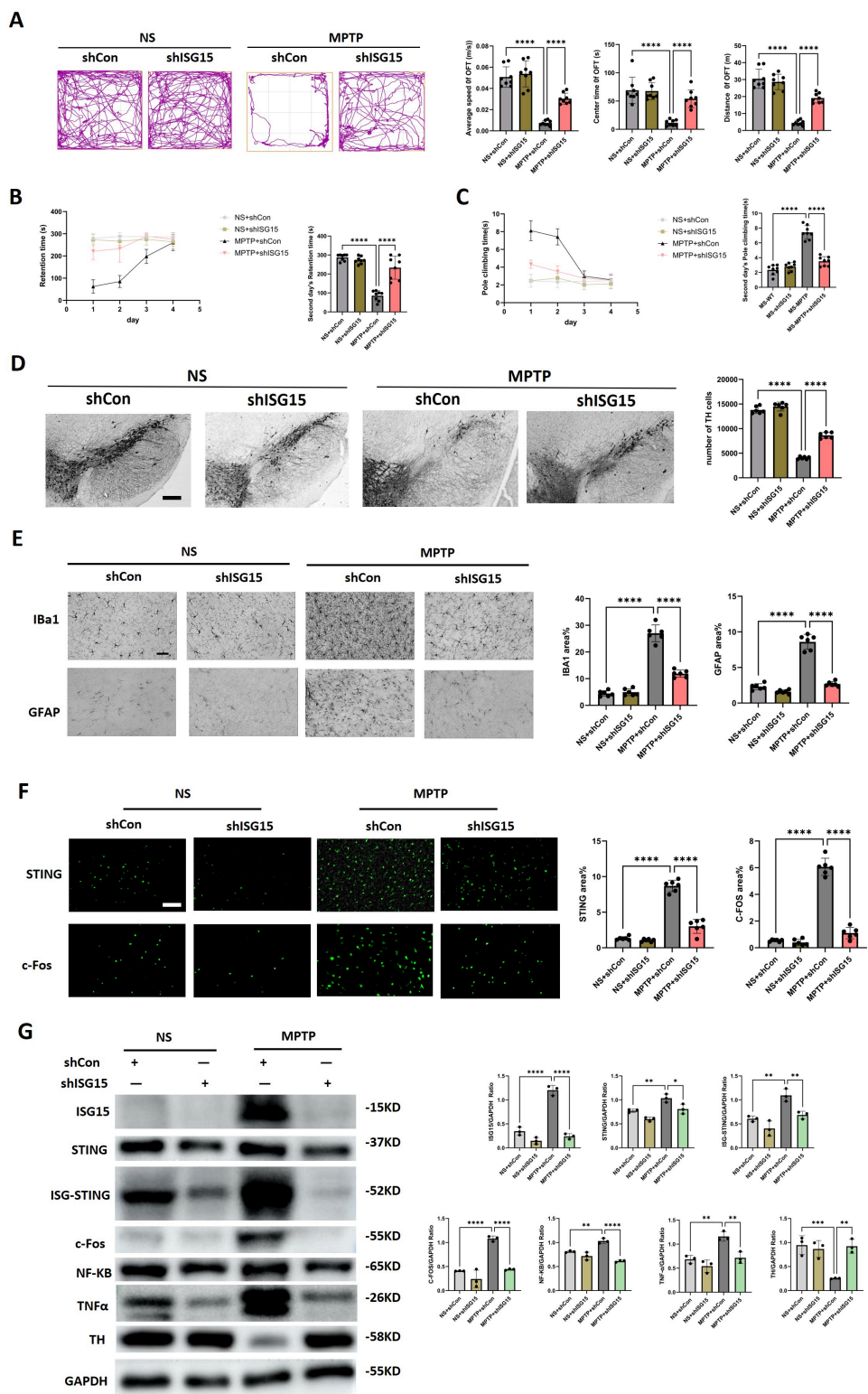


Figure 7

ISG15 knockdown protected against dopaminergic neuronal death via regulating STING-c-Fos axis in MPTP-injected mice. PLV-shCon and shISG15 were injected into the SNc of mice (n=10) for 3 weeks

prior to MPTP injection, and the SNc sections were processed to: (A-C) behavior test (n=10), including (A) the trajectory map, distance traveled, center time, and mean speed of mice in the open field test. (B) The retention time of mice at days 1-4 in the Rotarod test and the statistical analysis of day 2. (C) Pole climbing time of mice at day 1-4 in the Rotarod test and the statistical analysis on day 2. (D-F) After the behavior test at day 7, mice were sacrificed and processed to: (D) Immunohistochemistry for TH. n=6. Scale bar =250um. (E) Immunohistochemistry for GFAP and IBA1. n=6. Scale bar =20um. (F) immunofluorescence for STING and c-Fos. n=6. Scale bar =50um. (G) WB using the indicated antibodies. n=4.

