

MnB-RLG nanozyme ameliorates intervertebral disc degeneration via metabolic-redox modulation

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ABSTRACT: Intervertebral disc degeneration (IDD) is a leading cause of low back pain. Oxidative stress, mitochondrial dysfunction, and lipid peroxidation-driven ferroptosis act as the core pathological drivers, forming a vicious cycle that exacerbates nucleus pulposus cell (NPC) dysfunction and extracellular matrix (ECM) degradation. Conventional therapeutic strategies are limited by single-target intervention and poor clinical efficacy, highlighting the urgent demand for multifunctional nanotherapeutics that synergistically target the complex pathological environment of IDD. Herein, we developed an interfacial electron-regulated manganese boride nanozyme conjugated with resveratrol-lipoic acid-RGD nanomicelles (MnB-RLG), which exhibited multiple enzyme-mimetic activities, including superoxide dismutase (SOD)-like, catalase (CAT)-like and glutathione peroxidase (GPx)-like properties. *In vitro* experiments revealed that MnB-RLG possessed favorable biocompatibility and efficient cellular internalization capacity, which effectively promoted proliferation and migration of NPCs. Notably, MnB-RLG markedly restrained ferroptosis and enhanced sirtuin 1 (SIRT1)/PTEN induced kinase 1 (PINK1)/Parkin-related mitophagy markers, thereby restoring mitochondrial membrane potential and respiratory capacity, eliminating excessive reactive oxygen species (ROS) accumulation, and maintaining ECM homeostasis in degenerative NPCs. In a rat puncture-induced IDD model, MnB-RLG significantly alleviated disc structural collapse, maintained disc height, and preserved ECM components *in vivo*. Collectively, this work develops a multifunctional MnB-RLG hybrid nanozyme that exerts synergistic therapeutic effects against IDD through metabolic-redox regulation, providing a potential disease-modifying approach for the clinical management of IDD.

KEYWORDS: intervertebral disc degeneration, nanozyme, mitochondrial homeostasis, mitophagy, ferroptosis, oxidative stress

1 Introduction

Intervertebral disc degeneration (IDD) is a leading cause of low

back pain (LBP), characterized by progressive structural and functional deterioration of the intervertebral disc [1, 2]. Its high prevalence severely compromises quality of life and imposes an escalating global healthcare burden [3]. While conservative treatment and surgical intervention constitute the current clinical mainstays, the former offers only limited symptomatic relief, whereas the latter may predispose patients to adjacent segment degeneration and biomechanical disturbances [4, 5]. These limitations underscore the urgent necessity for more effective and sustainable therapeutic strategies targeting IDD.

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Substantial evidence indicates that oxidative stress, defined as an imbalance between reactive oxygen species (ROS) production and antioxidant defenses, serves as a central driver of cellular senescence, apoptosis, and matrix-degrading phenotypes within the disc [6, 7]. Notably, ferroptosis, an iron-dependent form of lipid peroxidation-driven cell death characterized by glutathione peroxidase 4 (GPX4)-centered antioxidant defense failure, has emerged as an important mechanism linking redox imbalance to disc degeneration [8, 9]. Previous studies have demonstrated that reduced GPX4 activity, impaired solute carrier family 7 member 11 (SLC7A11)-mediated antioxidant defense, abnormal iron metabolism, and excessive lipid peroxide accumulation in nucleus pulposus cells (NPCs) collectively drive uncontrolled lipid peroxidation, ultimately leading to deterioration of disc structure and extracellular matrix integrity [10–12]. Importantly, inhibition of ferroptosis significantly delays the progression of IDD [13]. Thus, therapeutic strategies aimed at restoring ferroptosis homeostasis hold considerable promise for IDD management.

Mitochondria are increasingly recognized as a pivotal hub linking redox homeostasis to disc function, with mitochondrial dysfunction acting as a critical contributor to IDD progression [14, 15]. Mitochondrial dysfunction is characterized by the loss of mitochondrial membrane potential, metabolic insufficiency, and excessive ROS generation [16]. Furthermore, impaired mitochondrial quality, including dysregulated fission-fusion dynamics and compromised electron transport chain activity, can trigger a self-reinforcing cycle that amplifies oxidative stress-driven inflammation and matrix catabolism [14, 17]. As an essential mitochondrial quality control (MQC) mechanism, mitophagy selectively removes damaged mitochondria and protects cells from persistent oxidative injury [18, 19]. Previous studies have revealed that activation of phosphatase and tensin homolog-induced kinase 1 (PINK1)/Parkin-dependent mitophagy helps maintain NPC viability and disc homeostasis [20, 21]. Meanwhile, sirtuin 1 (SIRT1), a NAD⁺-dependent deacetylase, has been identified as an upstream regulator of mitochondrial homeostasis and mitophagy-related signaling pathways in NPCs subjected to mechanical or inflammatory stress [22]. Recent studies indicate that SIRT1 activation alleviated NPC senescence and mitochondrial dysfunction by modulating PINK1-dependent mitophagy [23–25], suggesting that SIRT1 may serve as a potential therapeutic target for IDD progression. Thus, simultaneous regulation of oxidative stress, ferroptosis, and mitochondrial quality control may be more effective than targeting a single pathological process.

Nanozymes are nanomaterials with intrinsic enzyme-mimetic catalytic activity, which can effectively scavenge ROS under pathological conditions and have been widely applied in catalytic ROS scavenging [26]. However, most conventional nanozyme systems predominantly rely on single antioxidant pathways [27], and their catalytic repertoire remains insufficient to accommodate the complexity of mitochondrial bioenergetic regulation and lipid peroxidation dynamics. Transition metal borides (TMBs), characterized by strong metal-boron interactions and conductive frameworks, can integrate catalytic redox activity with electronically active scaffolds [28–30]. As two-dimensional derivatives of TMBs, MBenes possess prominent redox-active and conductive properties, and provide structural support for modulating the activity of mitochondrial respiratory chain [31–33]. These features make MBene-based composite nanozymes attractive candidates for

constructing multifunctional therapeutic systems through interfacial electron-transfer regulation in IDD treatment [34–36]. Thus, the development of multifunctional nanozymes that integrate ROS scavenging with mitochondrial stabilization and metabolic support, represents a promising therapeutic strategy for IDD treatment.

Here, we constructed an interfacial electron transfer functional composite consisting of metal boride-based manganese boride nanozyme (MnB) nanosheets and a resveratrol-lipoic acid-RGD nanomicelles (RLG), named MnB-RLG. As a two-dimensional metal boride, MnB exhibited prominent multi-enzyme-mimetic activity, which enabled efficient scavenging of ROS and suppression of lipid peroxidation, thereby inhibiting oxidative stress and ferroptosis-related processes. Coordination between the disulfide bonds of α -lipoic acid and the MnB surface establishes a stable interfacial electronic microenvironment, which enhances the catalytic antioxidant performance of MnB and amplifies resveratrol mediated activation of SIRT1 signaling. These effects collectively promoted mitophagy and mitochondrial biogenesis, improved mitochondrial function, and cellular energy metabolism. Through the coordinated regulation of oxidative stress, ferroptosis, and mitochondrial dysfunction, the MnB-RLG system exerts synergistic bioactivities and effectively mitigates IDD progression.

2 Experimental

2.1 Preparation of MnB

1.5 g of $N_{12}Mn-(Mo_{2/3}Y_{1/3})_2AlB_2$ powder was mixed with 20 mL of 48% hydrofluoric acid (HF), and etched at room temperature for 48 h. The mixture was then centrifuged at 3000 rpm for 3 min and repeatedly washed with deionized water until the supernatant reached neutral pH. For intercalation, 15 mL of 25% tetrabutylammonium hydroxide (TBAOH) was added to the sediment and stirred for 5 min, followed by centrifugation at 10,000 rpm for 5 min to isolate the multilayer precipitate. This precipitate was washed with deionized water and ultrasonically re-dispersed. The mixture was then centrifuged at 3500 rpm for 5 min. This process was repeated 6 or 7 times, and the upper black colloidal solution, which contained monolayer MnB nanosheets, was collected. Finally, the dispersion was freeze-dried to obtain $N_{12}Mn-Mo_{1.33}B$ powder.

