

PINK1 overexpression suppresses p38 MAPK/NF- κ B signaling to attenuate chondrocyte senescence in osteoarthritis

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Abstract. PTEN-induced putative kinase 1 (PINK1), a master regulator of mitophagy, is implicated in mitochondrial homeostasis, yet its role in knee osteoarthritis (OA) pathogenesis remains unclear. The present study investigated the mechanisms by which PINK1 modulates chondrocyte senescence during OA progression. Utilizing a destabilization of the medial meniscus-induced OA murine model, decreased PINK1 expression, impaired mitochondrial function and suppressed mitophagy were observed in OA cartilage. *In vitro*, lipopolysaccharide-induced chondrocyte senescence was exacerbated by PINK1 knockdown but mitigated by PINK1 overexpression, which restored mitophagy and reduced senescence-associated β -galactosidase activity, reactive oxygen species accumulation and mitochondrial membrane potential collapse. RNA sequencing and mechanistic studies identified the p38 MAPK/NF- κ B pathway as a downstream target; PINK1 knockdown amplified the phosphorylation of p38 MAPK/NF- κ B, promoting mitochondrial dysfunction and senescence. By contrast, pharmacological inhibition of p38 MAPK/NF- κ B rescued these effects in PINK1-deficient chondrocytes. Collectively, PINK1 attenuated OA progression by suppressing chondrocyte senescence via inhibition of the p38 MAPK/NF- κ B pathway, highlighting its potential as a therapeutic target for OA management.

Introduction

Knee osteoarthritis (KOA), a progressive degenerative joint disorder characterized by articular cartilage degradation, synovial inflammation and osteophyte formation, is a leading cause of age-associated mobility disability (1,2). Epidemiological projections suggest OA prevalence will double by 2030 compared with the levels in the 2010s, reflecting global demographic shifts toward aging populations (3). Studies suggest that chondrocyte senescence, a terminal cell cycle arrest state, drives OA progression (4,5). Senolytic elimination of senescent chondrocytes preserves cartilage integrity in murine post-traumatic OA models (6,7), while intra-articular injection of senescent cells induces OA pathology (8). Mechanistically, senescent chondrocytes exhibit high levels of senescence-associated secretory phenotype (SASP) proteins, including MMPs, pro-inflammatory cytokines and reactive oxygen species (ROS), which collectively promote extracellular matrix (ECM) degradation and chronic synovitis (9,10). However, the upstream molecular mechanisms governing chondrocyte senescence remain incompletely understood, hindering targeted OA therapy development.

Mitophagy, a selective autophagic process essential for mitochondrial quality control, serves a key role in age-related pathology research (11). OA chondrocytes exhibit impaired mitophagic activity, leading to pathological accumulation of dysfunctional mitochondria (12-14). This degradation mechanism is notably suppressed in senescent cells and associated with abnormal mitochondrial retention and elevated ROS generation (15). Notably, restoring mitophagic flux attenuates cell senescence markers (16), while mitophagy activation reduces oxidative stress in OA pathogenesis (17). These findings establish mitochondrial homeostasis disruption as a key link between chondrocyte senescence and OA progression.

PTEN-induced putative kinase 1 (PINK1), a master regulator of mitochondrial quality control, serves critical roles in age-related pathologies including Parkinson's disease and cardiac hypertrophy (18,19). PINK1 orchestrates mitochondrial homeostasis by phosphorylating ubiquitin chains at Ser65 to recruit Parkin, initiating ubiquitin-dependent

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clearance of damaged mitochondria (20). It also phosphorylates mitochondrial Tu translation elongation factor (TUFm) at Ser222, inhibiting autophagy-related 5-12 complex formation and suppressing non-canonical mitophagy (21). The role of PINK1 in chondrocyte senescence and K OA pathogenesis remains controversial: While Shin *et al* (22) reported that PINK1-mediated mitophagy contributes to cartilage degeneration, other studies (23,24) have demonstrated that PINK1/Parkin pathway activation exerts chondroprotective effects through enhanced mitochondrial quality control. This paradox highlights context-dependent regulatory functions of PINK1, potentially influenced by disease stage and microenvironmental factors, necessitating further mechanistic investigation.

The present study aimed to clarify PINK1-mediated mitophagy in chondrocyte senescence during OA progression. Using lipopolysaccharide (LPS)-induced inflammatory models, the PINK1 modulation effects on mitochondrial function, redox homeostasis and senescence-related phenotypes were investigated. RNA-sequencing (seq) with functional validation was performed to identify the downstream effectors of PINK1, particularly its interaction with the p38 MAPK/NF- κ B signaling axis. The present findings may advance molecular stratification of OA pathogenesis and establish a framework for mitochondria-targeted therapy.

Materials and methods

Mouse model generation. A total of 20 male C57BL/6J mice (weight, 25 ± 2 g; age, 6 weeks) were purchased from Jiangsu Huachuang Xinnuo Pharmaceutical Technology Co., Ltd. All experimental procedures were approved by The Animal Ethics Committee of Nanjing University of Chinese Medicine (Nanjing, China; approval no. 202409A051). Animals were housed under specific pathogen-free conditions (25°C, 50% relative humidity) with a 12/12-h light/dark cycle, free access to food and water and ≤ 5 mice/cage. Using SPSS software (IBM Corp.; version 22.0), the mice were randomly assigned to the control or the KOA model group ($n=10$ /group). After a 7-day acclimation period, destabilization of the medial meniscus (DMM) surgery was performed on the right hind limb according to established methods (25). Anesthesia was induced with 3% isoflurane and maintained with 1% isoflurane during surgery. The surgical area was shaved and disinfected with 75% ethanol, and a medial approach to the knee joint was used. After blunt layered separation of the muscles, the medial meniscus was transected with a scalpel to induce joint instability and establish the KOA model. The control group underwent a sham procedure, involving all surgical steps except for transection of the medial meniscus. All animals were given a 14-day postoperative recovery period to ensure model establishment. The animal experiment lasted a total of 3 weeks (7 days of acclimatization and 14 days for KOA model induction). During this period, animals exhibiting severe decline in mobility, difficulty moving or inability to access food and water, indicative of extreme debilitation, were designated for early euthanasia. No unexplained deaths occurred. Euthanasia was performed by intraperitoneal injection of sodium pentobarbital (150 mg/kg). Death was confirmed based on the absence of spontaneous respiration for >2 min,

no palpable heartbeat, no response to resuscitation and fixed dilated pupils with loss of the light reflex. The knee joints were dissected and articular cartilage specimens were snap-frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

Micro-computed tomography scanning. Micro-CT scanning (Skyscan; Bruker Corporation) was performed on knee joint samples. The scanning parameters included a voltage of 80 kV, current of 100 μA and resolution of 9 μm . The scan region was focused on the subchondral bone area beneath the tibial plateau in the weight-bearing zone. Subsequently, CTVox 3.3, Blue Scientific) to perform three-dimensional reconstruction of the knee joint and conduct structure model index of trabeculae) and bone volume fraction) analyses to assess its structural characteristics.

Histological observation. Articular cartilage from the knee joint was collected and fixed at room temperature in 4% paraformaldehyde for 48 h. The tissue was then decalcified in 10% EDTA solution at 37°C. After paraffin embedding at 65°C, sections were prepared at a thickness of 0.4 μm . The sections were stained at room temperature with hematoxylin and eosin (H&E) and with Safranin O-Fast Green (SO/FG). H&E staining was performed with hematoxylin for 5-10 min and counterstain cytoplasm with eosin for 1-3 min. For SO/FG staining, with Fast Green for 5 min, then stain with Safranin O for 30 min. The knee articular cartilage was examined under a light microscope. OA Research Society International (OARSI) score (26) was calculated to assess pathological degeneration.

ELISA. The concentrations of pro-inflammatory cytokines, including IL-1 β , IL-6 and tumor necrosis factor- α (TNF- α), in serum samples were quantified using commercially available mouse-specific ELISA kits according to the manufacturers' instructions [Mouse IL-1 β (cat. no. MLB00C), IL-6 (cat. no. M6000B) and TNF- α ELISA kit (cat. no. MTA00B; all R&D Systems, Inc.)]. Briefly, serum samples were collected by centrifugation of whole blood at 3,000 $\times$ g for 10 min at 4°C and stored at -80°C until analysis. Standards and samples were added to pre-coated 96-well plates in duplicate and incubated for 2 h at room temperature. After washing five times with wash buffer, biotinylated detection antibodies were added for 1 h at room temperature. The optical density was measured at 450 nm. Cytokine concentrations were calculated based on standard curves generated for each assay. All samples were analyzed in triplicate to ensure data reliability.

Cell isolation and culture. Immortalized human chondrocytes (SV40 cells; cat. no. T0021) were purchased from Applied Biological Materials, Inc. For primary cell culture, articular cartilage was aseptically isolated from the tibial plateau and washed three times with PBS. The cleaned cartilage fragments were minced into 1 mm³ pieces and digested in 0.2% type II collagenase at 37°C for 6 h with gentle agitation. The cell suspension was filtered through a 70- μm cell strainer and centrifuged at 300 $\times$ g for 6 min at room temperature to isolate chondrocytes. The harvested cells were cultured in DMEM supplemented with 10% fetal bovine serum (both Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.) under standard

Table I. Interference sequences for PINK1 short hairpin RNA and empty vector plasmid.