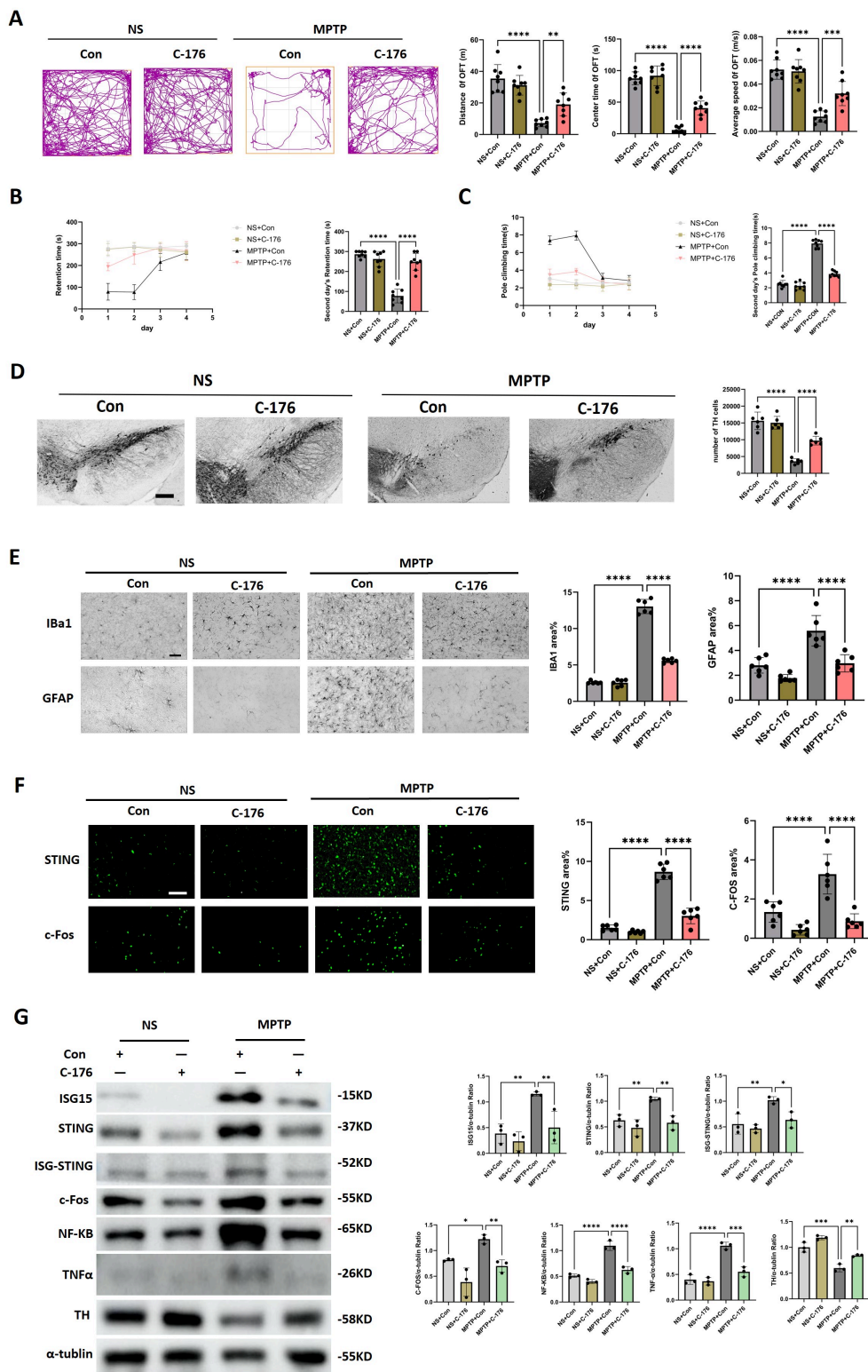


Figure 8

STING inhibitor C-176 protected dopaminergic neuronal death via downregulating c-Fos in MPTP-injected mice. C-176 and control were administered in MPTP-injected mice, and the SNc sections were processed to:(A-C) behavior test (n=10), including (A) the trajectory map, distance traveled, center time, and mean speed of mice in the open field test. (B) The retention time of mice at days 1-4 in the Rotarod test and the statistical analysis on day 2. (C) Pole climbing time of mice at day 1-4 in the Rotarod test

and the statistical analysis on day 2. (D-F) After the behavior test at day 7, mice were sacrificed and processed to: (D) Immunohistochemistry for TH. n=6. Scale bar =250um. (E) Immuno-histochemistry for GFAP and IBA1. n=6. Scale bar =20um. (F) immunofluorescence for STING and c-Fos. n=6. Scale bar = 50μm.(G) WB using the indicated antibodies. n=4.