2.2 Preparation of RLG

30 mg of lipoic acid and 3 mg of resveratrol were dissolved together in 1 mL of acetone. This solution was added dropwise to 10 mL of deionized water and mixed thoroughly under magnetic stirring. Acetone was then removed via rotary evaporation. To stabilize the self-assembled nanostructure, a cross-linking reaction was performed under 365 nm ultraviolet light irradiation for 10 min to induce thiol-mediated crosslinking. The cross-linked sample was purified by ultrafiltration centrifugation, and washed with deionized water. The nanoparticles were re-dispersed in phosphate-buffered saline (PBS) buffer and incubated with 1 mg of RGD peptide for 4 h. Unbound RGD peptides were removed by dialysis, obtaining final RLG nanomicelles.

2.3 Preparation of MnB-RLG

RLG nanomicelles and MnB nanosheet aqueous dispersions were mixed at a 1:1 mass ratio and stirred gently at room temperature for

6 h. Subsequently, unbound RLG nanomicelles were removed by repeated centrifugation and washing. The purified conjugate was lyophilized to obtain MnB-RLG composite nanomaterial powder.

2.4 Cy5.5 labeling of MnB, RLG, and MnB-RLG

For fluorescence tracking of cellular uptake, the materials were covalently labeled with Cy5.5. Briefly, MnB, RLG, or MnB-RLG (1 mg·mL⁻¹ in PBS) was activated by addition of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) under gentle stirring. Subsequently, Cy5.5-NHS, dissolved in dimethyl sulfoxide (DMSO), was added dropwise to the activated suspension in the dark, and the coupling reaction was allowed to proceed for 2–4 h at room temperature. After formation of stable amide linkage, the labeled nanoparticles (MnB-Cy5.5, RLG-Cy5.5, and MnB-RLG-Cy5.5) were purified by centrifugation to remove free dye and excess reagents, and used for subsequent fluorescence imaging experiments.

2.5 Transmission electron microscopy (TEM)

Droplets of MnB, RLG, and MnB-RLG suspension were deposited onto carbon-coated copper grids coated with carbon support films. After drying at room temperature, imaging was performed at 200 kV, and elemental distribution maps were acquired in scanning transmission electron microscopy (STEM) mode.

2.6 Scanning electron microscope (SEM)

Briefly, MnB or MnB-RLG powders were dispersed in ethanol by sonication. A droplet of each suspension was pipetted on clean conductive adhesive, air-dried, and then imaged by SEM at 5.0 kV.

2.7 X-ray photoelectron spectroscopy (XPS)

XPS spectra were recorded with a monochromatized Al K α X-ray source (1486.6 eV). Powder samples were uniformly pressed onto double-sided conductive adhesive, secured on the sample stage, and surveyed to identify all elements. High-resolution narrow scans were then acquired for B, Mn, O, and other relevant elements, and data were fitted with Avantage software.

2.8 X-ray diffraction (XRD)

Freeze-dried powder was packed into the sample chamber and leveled to align with the chamber rim. Diffraction patterns were collected on a diffractometer using Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) at 40 kV and tube current at 40 mA over 2θ range of 5°–90° (step size 0.02°, speed 5°·min⁻¹). Diffraction peak positions were identified with GraphPad software to assess sample crystallinity.

2.9 Fourier transform infrared spectroscopy (FTIR)

Freeze-dried powder (1 wt% in KBr) was ground, pressed into a tablet, and analyzed by Fourier transform infrared spectrometer within 4000–400 cm⁻¹ range (resolution 4 cm⁻¹, 32 scans). Characteristic absorption peaks were analyzed to determine chemical structure and intermolecular interactions.

2.10 Cell culture

NPCs were isolated from the caudal discs of 8-week-old male Sprague-Dawley rats. Briefly, nucleus pulposus tissues were carefully dissected under sterile conditions, minced into small fragments, and digested with 0.2% type II collagenase at 37 °C for 2–4 h with gentle agitation. The suspension was filtered through a

70- μm cell strainer, centrifuged, and the pellet was resuspended in Dulbecco's modified Eagle medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco, USA) and 1% Penicillin/Streptomycin (Gibco, USA). Cells were cultured at 37 °C, 5% CO₂, and the culture medium was replaced every 2–3 days and passages 1–3 were used for experiments.

2.11 Superoxide dismutase (SOD) activity assay

SOD activity was determined with the Total Superoxide Dismutase Assay Kit (Beyotime, S0109) using nitroblue tetrazolium (NBT) photochemical method. NPCs were harvested, washed once with pre-chilled PBS, centrifuged at 4 °C, and supernatant was collected for analysis. Reaction mixture (160 μL per well) contained sample or blank and NBT/enzyme working solution. Reaction was initiated by adding 1 $\times$ initiation working solution (40 $\times$ stock solution diluted 1:39 with detection buffer) and performed in a 96-well plate under the kit-specified conditions. Absorbance (*A*) was read at 560 nm and percentage of SOD inhibition was calculated using the following equation (Eq. (1)):

$$\text{Inhibition rate (\%)} = \frac{(A_{\text{blank control 1}} - A_{\text{blank control 2}}) - (A_{\text{sample}} - A_{\text{blank control 3}})}{A_{\text{blank control 1}} - A_{\text{blank control 2}}} \times 100 \quad (1)$$

2.12 Catalase (CAT) activity assay

CAT-like activity was assessed by measuring O₂ generated during hydrogen peroxide (H₂O₂) decomposition. Briefly, NPC lysates were incubated with H₂O₂ in neutral buffer at 37 °C and the dissolved O₂ concentration (mg·L⁻¹) was recorded at specified time points (0–10 min) with a Mettler Toledo oxygen probe. O₂ generation rates were normalized to total protein content, and data presented as O₂ generation (mg·L⁻¹).

2.13 Glutathione peroxidase (GPx) activity assay

GPx-like activity was determined with the Glutathione Peroxidase Assay Kit (Beyotime, S0056) by coupling the reduction of peroxides to nicotinamide adenine dinucleotide phosphate (NADPH) oxidation mediated by glutathione reductase. NPCs (1 $\times$ 10⁶ cells) were washed with PBS, lysed with 100–200 μL of lysis buffer, and centrifuged at 4 °C and 12,000g for 10 min. The supernatant was collected for enzyme activity measurement. In a 96-well plate, 190 μL GPx detection working solution and 10 μL sample were mixed and incubated at room temperature for 15 min to equilibrate the reaction system prior to initiation. 10 μL of 30 mM peroxidase reagent was then added, mixed thoroughly, and absorbance at 340 nm was recorded every 2 min for 8 min. GPx activity was calculated based on the rate of decrease in optical density at 340 nm (OD₃₄₀) within the linear range of 0–8 min.

2.14 Cellular uptake analysis

NPCs were seeded onto confocal microplates and incubated with Cy5.5-labeled nanoparticles (10 $\mu\text{g}\cdot\text{mL}^{-1}$) at 37 °C for 2, 6, or 12 h. After PBS washing, fixed with 4% paraformaldehyde (PFA) at room temperature for 15 min, and permeabilized with 0.1% Triton X-100 for 10 min. Cells were then stained with F-actin Alexa Fluor™ 488 (ThermoFisher, A12379) for 15 min, and nuclei were counterstained with Hoechst 33342 (Beyotime, C1027) for 5 min. Images were acquired on an inverted fluorescence microscope (Olympus, Japan).

2.15 Live-dead cell staining

NPCs viability was assessed with the LIVE/DEAD™ Viability/Cytotoxicity Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Briefly, NPCs were incubated with 1 μ M calcein-acetoxymethyl ester (Calcein-AM) and 10 μ M ethidium homodimer-1 (EthD-1) at 37 °C for 20 min. Subsequently, fluorescence images were acquired on an inverted fluorescence microscope (Olympus, Japan). The apoptotic cells of control were quantified using the following formula: Apoptotic cells (of control) = apoptotic cell area/total cell area.