Name	Sequence (5'→3')
NC-iF	gatccgTTCTCCGAACGTGTCACGTTtcaagagAACGTGACACGTTCCGAGAAAttttt
NC-iR	aattaaaaaTTCTCCGAACGTGTCACGTTtcttgaAACGTGACACGTTCCGAGAAAcg
PINK1-i1F	gatccgCGGCTGGAGGAGTATCTGATAAtcaagagTATCAGATACTCCTCCAGCCGttttt
PINK1-i1R	aattaaaaaCGGCTGGAGGAGTATCTGATActtctgaTATCAGATACTCCTCCAGCCGcg
PINK1-i2F	gatccGAAGCCACCATGCCTACATTGtcaagagCAATGTAGGCATGGTGGCTTctttt
PINK1-i2R	aattaaaaaGAAGCCACCATGCCTACATTGcttctgaCAATGTAGGCATGGTGGCTTCg
PINK1-i3F	gatccgCGGACGCTGTTCTCGTTATGtcaagagCATAACGAGGAACAGCGTCCGttttt
PINK1-i3R	aattaaaaaCGGACGCTGTTCTCGTTATG ctcttga CATAACGAGGAACAGCGTCCGcg

Lowercase, exogenous segments; uppercase letters denote the core functional segments. NC, Negative Control; iF, interference Forward; iR, interference Reverse; PINK1, PTEN-induced putative kinase 1.

culture conditions (37°C, 5% CO₂ and 95% humidity). Medium replacement was performed every 48 h until 80–90% confluence was achieved. KOA conditions were modeled by treating cells with 10 ng/ml IL-1 β or 10 ng/ml TNF- α for 24 h; or 1 μ g/ml LPS for 24 h. For inhibition of the p38 MAPK pathway, chondrocytes were treated with 1 μ M talmapimod for 24 h. To activate the p38 MAPK signaling pathway, chondrocytes were treated with 1 μ g/ml diprovocim for 24 h. All treatments were performed at 37°C.

PINK1 short hairpin (sh)RNA transfection. shRNA designs were sourced from Sigma-Aldrich website (27). Based on the PINK1 gene (transcript ID: NM_032409.1), three shRNAs were designed: TRCN0000007101 (PINK1-interference1) targeting 5'-CGGCTGGAGGAGTATCTGATA-3'), TRCN0000199193 (PINK1-i2) targeting GAAGCCACCATGCCTACATTG and TRCN0000199446 (PINK1-i3) targeting CGGACGCTGTTCTCGTTATG-3'). Negative control plasmid (NC-interference) targeted TTCTCCGAACGTGTCACGTT (5'→3'). The oligonucleotides (oligos) were designed using the stem-loop (loop) sequence TCAAGAG. The specific interference sequences are listed in Table I. A third-generation lentiviral system was used for transduction (lentiviral transfer plasmid, packaging plasmid, and envelope plasmid are mixed at a 4:3:1 ratio (12:9:3 μ g), employing a puromycin-resistant lentiviral shRNA transfer vector and 293T packaging cells (both Nanjing Kress Biotechnology Co., Ltd.). Transfection was performed at 37°C using Lipo293™ (Beyotime Institute of Biotechnology; cat. no. C0521) for 6 h, followed by a medium change 24 h post-transfection. Viral supernatants were collected at 24 and 48 h post-transfection, filtered through a 0.45 μ m sterile filter, concentrated and purified using a PEG Lentivirus Purification kit (Chongqing Inovogen Biotechnology Co., Ltd.; cat. no. P1201), then resuspended in DMEM. Aliquots of 200 μ l/tube were prepared and stored at -80°C. Infection was performed at an MOI of 70 at 37°C for 24 h, followed by selection with 2 μ g/ml puromycin for 48 h. Cells were then switched to puromycin-free DMEM (Gibco, cat. no. 11965092.) for expansion, and assessed knockdown efficiency using FITC labelling observed by fluorescence microscopy and quantitative PCR (qPCR). qPCR was performed on a LightCycler 480 system using the SYBR Green I Master kit (Roche, cat.

no. 04887352001). Primer sequences (5'→3') were as follows: human PINK1 (forward: GCCTCATCGAGGAAAAACAGG; reverse: GATCACTAGCCAGGGAACACG) and the house-keeping gene human GAPDH (forward: GGAGCGAGATCCCTCCAAAT; reverse: GGCTGTTGTCATACTTCTCATGG). Thermal cycling conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec, 60°C for 30 sec (annealing), and 72°C for 30 sec. Relative gene expression was calculated using the 2^{- $\Delta\Delta$ Cq} method (28), normalized to GAPDH, and reported as fold change relative to the control group. TRCN0000199446 was selected as the shRNA interference sequence for subsequent experiments.

Lentiviral transduction for PINK1 overexpression in primary mouse chondrocytes. For PINK1 overexpression, PINK1 gene oligos (IDs B5469-1-38) were designed and amplified by Shanghai GenePharma Co., Ltd. and transduction was performed using a third-generation lentiviral system. The control group received an empty-vector virus with no insert, using the LV5 (EF-1aF/GFP&Puro) backbone. For the overexpression group, the transcript ID was NM_026880.2 and the same LV5 (EF-1aF/GFP&Puro) vector was used. The packaging plasmids included pGag/Pol, pRev and the envelope plasmid pVSV-G. All vectors/plasmids were supplied by Shanghai GenePharma Co., Ltd. Packaging was performed in 293T producer cells (Shanghai GenePharma Co., Ltd.). Cells were transfected at 37°C using the RNAi-mate transfection reagent (Shanghai Genepharm Co., Ltd.) for 6 h, after which the medium was replaced with DMEM containing 10% FBS. The culture was maintained at 37°C for 72 h to collect viral supernatant. Cell debris was removed by low-speed centrifugation at 4°C, 500 x g for 4 min, followed by filtration through a 0.45 μ m sterile filter. Finally, the virus was concentrated by ultracentrifugation at 4°C, 3,000 x g for 2 h, aliquoted, and stored at -80°C. Primary mouse chondrocytes were used as target cells. Infection was performed at an MOI of 90 at 37°C for 24 h, followed by puromycin selection at 2 μ g/ml for 48 h. The cells were then expanded in puromycin-free medium for subsequent assays. Lentiviral transfer plasmid, packaging and envelope plasmid are mixed at a 4:3:1 ratio (12:9:3 μ g).

RNA-seq. RNA quality and concentration were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc.) and an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.), with RNA integrity number >8.0 considered acceptable for sequencing. mRNA bearing poly (A) tails was enriched from 1–4 μ g of total RNA using 20 μ l Dynabeads Oligo (dT)25 magnetic beads (Invitrogen, cat. no. 61002). The mRNA was then fragmented into 200–300 bp short fragments by incubating at 94°C for 5 min in a divalent cation-containing fragmentation buffer with the NEBNext Magnesium RNA Fragmentation Module (New England Biolabs, cat. no. E6150S). First-strand cDNA was synthesized using random hexamer primers (50 μ M; Invitrogen, cat. no. N8080127) and SuperScript III reverse transcriptase (200 U/ μ l; both Invitrogen, cat. no. 18080093) under the following conditions: 25°C for 10 min, 50°C for 50 min, and inactivation at 85°C for 5 min. Second-strand cDNA synthesis was performed with DNA Polymerase I (10 U/ μ l; NEB, cat. no. M0209L) and RNase H (2 U/ μ l; NEB, cat. no. M0297S) in a second-strand synthesis buffer containing dNTPs (10 mM each), incubated at 16°C for 2.5 h. The double-stranded cDNA was purified, end-repaired and ligated with sequencing adapters. Libraries were constructed using the NEBNext Ultra RNA Library Prep kit (cat. no. E7530L, New England BioLabs, Inc.) and subjected to quality control using the Agilent 2100 Bioanalyzer. Final library concentrations were quantified by qPCR and diluted to 150 pM. High-quality libraries were sequenced on an Illumina NovaSeq 6000 platform (Illumina, Inc.) in paired-end mode (150 bp reads). Raw sequencing data were preprocessed using FastQC (v0.11.9) for quality assessment and Trimmomatic (v0.39) for adapter trimming and low-quality base removal. Clean reads were aligned to the reference genome (GRCh38) using HISAT2 (v2.2.1) and gene expression levels were quantified using StringTie (v2.2.1).