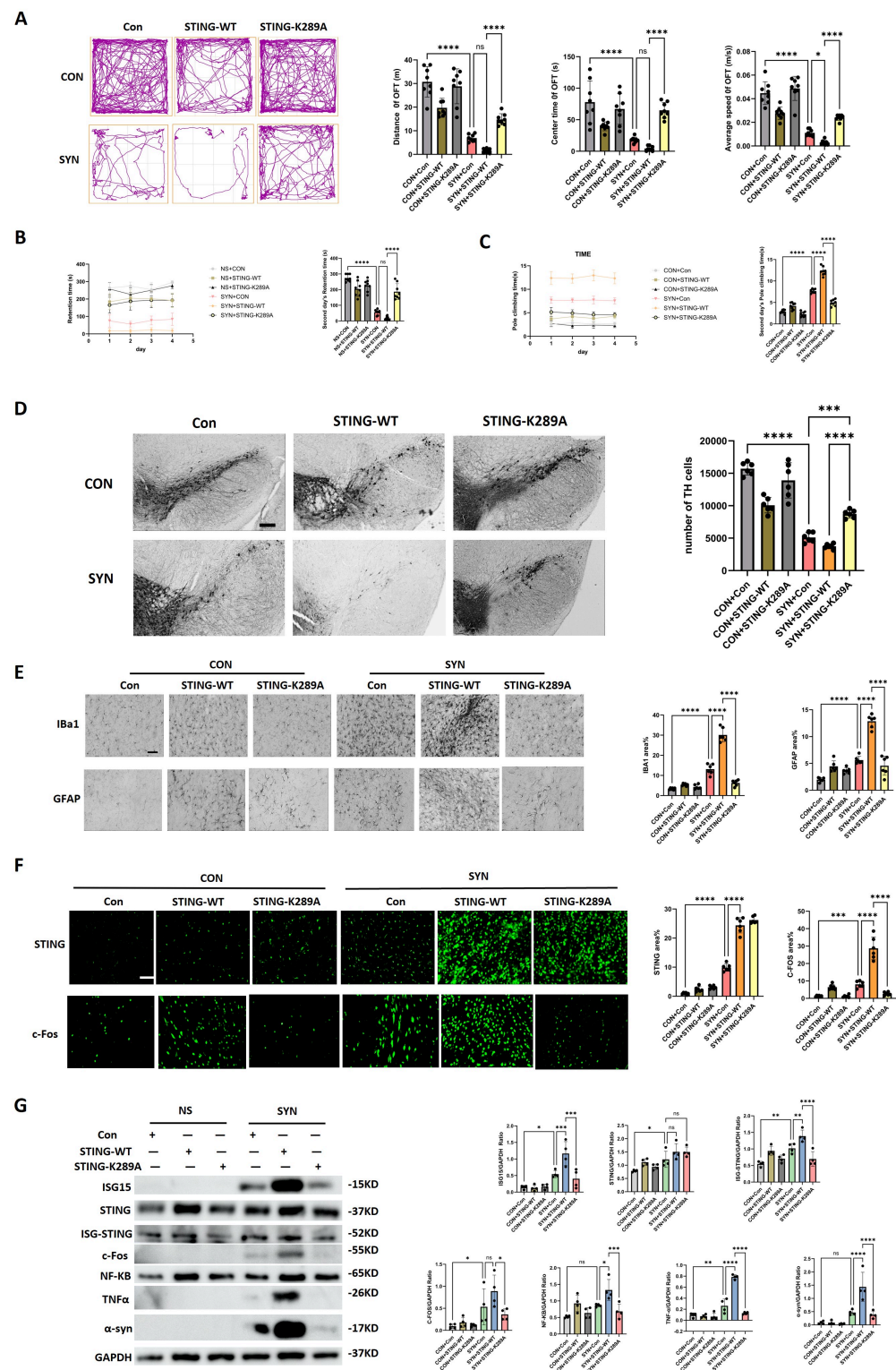


Figure 9

STING-K289A ameliorated dopaminergic neuronal death via regulating STING-c-Fos axis in AAV-SynA53T mice. PLV-Con, PLV-STING-WT, and PLV-STING-K289A with AAV-Con or AAV-SynA53T were injected into the SNc of mice (each group n=10) for 8 weeks and processed to: (A-C) behavior test (n=10), including (A) the trajectory map, distance traveled, center time, and mean speed of mice in the open field test. (B) The retention time of mice at days 1-4 in the Rotarod test and the statistical analysis on day 2. (C) Pole climbing time of mice at days 1-4 in the Rotarod test and the statistical analysis on day 2. (D-F) After behavior test at day 7, mice were sacrificed, and the SNc sections were processed to: (D) Immunohistochemistry for TH. n=6. Scale bar = 250 μ m. (E) Immunohistochemistry for GFAP and IBA1. n=6. Scale bar = 20 μ m. (F) immunofluorescence for STING and c-Fos. n=6. Scale bar = 50 μ m. (G) WB using the indicated antibodies. n=4.