2.16 Wound healing assay

For the wound healing assay, NPCs were seeded at 2×10^5 cells per well in 6-well plates until a confluent monolayer formed. A sterile pipette tip was used to create a straight scratch. Cell debris were removed by gentle washing with serum-free medium. Cells were then cultured in complete medium containing MnB, RLG, or MnB-RLG. Images of the wound area were acquired at 0, 6, 12, and 24 h and migration capacity was quantified using the following equation (Eq. (2)):

$$\text{Migration rate (\%)} = \frac{A_0 - A_n}{A_0} \times 100 \quad (2)$$

where A_0 denotes the initial wound area and A_n represents the residual area at each time point.

2.17 EdU assay

Cell proliferation was assessed with the 5-ethynyl-2'-deoxyuridine (EdU) kit (RiboBio, C10338). NPCs were incubated with 25 μ M EdU for 12 h at 37 °C, fixed with 4% PFA, permeabilized with 0.5% Triton X-100, and stained with Apollo488. Nuclei were counterstained with Hoechst 33342 for 5 min. Images were acquired using an inverted fluorescence microscope (Olympus, Japan).

2.18 Cellular toluidine blue (TB) and Safranin O (SO) staining

NPCs were stained with 0.1% TB (Solarbio, G3660) or 0.5% SO (Solarbio, G4810) solution for 1–2 min, rinsed gently with deionized water for 3–5 min. Subsequently, the samples were air-dried at room temperature and examined by a light microscope.

2.19 FerroOrange staining

Ferrous iron (Fe^{2+}) was detected with FerroOrange probe (Dojindo, Japan, F374). After PBS rinse, NPCs were incubated with 1 μ M probe for 30 min at 37 °C, and imaged by confocal microscopy (Olympus, Japan).

2.20 Lipid peroxidation measurement

Lipid peroxidation was determined with C11-BODIPY 581/591 (2 μ M; Invitrogen). NPCs were incubated with the probe for 30 min at 37 °C, counterstained with Hoechst 33342, and imaged by confocal microscopy (Olympus, Japan).

2.21 Measurement of malondialdehyde (MDA) and glutathione (GSH)

MDA content was quantified with a MDA kit (Beyotime, S0131S) according to the manufacturer's instructions. Briefly, NPCs were centrifuged at 12,000 rpm for 10 min at 4 °C, and the supernatant

was collected and incubated with a corresponding reagent for 15 min. Absorbance was read at 545 nm using a microplate reader. GSH and oxidized glutathione (GSSG) were determined with the Beyotime S0053 kit according to the manufacturer's instructions. The ratios of GSH/GSSG were calculated after normalization to protein content.

2.22 Mitochondrial and intracellular ROS detection

Mitochondrial superoxide was detected with MitoSOX™ Red (Solarbio, M4830), and intracellular ROS level was assessed with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA; Solarbio, D6470). For mitochondrial ROS detection, NPCs grown to 70%–80% confluence in 6-well plates were rinsed with PBS, and incubated with 5 μ M MitoSOX™ Red in 1 mL PBS at 37 °C for 10 min. For intracellular ROS detection, NPCs were incubated with 10 μ M DCFH-DA diluted in serum-free culture medium at 37 °C for 30 min. After three PBS washes, nuclei were counterstained with Hoechst 33342 for 10 min. Images were acquired by confocal fluorescence microscope (Olympus, Japan).

2.23 Mitochondrial TEM of NPCs

NPCs were fixed in 2.5% glutaraldehyde at 4 °C for 24 h, post-fixed in 1% osmium tetroxide for 1–2 h at room temperature, dehydrated through graded ethanol, and embedded in Epon 812 epoxy resin. Ultrathin sections (~ 70 nm) were cut, and stained with uranyl acetate and lead citrate. Mitochondrial ultrastructure was examined with a HT7700 TEM (Hitachi, Japan).

2.24 MitoTracker staining

Mitochondria were labeled with MitoTracker Red CMXRos (Beyotime, C1035). Briefly, NPCs were incubated with 200 nM MitoTracker working solution at 37 °C for 30 min. After a gentle PBS rinse, cells were counterstained with Hoechst 33342 for 10 min, and imaged on a fluorescence microscope (Olympus, Japan).

2.25 Mitochondrial membrane potential assay

Mitochondrial membrane potential was assessed with JC-1 kit (Beyotime, C2003S) according to manufacturer guidelines. NPCs were incubated with 5 $\mu\text{g}\cdot\text{mL}^{-1}$ JC-1 for 20 min at 37 °C. After PBS washes, cells were counterstained with Hoechst 33342 for 10 min, and imaged on a fluorescence microscope (Olympus, Japan). Red (aggregate) and green (monomer) fluorescence intensities were quantified using Image J, with the red/green emission ratio reflecting mitochondrial depolarization.

2.26 Immunofluorescence staining

NPCs were fixed with 4% PFA, blocked with QuickBlock™ Blocking Buffer (Beyotime, P0235), and incubated overnight at 4 °C with primary antibodies: collagen type II (COL II) (1:200; Proteintech, 28459-1-AP) and matrix metalloproteinase 13 (MMP13) (1:200; Proteintech, 18165-1-AP). After PBS washes, cells were incubated with Alexa Fluor-conjugated secondary antibodies (1:500; Proteintech) for 1 h at room temperature. Nuclei were counterstained with Hoechst 33342 and imaged by a confocal microscope (Olympus, Japan).

2.27 Measurement of oxygen consumption rate (OCR)

OCR was measured on the XFe96 analyzer (Agilent Technologies, USA) according to the manufacturer's instructions. NPCs were

seeded in the XFe96 plates, and treated with *tert*-butyl hydroperoxide (TBHP), TBHP + MnB, TBHP + RLG, or TBHP + MnB-RLG. The culture medium was replaced with the Seahorse XF assay medium, and the plate was incubated in a 37 °C CO₂-free incubator for 1 h. Basal OCR was recorded, followed by sequential injection of 1.5 μM mitochondrial inhibitors, namely oligomycin, 1 μM carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP), and 0.5 μM rotenone/antimycin A. Basal respiration, adenosine triphosphate (ATP)-linked respiration, maximum respiratory capacity, and spare respiratory capacity were calculated with Seahorse Wave software. Finally, OCR values were standardized by cell number and expressed in pmol·min⁻¹·10⁻⁴ cells.

2.28 Mitophagy determination

Mitophagy was visualized with Mtpagy Dye and Lyso Dye (Dojindo). NPCs were seeded on confocal plates and incubated with the Mtpagy Dye at 37 °C for 30 min. After PBS washes, cells were incubated with Lyso Dye at 37 °C for 30 min. Then, the cells were rinsed, counterstained with Hoechst 33342, and imaged by confocal microscope. Mitophagosomes were determined by co-localization of Mtpagy and Lyso signals.

2.29 Western blot analysis

Total proteins were extracted using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, P0013B) containing 1 mM phenylmethylsulfonyl fluoride (PMSF), and protein concentrations were determined via bicinchoninic acid (BCA) assay (Beyotime, P0011). Samples (30 μg·lane⁻¹) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene fluoride (PVDF) membranes, and blocked with 5% non-fat milk. Membranes were incubated overnight at 4 °C with primary antibodies: anti-NRF2 (1:1000, Proteintech, 16396-1-AP); anti-GPX4 (1:1000, Proteintech, 30388-1-AP); anti-TFR (1:1000, Proteintech, 11682-1-AP); anti-FTH (1:1000, Abcam, ab75973); anti-GAPDH (1:5000, Proteintech, 10494-1-AP), anti-xCT (1:1000, Proteintech, 26864-1-AP), anti-FPN1 (1:1000, Proteintech, 26601-1-AP), anti-HO-1 (1:1000, Proteintech, 10701-1-

AP), anti-SIRT1 (1:1000, Proteintech, 13161-1-AP), anti-COL II (1:1000, Proteintech, 28459-1-AP), anti-MMP13 (1:1000, Proteintech, 18165-1-AP), anti-PARK2 (1:1000, Proteintech, 14060-1-AP), anti-P62 (1:1000, Proteintech, 84826-1-RR), anti-LC3 (1:1000, Proteintech, 14600-1-AP), anti-PINK1 (1:1000, Abcam, ab186303). After washing, membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:5000; Proteintech) for 2 h, and visualized by enhanced chemiluminescence (ECL). Semi-quantification of protein expression was performed in Image J.