Transcriptome data analysis. Pearson correlation analysis were performed using the `cor()` function in R (version 4.3.0, The R Foundation for Statistical Computing). Pearson's correlation coefficients (r) were calculated for normally distributed continuous variables, while Spearman rank correlation was used for nonparametric data. The 'corrplot' package (version 0.92, Comprehensive R Archive Network), combined with hierarchical clustering, was used to visualize the correlation matrix. Two-sided tests were applied, with $P < 0.05$ indicating statistical significance, and Benjamini-Hochberg correction was used for multiple comparisons. Principal component analysis (PCA) was conducted with the 'prcomp()' function. Prior to analysis, sampling adequacy was assessed with the Kaiser-Meyer-Olkin test (threshold >0.6), and variable correlations were evaluated with Bartlett's test of sphericity ($P < 0.05$). The number of principal components was determined based on eigenvalues >1 (Kaiser criterion) and the scree plot. The 'factoextra' package (version 1.0.7, Comprehensive R Archive Network) was used to generate biplots showing both sample scores and variable loadings. The first two principal components (PC1 and PC2) explaining the cumulative variance were selected for visualization. Stability of the results was validated with 1,000 bootstrap iterations. Differential gene expression analysis was performed using DESeq2 (1.40.2, Bioconductor Project), with a significance threshold of $\log_2$ fold-change >1 and adjusted

$P < 0.05$. Functional enrichment analysis of differentially expressed genes was performed using Gene Ontology (GO, <https://www.geneontology.org/>) and Kyoto Encyclopedia of Genes and Genomes (KEGG, genome.jp/kegg/) databases. Gene set enrichment analysis was performed using the GSEA software (version 4.3.2, Broad Institute) in conjunction with the Molecular Signatures Database (MSigDB v7.5.1, Broad Institute). A pre-ranked gene list was generated based on $\log_2$ fold-change values from differential expression analysis. The Hallmark gene sets (H) were used for the analysis.

ROS. According to the manufacturer's instructions, the intracellular levels of ROS were quantitatively measured using an ROS Assay kit (cat. no. S0035M; Beyotime Institute of Biotechnology). Briefly, chondrocytes were plated in 6-well culture plates at a density of 1×10^6 cells/well and allowed to adhere overnight at 37°C. Culture medium was replaced with fresh DMEM (Gibco, cat. no. 11965092) containing the ROS-specific fluorescent probe working solution, and the cells were maintained at 37°C in a humidified 5% CO₂ incubator for 30 min. Cells were washed three times with PBS to remove excess probe solution and stained with DAPI for 10 min at 37°C. Fluorescence imaging for ROS detection was performed using an inverted fluorescence microscope (Leica DMI8; Leica Microsystems GmbH) equipped for excitation at 488 nm. All procedures were conducted under standardized conditions to ensure consistency and reproducibility of the results.

Measurement of mitochondrial membrane potential. According to the manufacturer's instructions, mitochondrial membrane potential changes were analyzed using the Mitochondrial Membrane Potential Assay kit with JC-1 (Beyotime Institute of Biotechnology). Chondrocytes were seeded at a density of 1×10^6 cells/well in 6-well plates and incubated with DMEM containing JC-1 working solution at 37°C for 20 min. Fluorescence images were captured using a fluorescence microscope (Leica DMI8; Leica Microsystems GmbH).

Senescence-associated β -galactosidase (SA- β -gal) staining. Cell senescence was assessed using a SA- β -gal Staining kit (cat. no. C0602; Beyotime Institute of Biotechnology) following established protocols (25). Briefly, chondrocytes were rinsed twice with PBS and fixed in 4% paraformaldehyde for 15 min at room temperature. Cells were incubated with freshly prepared staining solution containing X-gal in a humidified 37°C incubator for 16 h under CO₂-free conditions. Quantification was performed by calculating the percentage of SA- β -gal-positive cells relative to total cells in five randomly selected fields of view/sample using ImageJ software (v1.54f, National Institutes of Health).

Protein extraction and western blotting. Cells were lysed using RIPA buffer (Beyotime Institute of Biotechnology) supplemented with a protease inhibitor cocktail on ice for 30 min. The lysates were centrifuged at 12,000 $\times$ g for 15 min at 4°C, and the supernatants were collected. Protein concentration was measured using the BCA protein assay. Equal amounts of protein (20 μ g/lane) were separated by 4–20% SDS-PAGE and transferred onto polyvinylidene fluoride membranes

(MilliporeSigma). The membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated overnight at 4°C with the following primary antibodies: GAPDH (1:50,000; cat. no. 66004-1-Ig), β -tubulin (1:3,000; cat. no. 10094-1-AP), p65 (cat. no. 10745-1-AP), phosphorylated (p)-p65 (cat. no. 82335-1-RR), p38 (cat. no. 14064-1-AP), p-p38 (all Proteintech Group, Inc.; cat. no. 28796-1-AP), MMP-3 (Affinity Biosciences; cat. no. AF0217), iNOS (inducible nitric oxide synthase) (cat. no. 18985-1-AP), P16 (both Proteintech Group, Inc.; cat. no. 10883-1-AP), P21 (Affinity Biosciences; cat. no. AF6290), PINK1 (cat. no. 23274-1-AP), TUFm (cat. no. 26730.1-AP), BNIP3L (Bcl-2) interacting protein 3-like) (cat. no. 12986-1-AP), p62 (cat. no. 18420-1-AP) and LC3B (Microtubule-associated protein 1 light chain 3B) (all 1:1,000; all Proteintech Group, Inc.; cat. no. 18725.1-AP). Following washing three times with 0.1% TBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. The secondary antibodies were HRP-conjugated Goat Anti-Rabbit IgG (H+L) (cat. no. SA00001-2, Proteintech Group, Inc.) and HRP-conjugated Goat Anti-Mouse IgG (H+L) (both 1:5,000; cat. no. SA00001-1, Proteintech Group, Inc.). Chemiluminescent detection was performed with SuperSignal West Pico PLUS substrate (Thermo Fisher Scientific, Inc.; cat. no. 34580). Protein bands were visualized using an enhanced chemiluminescence detection system (cat. no. 1708265, Bio-Rad Laboratories, Inc.) and analyzed using ImageJ software (v1.53t, National Institutes of Health). All experiments were performed in triplicate to ensure reproducibility.

Statistical analysis. All data are presented as the mean $\pm$ standard deviation of ≥ 3 independent experimental repeats for continuous variables. Between-group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test or Bonferroni correction. $P < 0.05$ was considered to indicate a statistically significant difference. All analyses and data visualization were conducted using GraphPad Prism 5 (Dotmatics).

Results

Impaired mitochondrial autophagy and enhanced cell senescence in KOA cartilage. Micro-CT analysis confirmed the successful establishment of the KOA model. Compared with the sham-operated group, the KOA group showed a significant increase in osteophyte formation and more severe cartilage surface wear (Fig. 1A). Calculations of bone volume fraction and the structure model index of trabeculae showed that bone destruction was more pronounced in the KOA group than in the sham group (Fig. 1A). Histopathological evaluation revealed notable changes in the KOA group, characterized by marked chondrocyte loss, a posterior shift of the tidemark, and damage to the superficial cartilage structure. Further assessment to evaluate cartilage degeneration showed that the OARSI scores were significantly increased in the KOA group (Fig. 1B). Quantitative ELISA revealed significantly elevated levels of the pro-inflammatory cytokines IL-1 β , IL-6 and TNF- α in KOA compared with sham animals (Fig. 1C). Western blot analysis demonstrated a decline in mitophagy regulators (PINK1, TUFm, NIX, p62 and LC3) concomitant

with upregulation of senescence-associated proteins (MMP3, iNOS, p21 and p16) in KOA cartilage compared with sham tissue (Fig. 1D). These protein alterations were conserved at the transcriptional level, as evidenced by qPCR showing parallel modulation of corresponding mRNA expression patterns (Fig. 1E). Collectively, these findings demonstrated KOA progression is mechanistically linked to impaired mitochondrial clearance and exacerbated chondrocyte senescence in articular cartilage.

PINK1 overexpression decreases senescence and promotes mitophagy in chondrocytes. *In vitro*, LPS, IL-1 β and TNF- α significantly upregulated the protein expression levels of the senescence-associated markers p21, p16, iNOS and MMP3 (Fig. S1A), and increased the proportion of SA- β -gal-positive cells (Fig. S1B). To investigate the role of PINK1 in LPS-stimulated chondrocytes, overexpression was performed using adenoviral transfection. Western blot analysis revealed significant upregulation of mitophagy-associated proteins (PINK1, TUFm, BINP1, p62 and LC3B) in KOA chondrocytes compared with NC, which was markedly reversed by Lv-PINK1 infection (Fig. 2A). JC-1 and ROS fluorescence quantification demonstrated impaired mitochondrial membrane potential and elevated ROS levels in KOA chondrocytes relative to NC (Fig. 2B and C), both of which were significantly ameliorated by Lv-PINK1 treatment. SA- β -gal staining showed a significant increase in senescent chondrocytes during KOA progression compared with blank controls, with Lv-PINK1 infection decreasing senescence markers (Fig. 2D). Western blot analysis confirmed decreased levels of senescence-associated proteins (MMP3, iNOS, p21 and p16) following PINK1 overexpression (Fig. 2E). These coordinated changes demonstrated that enhancing PINK1-mediated mitophagy mitigated chondrocyte senescence through restored mitochondrial quality control under inflammatory stress.