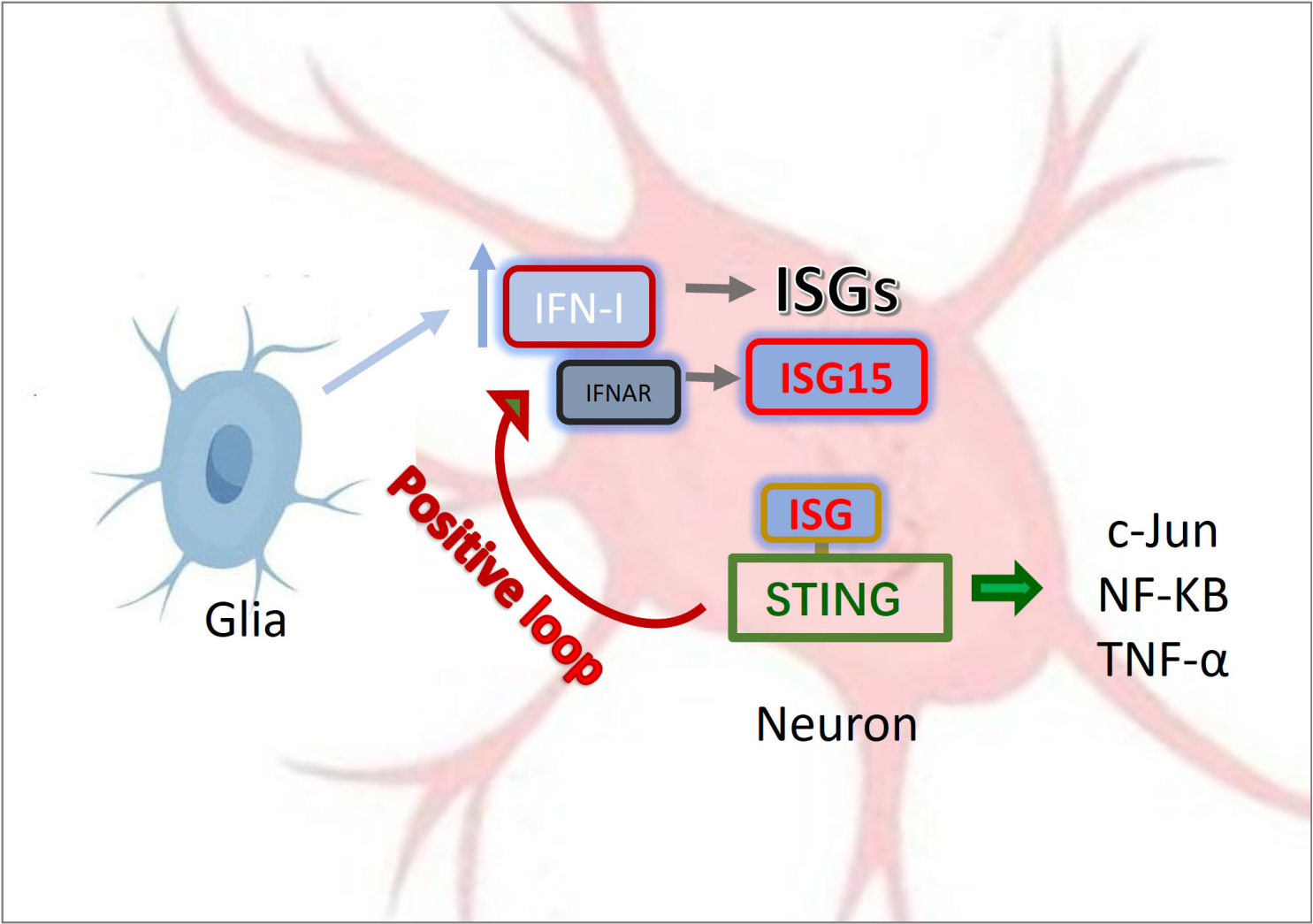


Figure 10

Schematic model of the mechanism underlying ISGylation of STING, which promoted dopaminergic neuronal death in PD models via regulating the STING-c-Fos axis. IFNs from glial cells induces 1SG15 production, which mediates ISGylation of STING, augmenting STING function and activity (gain of function) to induce downstream IFN and ISG15, creating a feedback loop and potentiating pathogenesis.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigure.pdf](#)