2.30 Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted with TRIzol reagent (Invitrogen, USA), and RNA concentration and purity were determined with Nanodrop. 1 μg RNA was reverse-transcribed into complementary DNA (cDNA) with the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). qRT-PCR was performed in a 10 μL reaction volume (5 μL of SYBR Green Master Mix (Vazyme), 0.25 μL of each primer, and 4.5 μL of cDNA) with standard cycling. Relative gene expression levels were calculated by the 2^{-ΔΔCt} method with *Gapdh* as endogenous control. The primer sequences used in this study are listed in Table 1.

2.31 In vivo disc degeneration model

All animal procedures were approved by the Institutional Animal Care Committee of Taizhou Hospital (Approval No. TZYY-2026002), in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague-Dawley rats (200–250 g) were anesthetized with 2% isoflurane, and the coccygeal Co7/8 disc was punctured with a 22 G needle to induce disc degeneration. Postoperative analgesia was administered for 72 h.

2.32 Magnetic resonance imaging (MRI)

T₂-weighted sagittal MRI scans were performed on a 3.0 T GE

Table 1 Primer sequences used for qRT-PCR

Gene	Forward (5'–3')	Reverse (5'–3')
<i>Col2a1</i>	ACCTCAGCCTTCCCATTTGTT	TGGGTCCTTTGGGTTTCGCAAT
<i>Mmp13</i>	TCCATCCCGAGACCTCATGT	AGCATCATCACTCCACACAG
<i>Gpx4</i>	GAGGCAAGACCGAAGTAACTAC	CCGAAGTGTTACACGGGAA
<i>Slc7a11</i>	TCTCCAAAGGAGGTTACCTGC	AGACTCCCCTCAGTAAAGTGAC
<i>Slc40a1</i>	GGATTGTTGTTGTCAGGAGAA	ACCAAGTCCATCCCGAAATAAA
<i>Nfe2l2</i>	TCCAGTCAGAAACCACTGGAT	GAATGTCTGCGCCAAAAGCTG
<i>Hmox1</i>	TTCAGCATCCTCAGTTCC	CCGTGTCAACAAGGATAC
<i>Fth1</i>	CGAGGTGGCCGAATCTTCC	GTTTGTGCAGTTCAGTAGTGA
<i>Tfrc</i>	GGCTACTTGGGCTATTGTAAAGG	CAGTTTCTCCGACAACCTTCTCT
<i>Tnf</i>	AGTGACAAGCCTGTAGCCC	GAGGTTGACTTTCTCCTGGTAT
<i>Il1β</i>	CTGTGACTCATGGGATGATGATG	CGGAGCCTGTAGTGCAGTTG
<i>Il6</i>	TAGTCCTTCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
<i>Ifng</i>	ACCCACAGATCCAGCACAAAGC	TGTGGTTGTTGACCTCAAAGC
<i>Sdhb</i>	GCTCCCCGTATCAAGAAATTTGCC	CCAATACCATGGGGCCACATTTAT
<i>Uqcrc1</i>	AGACCCAGGTCAGCATCTTG	GCCGATTCTTTGTTCCCTTGA
<i>Atp5f1a</i>	ATGACGACTTATCCAAACAGGC	CGGGAGTGTAGGTAGAACACAT
<i>Gapdh</i>	GCAAGTTCAACGGCACAG	CGCCAGTAGACTCCACGAC

Signa HDX (TR 2275 ms, TE 80 ms, slice thickness 1.5 mm) at 4 or 8 weeks post-surgery. Nucleus pulposus signal intensity was quantified in OsiriX MD v.12.0.

2.33 X-ray

Plain tail radiographs were acquired on Convix 80 Universix 120° system (1 m collimator-to-film distance, 20–30 mAs, 70–85 kV) after 4 or 8 weeks post-surgery. Disc height index (DHI) was calculated and normalized to corresponding adjacent discs (DHI%).

2.34 Histological analysis

Caudal Co7/8 discs were harvested, fixed in 4% PFA for 48 h, and decalcified in 10% ethylenediaminetetraacetic acid (EDTA) for 8 weeks. After dehydration and paraffin embedding, 5 µm sections were prepared and stained with hematoxylin and eosin (H&E), safranin O-fast green (S.O.), and Alcian blue (A.B.) to visualize nucleus pulposus, annulus fibrosus, and cartilage endplate morphology. Images were captured on a light microscope (Olympus).

2.35 Immunohistochemistry (IHC) analysis

Sections were deparaffinized, rehydrated through graded ethanol, and subjected to antigen retrieval (citrate buffer, pH 6.0) in microwave. After blocking with 5% goat serum albumin for 1 h at 37 °C, sections were then incubated overnight at 4 °C with the following primary antibodies: anti-COL II (28459-1-AP, Proteintech; 1:150), anti-MMP13 (18165-1-AP, Proteintech; 1:150), anti-ACSL4 (22401-1-AP, Proteintech; 1:150), and anti-8-OHdG (sc-66036, Santa Cruz Biotechnology; 1:150). After PBS washes, sections were incubated with corresponding HRP-conjugated secondary antibodies (1:1000) for 1 h at 37 °C, and immunoreactivity was visualized with 3,3'-diaminobenzidine (DAB; ZSGB-BIO). Slides were counterstained with hematoxylin, and imaged on a light microscope. Staining intensity was quantified using Image J.

2.36 Statistical analysis

Data are presented as the mean ± standard deviation (SD). Differences between two groups were analyzed by unpaired Student's *t*-test, while multiple comparisons were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. All statistical analyses were conducted in GraphPad Prism software 9.0. *P* < 0.05 was considered statistically significant.

3 Results and discussion

3.1 Preparation and characterization of MnB-RLG

MnB MBene nanosheets were synthesized through selective removal of the Al component from the layered N,Mn-(Mo₂₃Y₁₃)₂AlB₂ precursor, followed by intercalation-assisted exfoliation to obtain few-layer N,Mn-Mo_{1.33}B nanosheets. TEM analysis revealed that MnB exhibited a typical two-dimensional lamellar morphology with high transparency, indicative of an ultrathin nanostructure (Fig. 1(a)). Elemental mapping analysis confirmed the homogeneous distribution of Mo, B, N, and Mn throughout the nanosheets, suggesting successful incorporation of N and Mn into the Mo_{1.33}B lattice without observable phase separation (Fig. 1(b)). SEM images consistently further revealed well-defined layered architectures with relatively smooth surfaces

(Fig. 1(c)), providing a robust structural foundation for subsequent surface functionalization and cargo loading.

α-Lipoic acid was selected as a molecular linker due to its distinctive dithiolane ring structure, which enables strong coordination with metal-based surfaces and facilitates efficient interfacial electron transfer [37, 38]. Furthermore, α-lipoic acid possesses both a hydrophobic dithiolane structure and a hydrophilic terminal carboxyl group, thereby conferring remarkable amphiphilic properties to the molecule [39, 40]. Accordingly, the resveratrol-lipoic acid-RGD nanomicelles (RLG) were fabricated through a self-assembly process driven by the amphiphilic nature of α-lipoic acid. TEM images showed that RLG formed uniform spherical micelles with a narrow size distribution, predominantly ranging from 100 to 200 nm (Fig. 1(d)). The distinct core-shell contrast indicated the formation of a hydrophobic core enriched with resveratrol molecules and a hydrophilic outer shell modified with RGD peptides, a structural configuration that significantly enhances aqueous dispersibility and promotes cellular interactions [41]. Notably, the particle size 100–200 nm falls within the optimal range for nanomedicine delivery systems, as it favors favorable circulation stability and enhances cellular internalization efficiency. Thus, the core-shell RLG micelles allow for efficient encapsulation of hydrophobic drugs while maintaining excellent aqueous dispersibility.