Transcriptomic analysis of PINK1 overexpression in OA chondrocytes. RNA-seq of chondrocytes revealed the anti-senescence mechanism of PINK1. Correlation (Fig. 3A) and principal component analysis (Fig. 3B) demonstrated distinct clustering between KOA and control groups, while Lv-PINK1-infected chondrocytes exhibited transcriptional profiles comparable with controls. There were 6,420 dysregulated genes in KOA vs. controls (2,425 up- and 3,995 downregulated), with Lv-PINK1 intervention altering 6,559 transcripts (3,877 up and 2,682 downregulated; Fig. 3C and D). The volcano plots using criteria of $|\log_2 \text{FC}| > 1$ and $P < 0.05$ confirmed these genome-wide alterations. KEGG pathway analysis (Fig. 3G and J) and scatter plots (Fig. 3H and K) highlighted the top 10 pathways and revealed that 'MAPK signaling pathway', 'TNF signaling pathway' and 'IL-17 signaling pathway' were associated with KOA. Lv-PINK1 treatment predominantly modulated 'focal adhesion' and 'IL-17 signaling pathway'. GSEA (Fig. 3I and L) confirmed the involvement of the MAPK signaling pathway in KOA development and its modulation by Lv-PINK1. Changes in MAPK pathway genes (Fig. 3M and N) showed that p38 expression was increased during KOA but decreased by Lv-PINK1 infection. These multi-omics findings demonstrated that PINK1 overexpression may mitigate chondrocyte senescence and

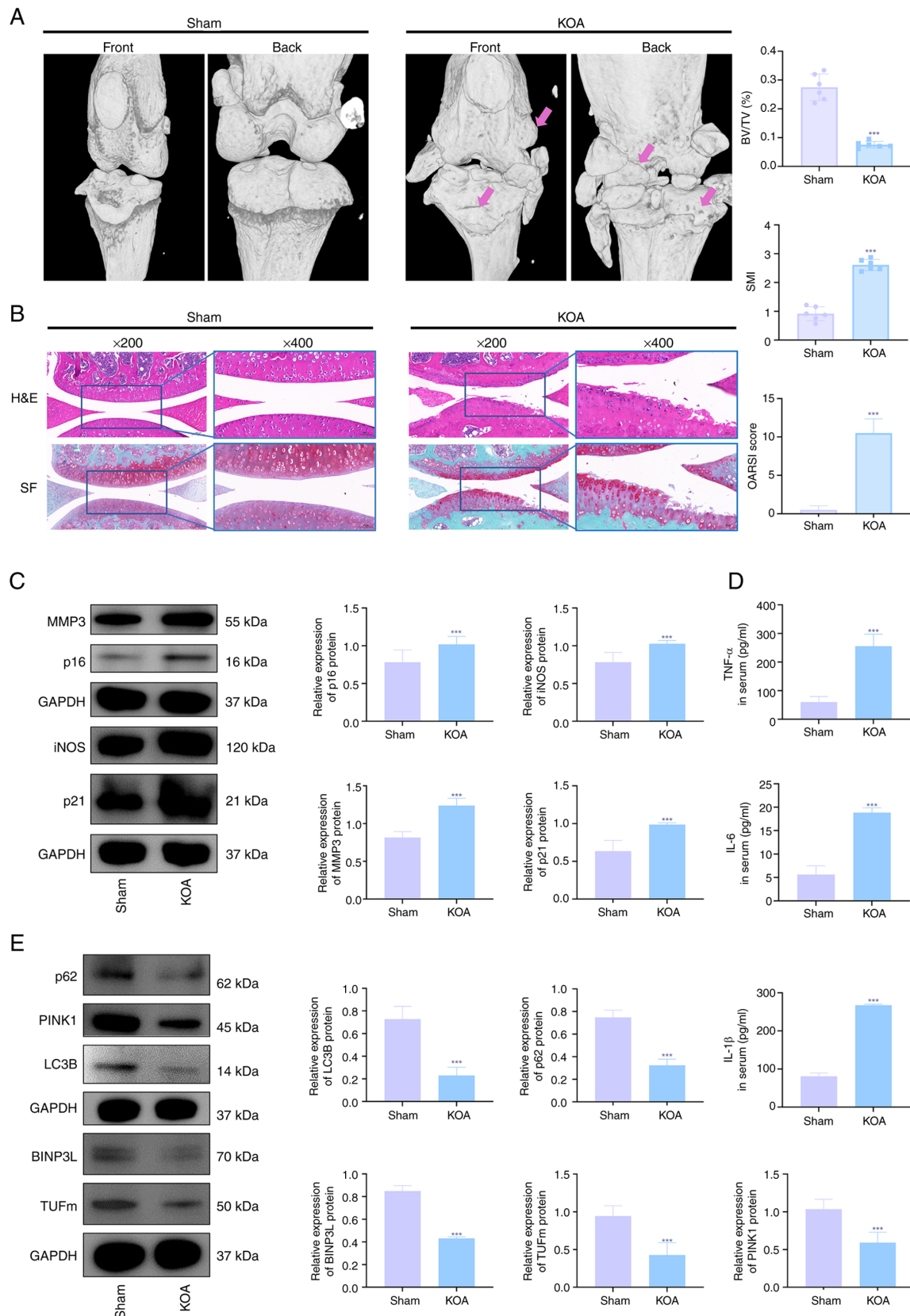


Figure 1. Impaired mitochondrial autophagy and enhanced cell senescence in KOA cartilage. (A) Representative microcomputed tomography 3D reconstructions of knee joints from sham-operated and destabilization of the Medial Meniscus induced KOA mice 14 days post-surgery. (B) Histopathological evaluation and OARSI scoring of knee joint sections ($n=6$), stained with HE and SF. Scale bar, $50\ \mu\text{m}$. (C) Western blot analysis and densitometry of senescence-associated markers (MMP3, iNOS, p21, p16) in cartilage, normalized to GAPDH ($n=3$). (D) Serum levels of proinflammatory cytokines (TNF- α , IL-6, IL-1 β) measured by ELISA using samples collected via cardiac puncture ($n=6$). (E) Western blot analysis and densitometry of mitophagy-related proteins (PINK1, LC3B ratio, p62, BNIP3L, TUFm) in cartilage, normalized to GAPDH ($n=3$). *** $P<0.001$ vs. sham. KOA, knee osteoarthritis; HE, hematoxylin and eosin; TNF- α , tumor necrosis factor- α ; PINK1, PTEN-induced putative kinase 1; TUFm, Tu translation elongation factor; OARSI, Osteoarthritis Research Society International; SO/FG, Safranin O-Fast Green; iNOS, inducible nitric oxide synthase; BNIP3L, BCL2-interacting Protein 3 Like; SMI, structure model index of trabeculae; BV/TV, bone volume fraction.