As a two-dimensional scaffold material, MnB nanosheets exhibit a large specific surface area and abundant surface-active sites, which provide sufficient anchoring sites for efficient loading of RLG [42]. Meanwhile, the two-dimensional layered structure helps to inhibit nanoparticle aggregation and promotes homogeneous dispersion, further improving the structural stability of the composite system [43, 44]. To construct the MnB-RLG hybrid system, RLG micelles were incubated with MnB nanosheets under mild conditions, facilitating interfacial assembly through coordination interactions between the disulfide moieties of α-lipoic acid and the MnB surface. TEM analysis demonstrated that spherical RLG nanoparticles were successfully anchored onto the MnB nanosheets, while the two-dimensional morphology of MnB remained intact (Fig. 1(e)). SEM images further confirmed a layered structure decorated with uniformly distributed nanoparticles across both the surface and interlayer regions (Fig. 1(f)), indicating efficient loading of RLG onto the MnB scaffold. Such sulfur-metal interactions not only effectively promote the stable anchoring of RLG nanoparticles onto the MnB surface, but also preserve the inherent structural integrity of MnB nanosheets.

The tight interfacial contact established between MnB nanosheets and RLG micelles shortens the transport pathways for electrons and active species, thereby constructing an efficient interfacial reaction microenvironment. This synergistic architecture provides additional active sites for subsequent catalytic reactions and biological regulation processes, which is conducive to enhancing the enzyme-like catalytic activity and antioxidant capacity of MnB nanomaterials. Collectively, this hierarchically MnB-RLG hybridized structure, synergistically assembled from two-dimensional nanosheets and nanoparticles, not only achieves the stable loading of RLG, but also provides a crucial structural foundation for the multifunctional biological functions.

3.2 Chemical composition and structural characterization of MnB-RLG

To further elucidate the elemental composition, chemical states,

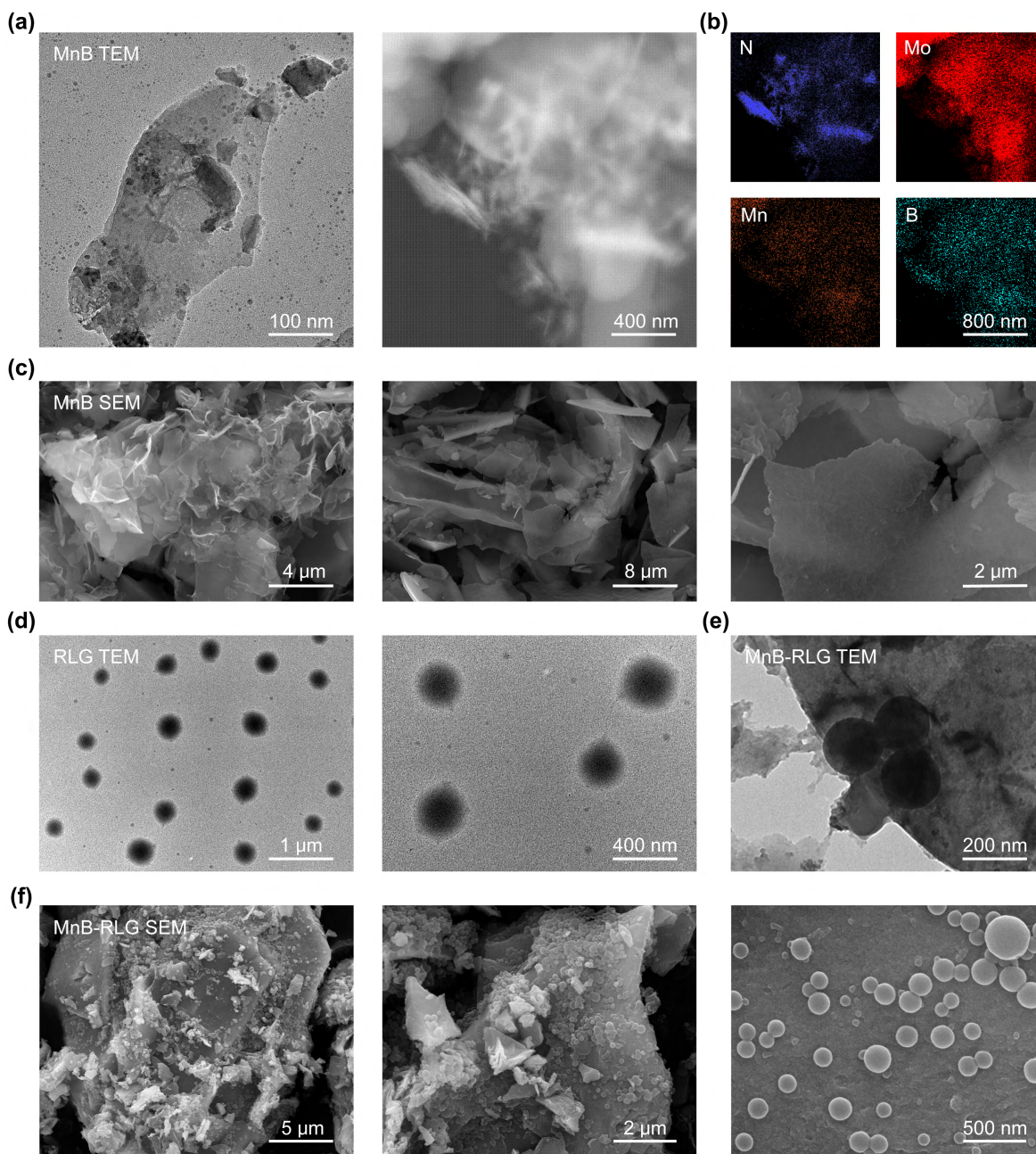


Figure 1 Fabrication and characteristics of MnB, RLG, and MnB-RLG nanozymes. (a) Representative TEM image of MnB at different magnifications. (b) Corresponding elemental mapping of MnB. (c) Representative SEM image of MnB at different magnifications. (d) Representative TEM image of RLG at different magnifications. (e) Representative TEM image of MnB-RLG. (f) Representative SEM images of MnB-RLG at different magnifications.

and interfacial interactions within the MnB-RLG systems, XPS, FTIR, and XRD analyses were performed. In the high-resolution XPS spectra of MnB, the Mn 2p region exhibited characteristic peaks at approximately 641 eV (Mn 2p_{3/2}) and 653 eV (Mn 2p_{1/2}), indicating that Mn was predominantly present in the +2-oxidation state (Fig. 2(a)). The N 1s spectrum exhibited a dominant peak at ~ 398–399 eV, which is consistent with metal-nitrogen bonding and confirms the successful incorporation of N into the MBene lattice (Fig. 2(b)). Moreover, the B 1s peak centered at ~ 193 eV implied partial surface oxidation of boron. Meanwhile, the Mo 3d_{5/2} and Mo 3d_{3/2} peaks appeared at ~ 232 and ~ 235 eV respectively, indicating that Mo mainly exists in a low-valence state, which is consistent with the preserved boride framework (Figs. 2(c)

and 2(d)). Collectively, these results confirm the successful formation of N, Mn co-doped Mo_{1.33}B MBene with well-defined chemical states.