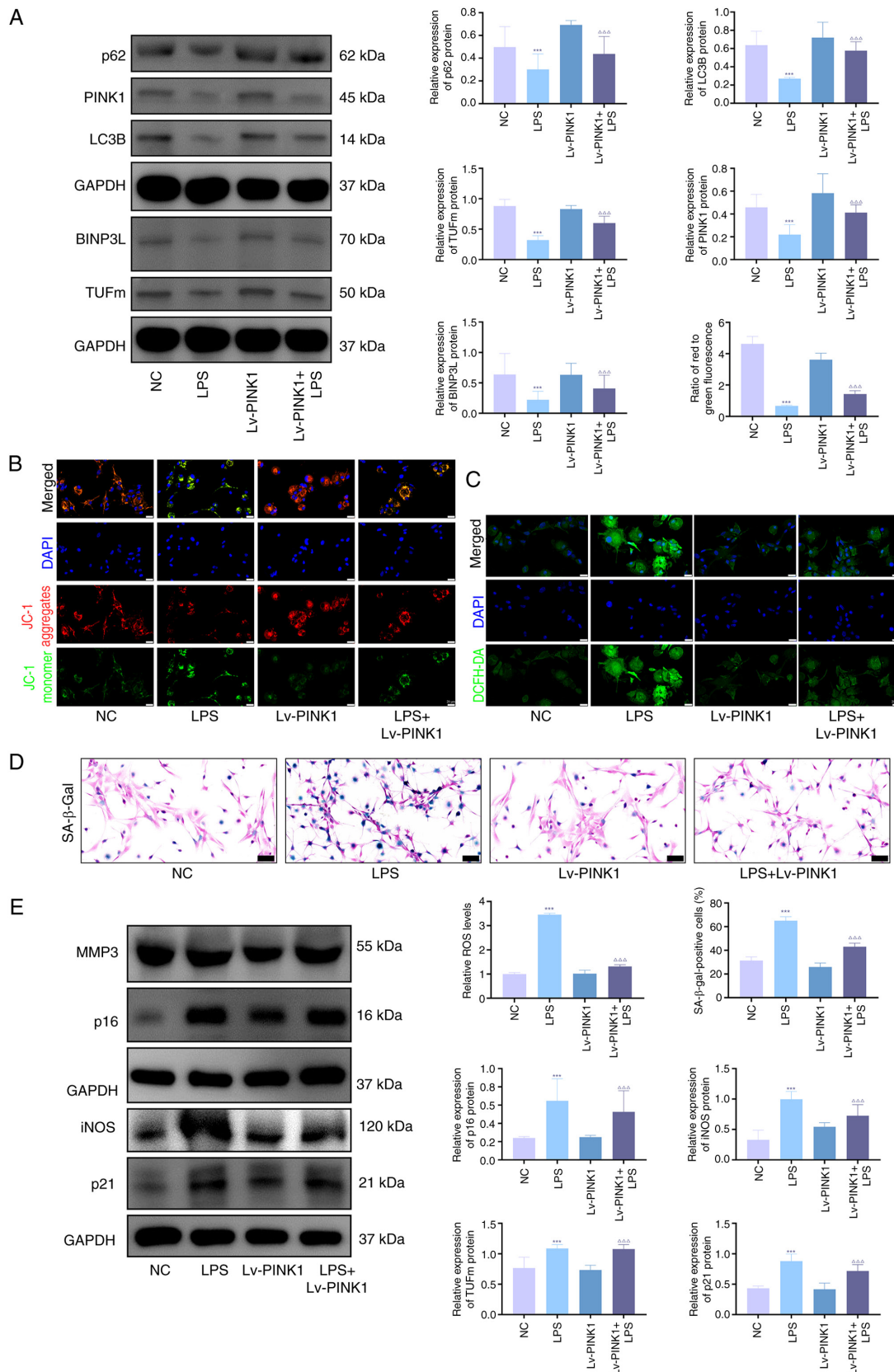


Figure 2. PINK1 overexpression decreases senescence and promotes mitophagy in chondrocytes. (A) Western blot analysis and densitometric quantification of mitophagy markers (PINK1, LC3B, p62, BNIP3L, TUFm) in primary mouse chondrocytes following transduction with Lv-PINK1, followed by LPS treatment (1 μ g/ml, 24 h). Protein signals were normalized to GAPDH. (B) Mitochondrial membrane potential was assessed using JC-1 staining. Red, healthy mitochondria (J-aggregates); green, depolarized mitochondria (J-monomers). (C) Levels of intracellular ROS were detected using the DCFH-DA probe. Green fluorescence intensity was quantified in ≥ 100 cells/group. Scale bar, 20 μ m. (D) Cellular senescence (blue) was evaluated by SA- β -gal staining. The percentage of SA- β -gal-positive cells was quantified (scale bar, 50 μ m). (E) Western blot quantification of senescence-associated secretory phenotype markers (MMP3, iNOS) and cell cycle inhibitors (p21, p16). Protein signals were normalized to GAPDH. n=3. ***P<0.001 vs. NC; $\Delta\Delta\Delta$ P<0.001 vs. LPS. PINK1, PTEN-induced putative kinase 1; TUFm, Tu translation elongation factor; ROS, reactive oxygen species; SA- β -gal, senescence-associated β -galactosidase; LPS, lipopolysaccharide; BNIP3L, BCL2-interacting Protein 3 Like; Lv, Lentiviral Vector; iNOS, inducible nitric oxide synthase; NC, negative Control.

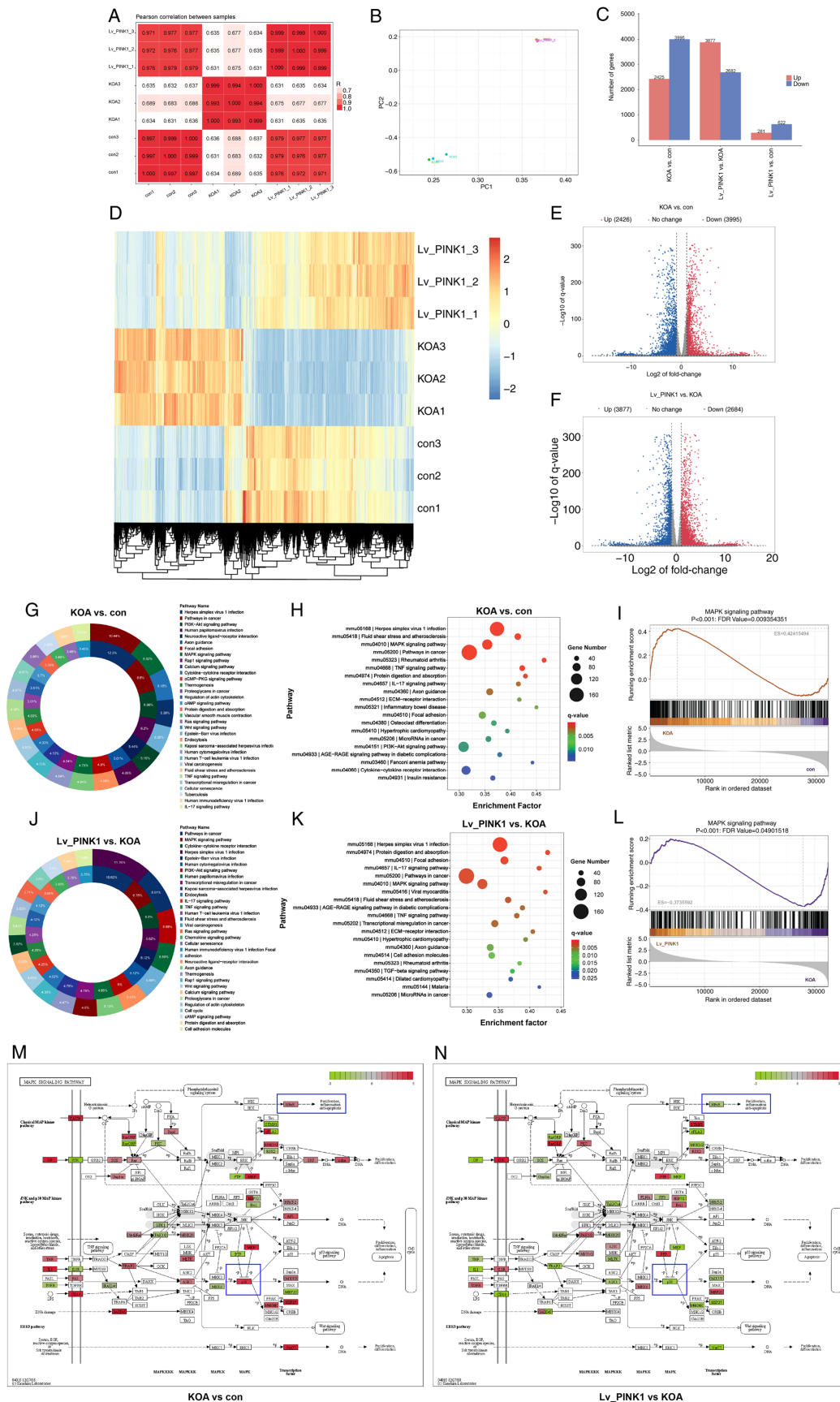


Figure 3. Transcriptomic analysis of PINK1 overexpression in OA chondrocytes. (A) Correlation analysis, (B) PCA and (C) number and (D) heatmap of differentially expressed genes between groups. Top 10 differentially expressed genes in (E) KOA vs. con and (F) Lv-PINK1 vs. KOA group. KEGG enrichment analysis (G) donut chart and (H) bubble plot of differentially expressed genes in the KOA vs. con group. (I) GSEA of the MAPK signaling pathway in the KOA vs. con group. KEGG enrichment analysis (J) donut chart and (K) bubble plot of differentially expressed genes in the Lv-PINK1 vs. KOA group. (L) GSEA of the MAPK signaling pathway in the Lv-PINK1 vs. KOA group. (M) Changes in MAPK signaling pathway-related target genes in the KOA vs. con and (N) Changes in MAPK signaling pathway-related target genes in the Lv-PINK1 vs. KOA group. PINK1, PTEN-induced putative kinase 1; PCA, principal component analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis; KOA, knee osteoarthritis; Lv, lentiviral vector; con, control.

maintain mitochondrial function via regulation of the MAPK signaling pathway.

Activation of the p38 MAPK/NF- κ B pathway induces senescence and mitochondrial damage in LPS-treated chondrocytes. To validate the transcriptomic findings, diprovocim, a TLR (Toll like receptors)7/8 agonist that indirectly activates p38 MAPK and NF- κ B pathways, was used to investigate its effects on KOA chondrocytes. ELISA demonstrated that diprovocim significantly elevated IL-6 and TNF- α levels in the culture medium (Fig. 4A). Western blotting (Fig. 4B) confirmed that diprovocim upregulated the expression of mitophagy-(PINK1, TUFm, BNIP3L, p62 and LC3B) and senescence-associated proteins (MMP3, iNOS, p21 and p16), while promoting phosphorylation of p38 MAPK and NF- κ B. SA- β -gal staining revealed a marked increase in senescent cells following diprovocim treatment (Fig. 4F). JC-1 staining (Fig. 4C) and ROS assays (Fig. 4D) further indicated decreased mitochondrial membrane potential and elevated ROS accumulation in treated chondrocytes.

PINK1 gene knockdown enhances p38 MAPK/NF- κ B phosphorylation and exacerbates chondrocyte senescence. To validate the preliminary findings, PINK1 knockdown models in KOA chondrocytes were established to investigate the activation of the p38 MAPK/NF- κ B pathway and cell senescence progression. Successful shRNA transfection was demonstrated (Fig. 5A). Western blot analysis demonstrated that PINK1 deficiency significantly elevated phosphorylation levels of p38 MAPK and NF- κ B compared with the control (Fig. 5B). In addition, senescence-associated proteins (MMP3, iNOS, p21 and p16) were upregulated (Fig. 5C), whereas mitochondrial autophagy markers (PINK1, TUFm, NIX, p62 and LC3B) exhibited downregulation (Fig. 5D). LPS stimulation exacerbated these phenotypical alterations and SA- β -gal staining revealed a pronounced increase in senescent cells following PINK1 knockdown, particularly in LPS-treated cells (Fig. 5E). JC-1 assays demonstrated diminished mitochondrial membrane potential (Fig. 5F), while ROS quantification confirmed elevated oxidative stress in PINK1-deficient cells (Fig. 5G).

Inhibition of the p38 MAPK/NF- κ B pathway ameliorates senescence and mitochondrial damage in PINK1-silenced chondrocytes. To investigate whether PINK1 knockdown exacerbates chondrocyte senescence via activation of the p38 MAPK/NF- κ B pathway, talmapimod, a specific p38 MAPK inhibitor, was used in PINK1-silenced chondrocytes to evaluate its potential therapeutic effects. Western blot analysis demonstrated that pharmacological inhibition of p38 MAPK significantly decreased phosphorylation levels of both p38 MAPK and NF- κ B, while upregulating senescence-associated proteins such as MMP3, iNOS, p21 and p16 (Fig. 6A and D). By contrast, treatment with diprovocim produced opposing effects on these biomarkers. Consistent with these findings, SA- β -gal staining revealed a marked decrease in senescent cells following inhibitor treatment, whereas agonist intervention significantly increased the proportion of senescent cells (Fig. 6E). JC-1 staining indicated that p38 MAPK activation restored mitochondrial membrane potential and markedly

decreased ROS accumulation (Fig. 6B and C). Collectively, these results suggested modulation of the p38 MAPK/NF- κ B pathway exerted regulatory effects on chondrocyte senescence through the maintenance of mitochondrial homeostasis. These results collectively indicated that compromised PINK1-mediated mitophagy exacerbated mitochondrial dysfunction and promoted cellular senescence under inflammatory conditions, potentially via activation of the p38 MAPK/NF- κ B pathway.

Discussion

As the sole resident cells of articular cartilage, chondrocytes serve a key role in maintaining ECM homeostasis. However, under chronic stress or inflammation, these cells progressively transform into a senescent phenotype, culminating in irreversible cell cycle arrest characterized by distinct morphological changes including enlarged and flattened cell morphology and functional decline (9). This senescence-associated metabolic impairment compromises cartilage structural integrity, leading to ECM degradation and tissue degeneration. Notably, senescent chondrocytes exhibit paradoxical biological activity through sustained secretion of proinflammatory cytokines (IL-1 β , IL-6 and TNF- α), MMPs and other catabolic mediators, as a phenomenon collectively termed the SASP (29). The SASP propagates senescence via paracrine signaling, disrupts ECM biosynthesis and activates matrix-degrading enzymes, creating a self-amplifying loop that accelerates cartilage destruction. Clinical evidence reveals elevated senescence biomarkers, including telomere shortening and increased senescent cell burden, in joint tissue of young patients with post-traumatic OA (30). Complementary studies demonstrate upregulation of SASP factors across multiple articular compartments, particularly in subchondral bone, synovium and infrapatellar fat pads (31-33). Here, male mice were used for DMM modeling and mechanistic experiments to avoid hormone fluctuations from the estrous cycle in females, which can confound inflammatory phenotypes and metabolic endpoints. This approach enhances internal consistency and decreases variability. The present study corroborated the aforementioned clinical observations, showing significant upregulation of cyclin-dependent kinase inhibitors p16 and p21 alongside marked elevation of serum IL-1 β , IL-6 and TNF- α levels compared with control groups. This multisystemic manifestation of senescence markers across articular tissue underscores the systemic nature of cell aging in K OA pathogenesis.

The molecular mechanisms underlying cell senescence have been extensively characterized, with mitochondrial dysfunction emerging as a key determinant following Harman's free radical theory of aging (34-36). Substantial evidence has established that mitochondrial impairment orchestrates intracellular ROS accumulation, disrupts adenosine triphosphate biosynthesis and ultimately initiates cellular senescence through defined molecular cascades (37-39). Evolutionary conservation has equipped eukaryotic cells with sophisticated quality control systems, including mitophagy, a selective autophagy mechanism responsible for clearing depolarized mitochondria to preserve organellar homeostasis (40). Nevertheless, compromised mitophagic flux induces progressive accumulation of dysfunctional mitochondria, culminating

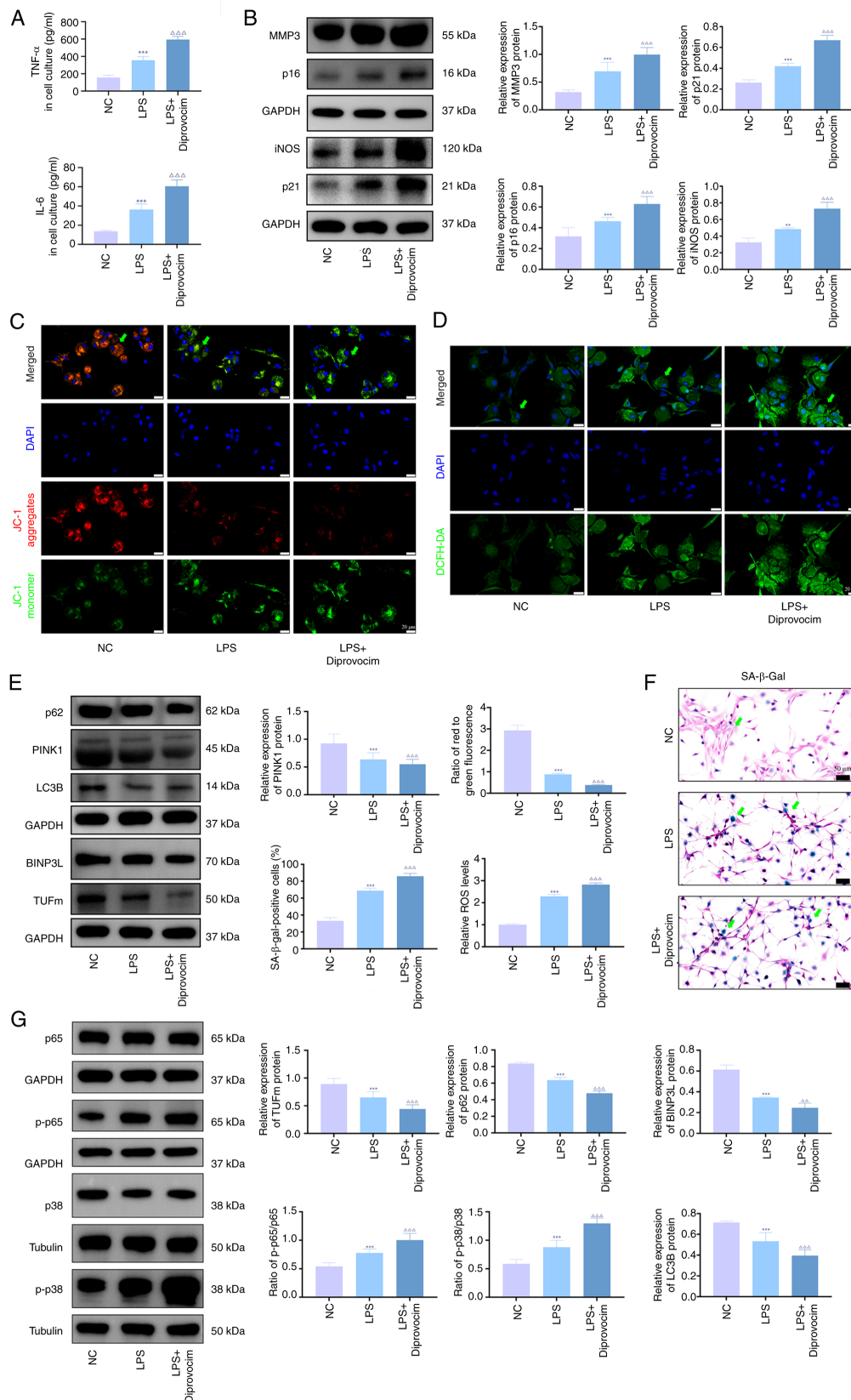


Figure 4. Activation of the p38 MAPK/NF- κ B pathway induces senescence and mitochondrial damage in LPS-treated chondrocytes. (A) ELISA was performed to detect the levels of TNF- α and IL-6 in the culture medium of immortalized human chondrocytes. (B) Western blotting was performed to detect the protein expression of MMP3, iNOS, p21 and p16 in immortalized human chondrocytes. Representative (C) JC-1 staining showing mitochondrial membrane potential in immortalized human chondrocytes. (D) DCFH-DA staining showing ROS levels in immortalized human chondrocytes. (E) Western blotting was performed to detect the protein expression of PINK1, LC3B, p62, BINP3L and TUFm in immortalized human chondrocytes. (F) Representative SA- β -Gal staining showing senescence levels in each group of immortalized human chondrocytes. (G) Western blotting was performed to detect the protein expression levels of p-p65, p65, p-p38 and p38. n=3. **P<0.01, ***P<0.001 vs. sham; $\Delta\Delta\Delta$ P<0.001 vs. LPS. LPS, lipopolysaccharide; ROS, reactive oxygen species; PINK1, PTEN-induced putative kinase 1; TUFm, Tu translation elongation factor; SA- β -gal, senescence-associated β -galactosidase; iNOS, inducible nitric oxide synthase; p-, phosphorylated; BINP3L, BCL2 Interacting Protein 3 Like.