Following hybridization with RLG nanomicelles, the XPS spectra of MnB-RLG largely retained the intrinsic metal-related features of MnB, indicating that the MBene framework maintained chemical stability during the assembly process. Meanwhile, newly appeared C 1s components assigned to C–C/C–H and C–N/C–O bonds, along with the S 2p signals derived from α -lipoic acid, confirmed the successful immobilization of organic moieties onto the MnB surface (Figs. 2(e) and 2(f)). In addition, the B 1s and Mo 3d core-level spectra of MnB-RLG exhibited binding energies comparable to those of pristine MnB (Figs. 2(g) and 2(h)), with only minor peak

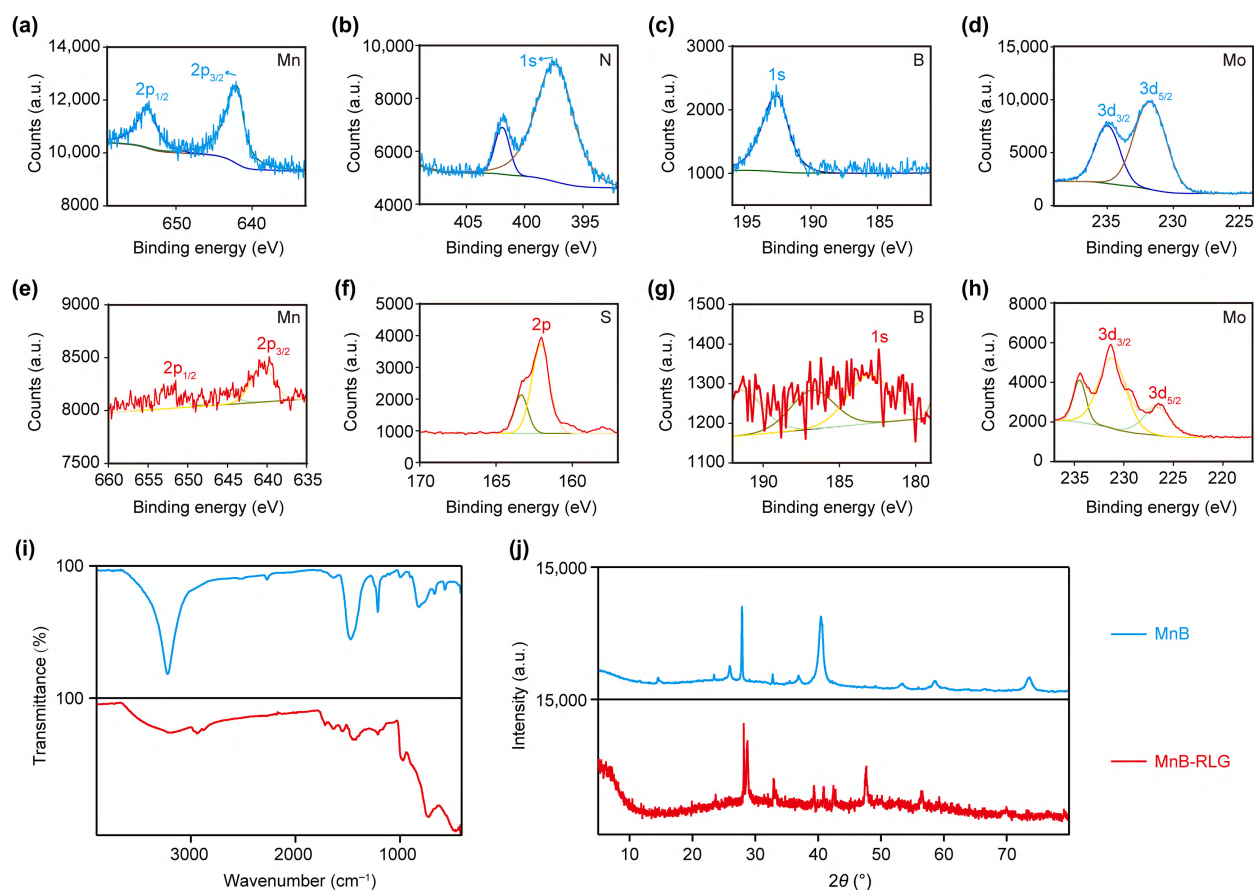


Figure 2 Chemical composition and structural characterization of MnB and MnB-RLG nanozymes. XPS spectra of MnB at (a) Mn 2p, (b) N 1s, (c) B 1s, and (d) Mo 3d. XPS spectra of MnB-RLG at (e) Mn 2p, (f) S 2p, (g) B 1s, and (h) Mo 3d. (i) FTIR spectra of MnB and MnB-RLG. (j) XRD patterns of MnB and MnB-RLG.

broadening and intensity variation, indicating that the local chemical environments of the B–M ($M = \text{Mn}, \text{Mo}$) bonds were largely preserved after hybridization. These results demonstrate that RLG nanomicelles are stably anchored onto MnB through interfacial interactions rather than simple physical adsorption, which is indispensable for mediating biological interactions.

FTIR spectra further corroborate the successful fabrication of the MnB-RLG hybrid system. Compared with pristine MnB, MnB-RLG exhibited characteristic absorption bands corresponding to C–H and C=O stretching vibrations, which are indicative of organic components from RLG, while the modified O–H/N–H stretching region implied the formation of intermolecular interactions at the organic-inorganic interface (Fig. 2(i)). Notably, XRD analysis revealed that MnB-RLG maintained the characteristic diffraction peaks of MnB, confirming the preservation of crystalline structure of the MBene which is essential for maintaining intrinsic electronic and catalytic properties (Fig. 2(j)).

The MnB-RLG hybrid system establishes a favorable balance between framework integrity and interfacial adaptability through hierarchical assembly across multiple length scales. At the inorganic backbone level, the well-preserved Mn–B and Mo–B bonding environments ensure structural robustness and electronic continuity of the MBene nanosheets. Meanwhile, the interfacial anchoring of RLG generates a dynamic organic layer that does not disrupt the underlying lattice, but rather synergistically integrates with it through noncovalent coordination and interfacial coupling. Such a hybrid architecture enables the material to maintain its intrinsic physicochemical characteristics, while acquiring enhanced

surface functionality. Importantly, this multiscale structural integration underscores the significance of interfacial hierarchy in the design of biofunctional hybrid materials.

Collectively, these results highlight a rational design strategy for integrating structural stability with interfacial functionality in the MnB-RLG hybrid system.

3.3 Multiple enzyme-like activities of MnB-RLG

Oxidative stress is a central pathological feature of the degenerative microenvironment in NPCs, characterized by excessive accumulation of ROS and insufficient endogenous antioxidant defense [45]. Notably, SOD, CAT, and GPx form a hierarchical antioxidant network, which is responsible for sequential detoxification of superoxide anions, hydrogen peroxide, and lipid peroxides [46]. To evaluate the intrinsic antioxidant catalytic properties of MnB-RLG, the SOD-, CAT-, and GPx-like activities are systematically assessed.

Given the transition-metal-rich composition and engineered electronic structure of MnB, its superoxide scavenging capability was first evaluated using an SOD activity assay. Both MnB and MnB-RLG exhibited pronounced SOD-like activity, as evidenced by the concentration-dependent inhibition of $\cdot\text{O}_2^-$ generation (Fig. S1(a) in the Electronic Supplementary Material (ESM)). Notably, MnB-RLG showed enhanced superoxide scavenging efficiency compared with pristine MnB, suggesting that interfacial integration with RLG further promotes redox catalytic performance. This result indicates that the incorporation of Mn into the MBene framework endows the material with efficient SOD-mimicking capability,

which may help attenuate early ROS accumulation in degenerative NPCs.

The CAT-like activity of MnB-based nanozymes was subsequently evaluated by monitoring oxygen generation during H_2O_2 decomposition. As shown in Fig. S1(b) in the ESM, both MnB and MnB-RLG catalyzed the rapid conversion of H_2O_2 into O_2 and H_2O in a time-dependent manner, with MnB-RLG exhibiting higher catalytic efficiency and generating significantly higher dissolved oxygen levels within a short reaction period. This enhanced CAT-like activity can be attributed to the synergistic effect between the MBene catalytic surface and the interfacial electronic modulation induced by RLG, which may facilitate adsorption and decomposition of H_2O_2 .