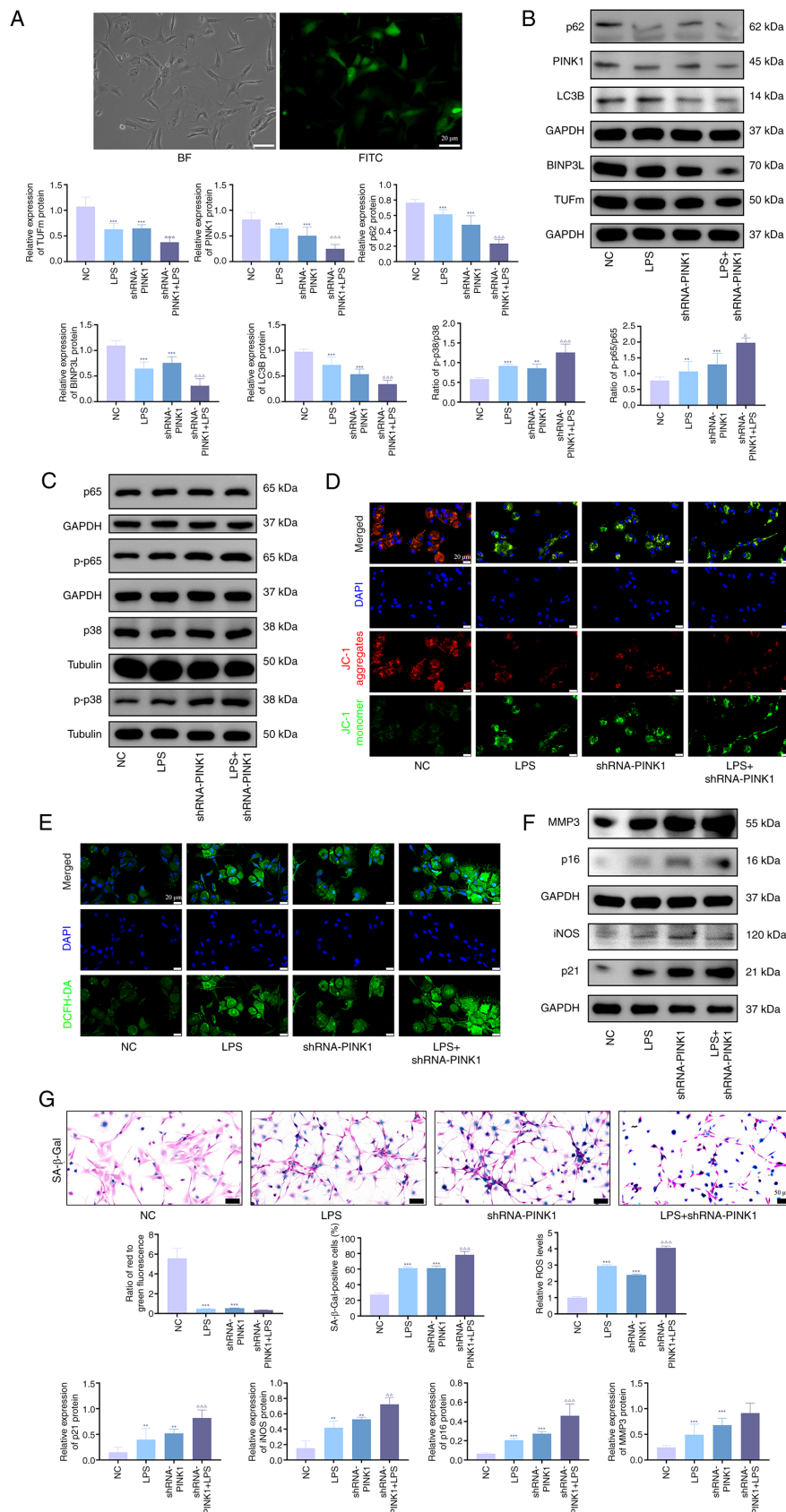


Figure 5. PINK1 gene knockdown enhances p38 MAPK/NF- κ B phosphorylation and exacerbates chondrocyte senescence. (A) Adenoviral infection efficiency of immortalized human chondrocytes observed under a fluorescence microscope. Western blot analysis of (B) PINK1, LC3B, p62, BINP3L and TUFm and (C) p-p65, p65, p-p38 and p38 protein expression. Representative (D) JC-1 staining showing mitochondrial membrane potential and (E) DCFH-DA staining showing ROS levels in immortalized human chondrocytes. (F) Western blot analysis of MMP3, iNOS, p21 and p16 protein expression in immortalized human chondrocytes. (G) Representative SA- β -Gal staining showing senescence levels in immortalized human chondrocytes. $n=3$. ** $P<0.01$, *** $P<0.001$ vs. sham; $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.01$, $^{\Delta\Delta\Delta}P<0.001$ vs. LPS. PINK1, PTEN-induced putative kinase 1; TUFm, Tu translation elongation factor; ROS, reactive oxygen species; SA- β -Gal, senescence-associated β -galactosidase; BINP3L, BCL2-interacting Protein 3 Like; p-, Phosphorylation; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NC, Negative Control; sh, short hairpin; BF, Bright field.