In addition to superoxide and hydrogen peroxide, lipid peroxides are critical mediators of oxidative damage and ferroptosis [47]. Thus, the GPx-like activity of MnB-based nanozymes was further investigated. Both MnB and MnB-RLG effectively reduced lipid peroxides in a dose- and time-dependent manner, as reflected by the accelerated decrease in absorbance associated with NADPH consumption (Fig. S1(c) in the ESM). Notably, MnB-RLG showed superior GPx-like activity compared with MnB alone, indicating an enhanced ability to suppress lipid peroxidation and maintain cellular redox balance.

These findings demonstrate that MnB-based nanozymes possess intrinsic multi-enzyme-like activities, including SOD-, CAT-, and GPx-like functions, while interfacial hybridization with RLG further amplifies their antioxidant catalytic efficiency. Such comprehensive ROS-scavenging capacity provides a physicochemical basis for mitigating oxidative stress-driven cellular dysfunction and IDD progression.

3.4 Cellular uptake and biocompatibility of MnB-RLG in NPCs

Efficient cellular internalization is a prerequisite for nanomaterial-mediated regulation of intracellular functions [48]. To visualize the uptake behavior of different formulations, MnB, RLG, and MnB-RLG were fluorescently labeled with Cy5.5 and incubated with NPCs for different time periods. All Cy5.5-labeled materials exhibited time-dependent cellular internalization, with red fluorescence signals gradually increasing from 2 to 12 h (Figs. 3(a)–3(d)). Moreover, Live/dead staining revealed that NPCs treated with MnB, RLG, or MnB-RLG maintained high cell viability, with no obvious increase in propidium iodide (PI)-positive cells compared with the control group (Figs. 3(e) and 3(f)), indicating favorable biocompatibility.

These results reveal that the MnB-RLG can be efficiently internalized by NPCs, and does not induce nonspecific cytotoxic stress, providing a necessary intracellular basis for subsequent regulation of cellular functions.

3.5 MnB-RLG promotes proliferation and migration in NPCs

Given the critical role of NPC proliferation and migration in maintaining disc cellularity and intrinsic repair capacity, we next evaluate the effects of MnB, RLG, and MnB-RLG on NPC proliferative activity, and migratory behavior. EdU incorporation assays were performed to assess the cell proliferation. As shown in Figs. 3(g) and 3(h), MnB and RLG treatment moderately increased the proportion of EdU-positive cells, whereas MnB-RLG treatment

resulted in a significantly higher proliferative efficiency, suggesting a synergistic pro-proliferative effect of the composite system. In addition, scratch wound assays demonstrated a time-dependent increase in wound closure in all treated groups, with MnB-RLG exhibited the best migratory-promoting effect (Figs. 3(i)–3(k)).

Together, these findings suggest that the combined MnB-RLG composite effectively facilitates proliferation and migration of NPCs.

3.6 MnB-RLG maintains ECM homeostasis in NPCs

Excessive ROS can disrupt the ECM homeostasis of NPCs [49]. Therefore, we investigate the protective effects of MnB-RLG on ECM homeostasis. TB and SO staining showed a marked loss of proteoglycan content and severe destruction of ECM structure in TBHP-treated NPCs compared to the control group (Fig. 4(a)). Treatment with MnB or RLG alone partially restored matrix staining intensity, whereas MnB-RLG treatment most effectively preserved the proteoglycan-rich matrix (Fig. 4(a)). Consistent with the histological findings, Western blot and qPCR analyses revealed that TBHP significantly downregulated COL II expression, while markedly upregulating the expression of the matrix-degrading enzyme MMP13. Notably, MnB-RLG treatment significantly reversed these effects (Figs. 4(b)–4(f)). Immunofluorescence analysis of COL II and MMP13 further confirmed these findings (Figs. 4(g)–4(j)). Collectively, these results demonstrate that MnB-RLG effectively mitigates oxidative stress-induced ECM degradation in NPCs.

Oxidative stress can promote the inflammatory response, which also serves as a key driver of matrix catabolism in NPCs [49, 50]. Beyond matrix remodeling, we further evaluated the expression of inflammatory gene expression. TBHP treatment significantly upregulated the mRNA levels of *Tnf*, *Il6*, *Il1 β* , and *Ifng* (Figs. 4(k)–4(n)). In contrast, compared to MnB or RLG alone, MnB-RLG treatment exerted the most pronounced inhibitory effect on the expression of pro-inflammatory cytokines.

These results indicate that MnB-RLG exerts superior protective effects on ECM homeostasis in NPCs by enhancing anabolic matrix synthesis, suppressing the expressions of matrix-degrading enzymes, and attenuating oxidative stress-induced inflammation.

3.7 MnB-RLG attenuates ferroptosis and lipid peroxidation in NPCs

Ferroptosis, characterized by iron accumulation and the massive generation of lipid peroxides, could further exacerbate oxidative stress [51, 52]. Emerging evidence reveals that ferroptosis is a critical contributor to NPC degeneration and IDD progression [8, 11, 53]. Given that MnB-RLG possesses the GPx-like activity, we next investigated the protective effects of MnB-RLG on ferroptosis-related pathways in NPCs. Western blot and qPCR analyses revealed that TBHP stimulation profoundly disrupted mRNA and protein expression of ferroptosis-regulatory molecules, as evidenced by the marked downregulation of GPX4, xCT, ferroportin 1 (FPN1), nuclear factor erythroid 2-related factor 2 (NRF2), and heme oxygenase-1 (HO-1) (Figs. 5(a)–5(d)). This expression pattern is characteristic of a ferroptosis-prone cellular state, in which impaired cystine uptake, reduced glutathione-dependent detoxification of lipid peroxides, and disturbed iron export collectively facilitate lipid oxidative damage. Treatment with MnB or RLG alone partially restored the expression of these ferroptosis-related molecules, whereas MnB-RLG treatment achieved the most