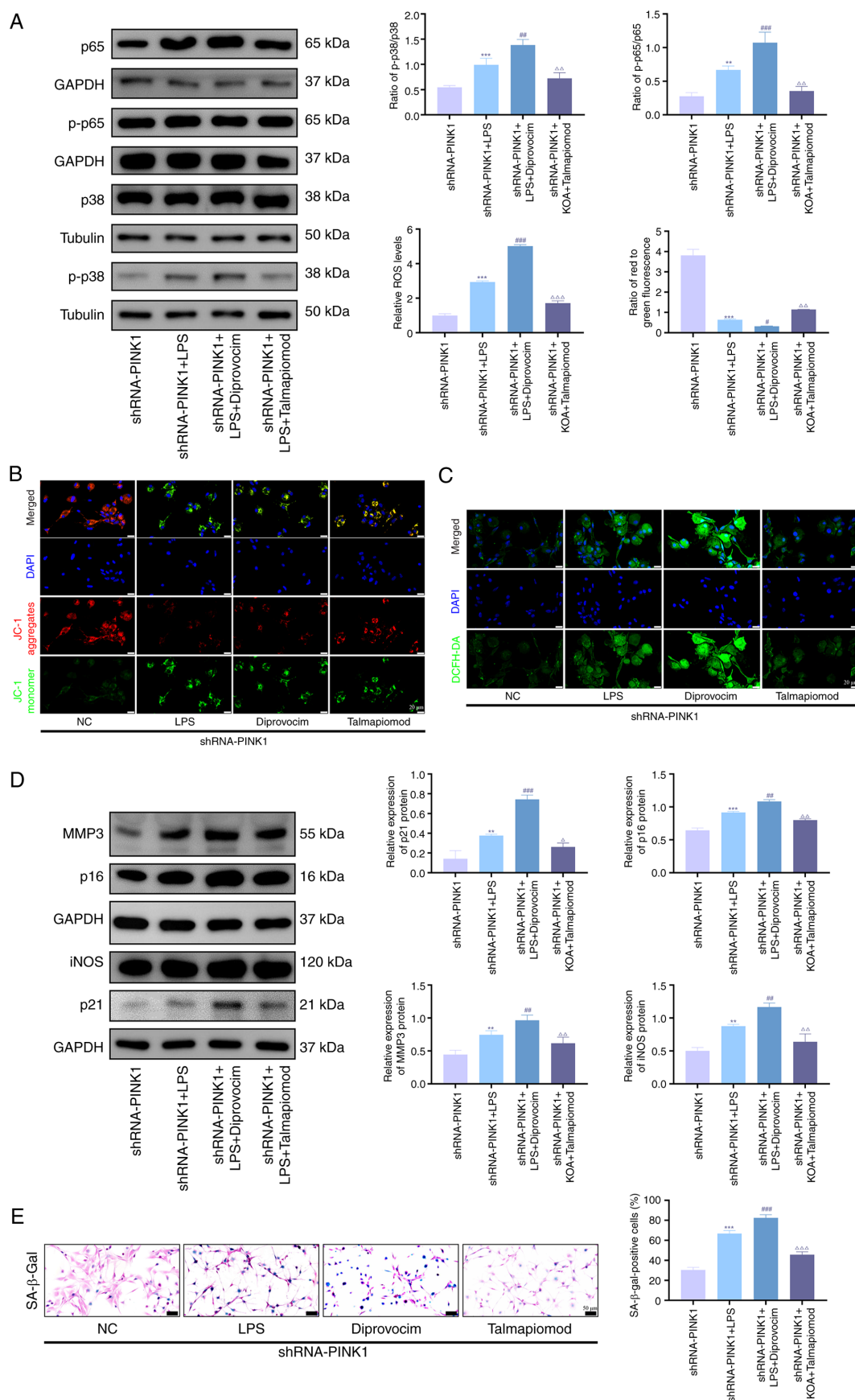


Figure 6. Inhibition of p38 MAPK/NF- κ B pathway ameliorates senescence and mitochondrial damage in PINK1-silenced chondrocytes. (A) Western blot analysis of protein expression of p-p65, p65, p-p38 and p38. (B) Representative JC-1 assay showing mitochondrial membrane potential and (C) DCFH-DA staining showing ROS levels, (D) Western blot analysis of protein expression of MMP3, iNOS, p21 and p16 and (E) representative SA- β -Gal staining showing senescence levels in immortalized human chondrocytes. $n=3$. ** $P<0.01$, *** $P<0.001$ vs. sham; # $P<0.05$, ### $P<0.01$, ### $P<0.001$ vs. LPS; $\Delta P<0.05$, $\Delta\Delta P<0.01$, $\Delta\Delta\Delta P<0.001$ vs. diprovocim. ROS, reactive oxygen species; PINK1, PTEN-induced putative kinase 1; SA- β -gal, senescence-associated β -galactosidase; p-, Phosphorylation; iNOS, inducible nitric oxide synthase; sh, short hairpin; NC, Negative Control; LPS, Lipopolysaccharide.

in bioenergetic collapse and accelerated chondrocyte senescence. The present study systematically investigated this mitophagy-senescence axis through multimodal validation. Immunoblotting demonstrated significant decreases in PINK1 and LC3-II expression in osteoarthritic cartilage compared with healthy donor-matched controls. Complementary *in vitro* analyses using LPS-induced senescence models revealed mitochondrial membrane potential dissipation accompanied by notable ROS elevation vs. untreated chondrocytes. These mechanistically interlinked findings substantiate impaired mitochondrial quality control as a pathognomonic feature of K OA-associated chondrocyte senescence, consistent with a prior demonstration of mitophagic failure in this pathology (25).

PINK1 serves as a key regulator of mitophagy, operating through both canonical and non-canonical molecular pathways. In the canonical pathway, PINK1 orchestrates mitochondrial quality control by phosphorylating ubiquitin chains at Ser65 residues, thereby recruiting parkin to initiate ubiquitination-mediated marking of damaged mitochondria (41,42). *In vitro* models of K OA (25), demonstrated PINK1-dependent PARKIN regulation, with specific inhibition decreasing PARKIN expression, while PINK1 overexpression restored physiological expression levels. These findings confirm the essential role of the PINK1-PARKIN axis in OA pathogenesis (23). Notably, the present investigation revealed a novel non-canonical regulatory mechanism: In LPS-induced senescent chondrocytes, PINK1 overexpression upregulated TUFm expression, as determined by western blot analysis. This finding aligns with the report by Lin *et al* (21) in 293T cells, where activated PINK1 promotes phosphorylation of TUFm at Ser222, which hinders the formation of the Atg5-Atg12 complex, thereby maintaining mitophagic flux homeostasis. Mechanistically, this dual-axis regulatory mechanism explains the mechanism by which PINK1 overexpression synchronously enhances mitochondrial clearance efficiency while mitigating autophagic stress-induced cellular damage.

Notably, the present study provided novel mechanistic insights into the anti-senescence effects of PINK1 overexpression, demonstrating its dependence on suppression of the p38 MAPK/NF- κ B signaling pathway through integrated omics and molecular analyses. Transcriptomic profiling revealed marked upregulation of the MAPK pathway components in K OA progression, which was attenuated by lentiviral-mediated PINK1 overexpression. Molecular validation demonstrated elevated p38 phosphorylation levels in KOA chondrocytes, a phenomenon exacerbated by PINK1 inhibition. This regulatory association corroborates recent cardiovascular research where cardiac-specific PINK1 overexpression suppressed p38 signaling activation, thereby ameliorating pathological remodeling in heart failure models (43-45). Subsequent investigations have revealed that PINK1 deficiency exacerbates LPS-induced pathological cascades, characterized by ROS overproduction, aberrant activation of the p38 MAPK/NF- κ B signaling pathway and mitochondrial dysfunction (46,47). This establishes a self-perpetuating triad of oxidative stress, inflammatory activation and autophagic suppression (48). Notably, pharmacological inhibition of p38 signaling significantly ameliorated these pathological manifestations and disrupted this degenerative cycle, thereby underscoring the key role of PINK1 in mitigating senescence through suppression of the p38 MAPK/NF- κ B pathway. This mechanism demonstrates cross-organ corroboration with

the findings of Luo *et al* (49) and Qigen *et al* (50) in premature testicular failure research, who demonstrated that p38 MAPK mediates age- and obesity-induced Leydig cell senescence. The dual regulatory capacity of PINK1, which simultaneously enhances mitochondrial clearance and mitigates inflammatory stress, positions it as a key coordinator of cell homeostasis.

Overexpression of PINK1 promotes cartilage repair and regeneration through multiple mechanisms. As a key regulator of mitochondrial quality control, PINK1 overexpression enhances mitophagy in chondrocytes, clearing damaged mitochondria and maintaining cellular energy homeostasis (51,52). Studies have shown that PINK1 activates the PARKIN-dependent mitophagy pathway, decreases the accumulation of ROS, and inhibits chondrocyte apoptosis and matrix degradation (25,53). PINK1 overexpression upregulates the synthesis of type II collagen and proteoglycans, facilitating reconstruction of the cartilage ECM (54). In addition, PINK1 modulates inflammatory responses by lowering the expression of inflammatory cytokines such as IL-1 β and TNF- α , thereby creating a favorable microenvironment for cartilage repair (55). These mechanisms make PINK1 a potential therapeutic target for degenerative cartilage disease.

PINK1-mediated mitophagy and its downstream p38 MAPK/NF- κ B signaling pathway are highly conserved in mammals. This evolutionary conservation provides a biological basis for translating findings from animal models to human clinical applications (56,57). The PINK1/PARKIN pathway, as a core mechanism of mitochondrial quality control, has been shown to play a serve role in multiple age-related diseases, including human Parkinson's disease and cardiac disorder (58,59). These observations suggest that the mechanism by which PINK1 alleviates chondrocyte senescence by inhibiting the p38 MAPK/NF- κ B signaling pathway, may be present in human patients with KOA as well (60).

However, the present study had limitations and potential constraints. First, it lacked direct validation using human clinical samples. Although immortalized human chondrocytes were used for *in vitro* experiments, these cell lines may lose some physiological characteristics of primary chondrocytes after multiple passages, making it difficult to reflect the true pattern of PINK1 expression in cartilage from patients with primary KOA and its association with disease severity. Second, the present study primarily focused on changes in cartilage tissue, with insufficient overall assessment of other joint tissue involved in KOA and the whole-knee pathological changes. As KOA is a disease of the entire joint, interactions between these tissues are key to disease progression. In addition, the DMM model is a post-traumatic OA model that primarily simulates secondary OA caused by mechanical instability, which may not represent the pathophysiological processes of age-related primary KOA, particularly with respect to aging-associated metabolic and inflammatory mechanisms. Future studies should integrate clinical sample analyses, extend the observation period, increase sample size and adopt multi-species validation strategies to evaluate the clinical value of PINK1 in the prevention and treatment of human KOA.

In summary, the present study demonstrated that PINK1, a critical regulator of mitophagy, significantly alleviated the pathological changes in K OA by ameliorating chondrocyte senescence. This effect was mediated by inhibition of the p38 MAPK/NF- κ B signaling pathway. Further studies are

warranted to elucidate the detailed molecular mechanisms underlying the regulation by PINK1 on this signaling pathway.

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Availability of data and materials

The data generated in the present study may be found in the National Center for Biotechnology Information) under accession number PRJNA1331976 or at the following URL: dataview.ncbi.nlm.nih.gov/object/PRJNA1331976

Authors' contributions

SY and PW confirm the authenticity of all the raw data. LJ conceived and designed the study. YZ performed experiments; JL performed experiments; ZH performed experiments; HF performed experiments; ZH contributed to analysis and interpretation of data; ZZ contributed to analysis and interpretation of data; XS contributed to analysis and interpretation of data; PW contributed to analysis and interpretation of data. SY edited the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Ethical approval was obtained from Institutional Animal Care and Use Committee at Nanjing University of Chinese Medicine, Nanjing, China. (approval no. 202409A051).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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