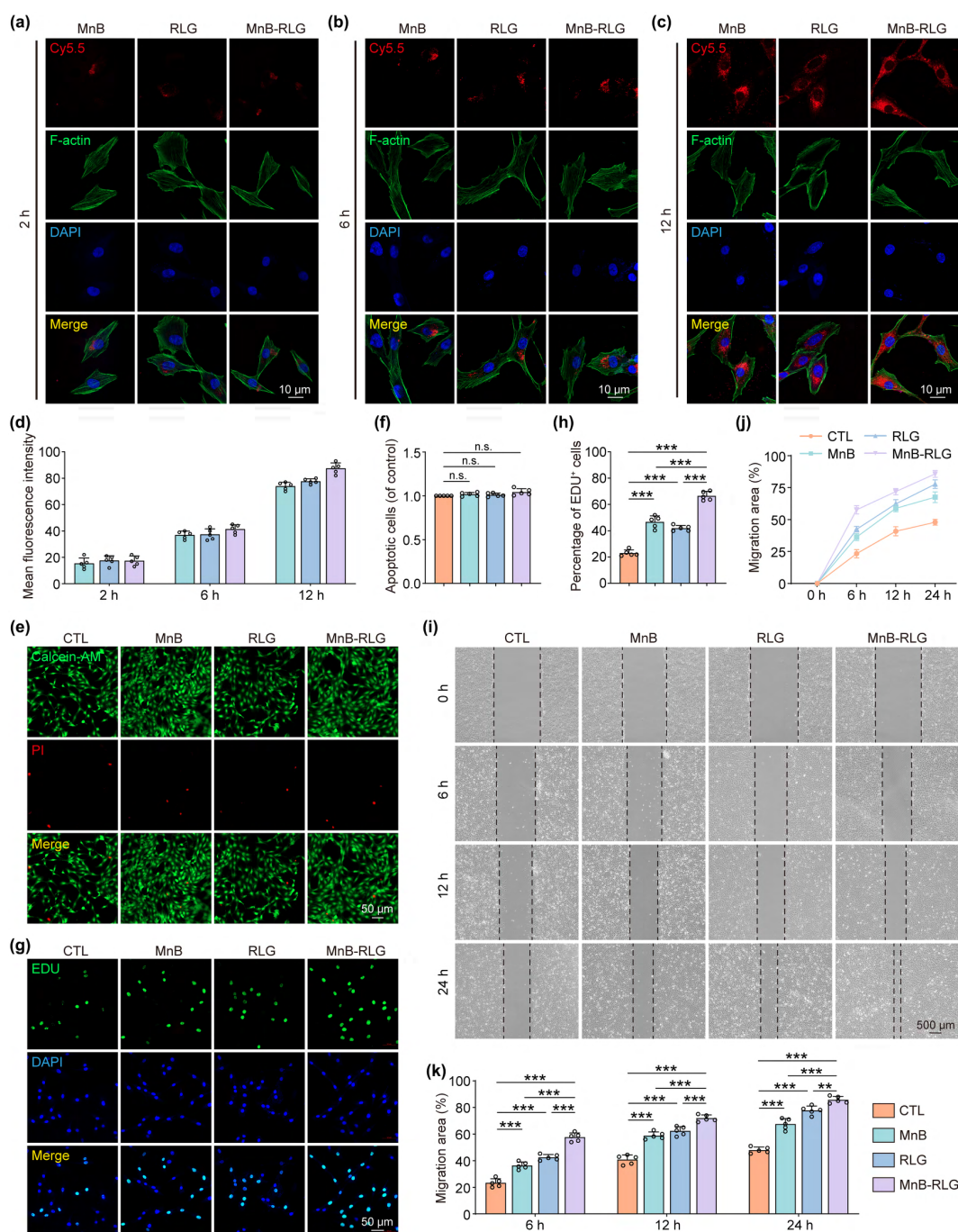


Figure 3 MnB-RLG promotes proliferation and migration in NPCs. (a)–(d) Representative images and quantification of MnB, RLG, or MnB-RLG internalization in NPCs ($n = 5$). (e) Representative images of Live/dead staining in NPCs, with live cells stained by calcein-AM (green) and dead cells stained by PI (red). (f) Quantification of apoptotic cells ($n = 5$). (g) Representative images and (h) quantification of EdU staining in NPCs ($n = 5$). (i) Representative images of wound healing assay at 0, 6, 12, and 24 h. (j) and (k) Quantification of migration area at the indicated time points ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups in panels (f), (h), and (k).

substantial recovery of their expression levels (Figs. 5(a)–5(d)), indicating attenuation of ferroptosis-associated molecular alterations. Consistent with these findings, TBHP-induced upregulation of TFR expression at both protein and mRNA levels was most effectively inhibited by MnB-RLG treatment (Figs. 5(a), 5(c), 5(e)), suggesting that the protective effect of MnB-RLG was accompanied by reduced iron import.

Iron overload is a central driver of ferroptosis [47, 52]; thus, we further evaluated intracellular ferrous iron (Fe^{2+}) accumulation in

NPCs. FerroOrange staining revealed a marked increase in intracellular Fe^{2+} signals in TBHP-treated NPCs compared with control group (Figs. 5(f) and 5(g)). Compared with MnB or RLG treatment alone, MnB-RLG treatment exhibited the most pronounced attenuation of FerroOrange fluorescence, indicating improved intracellular iron homeostasis (Figs. 5(f) and 5(g)). As a hallmark event of ferroptotic cell death, lipid peroxidation was subsequently assessed using BODIPY C11 staining. TBHP exposure significantly induced extensive lipid oxidative damage (Figs. 5(h)

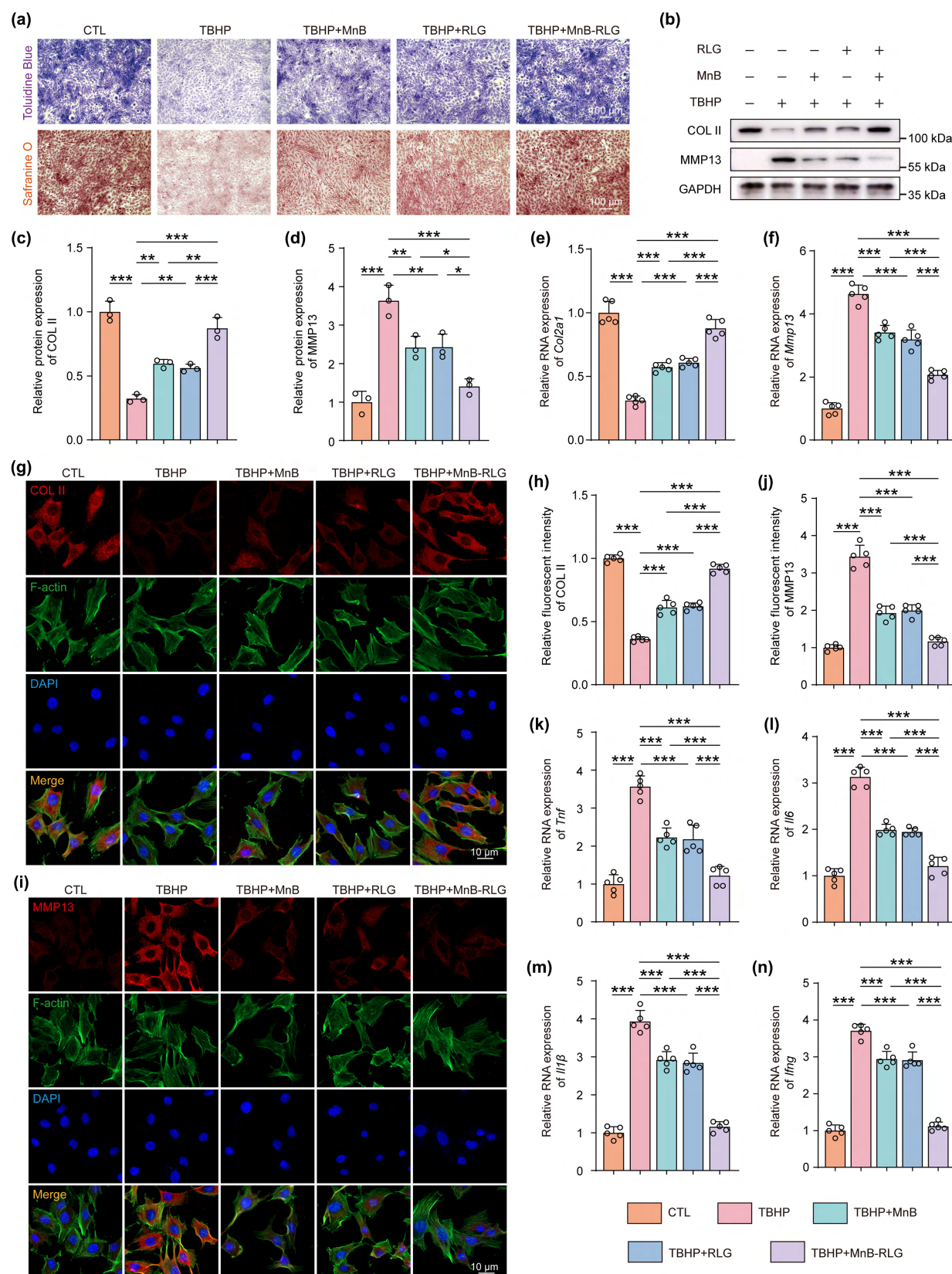


Figure 4 MnB-RLG maintains ECM homeostasis in NPCs. (a) Representative images of TB and SO staining in NPCs. (b) Representative blots and quantification of COL II and (d) MMP13 protein expressions in NPCs ($n = 3$). Quantitative RT-PCR of (e) *Col2a1* and (f) *Mmp13* mRNA expressions in NPCs ($n = 5$). (g) Representative images and (h) quantification of COL II staining in NPCs ($n = 5$). (i) Representative images and (j) quantification of MMP13 staining in NPCs ($n = 5$). Quantitative RT-PCR of (k) *Tnf*, (l) *Il6*, (m) *Il1 β* , and (n) *Ifng* mRNA expressions in NPCs ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups (panels (c)–(f), (h), and (j)–(n)).

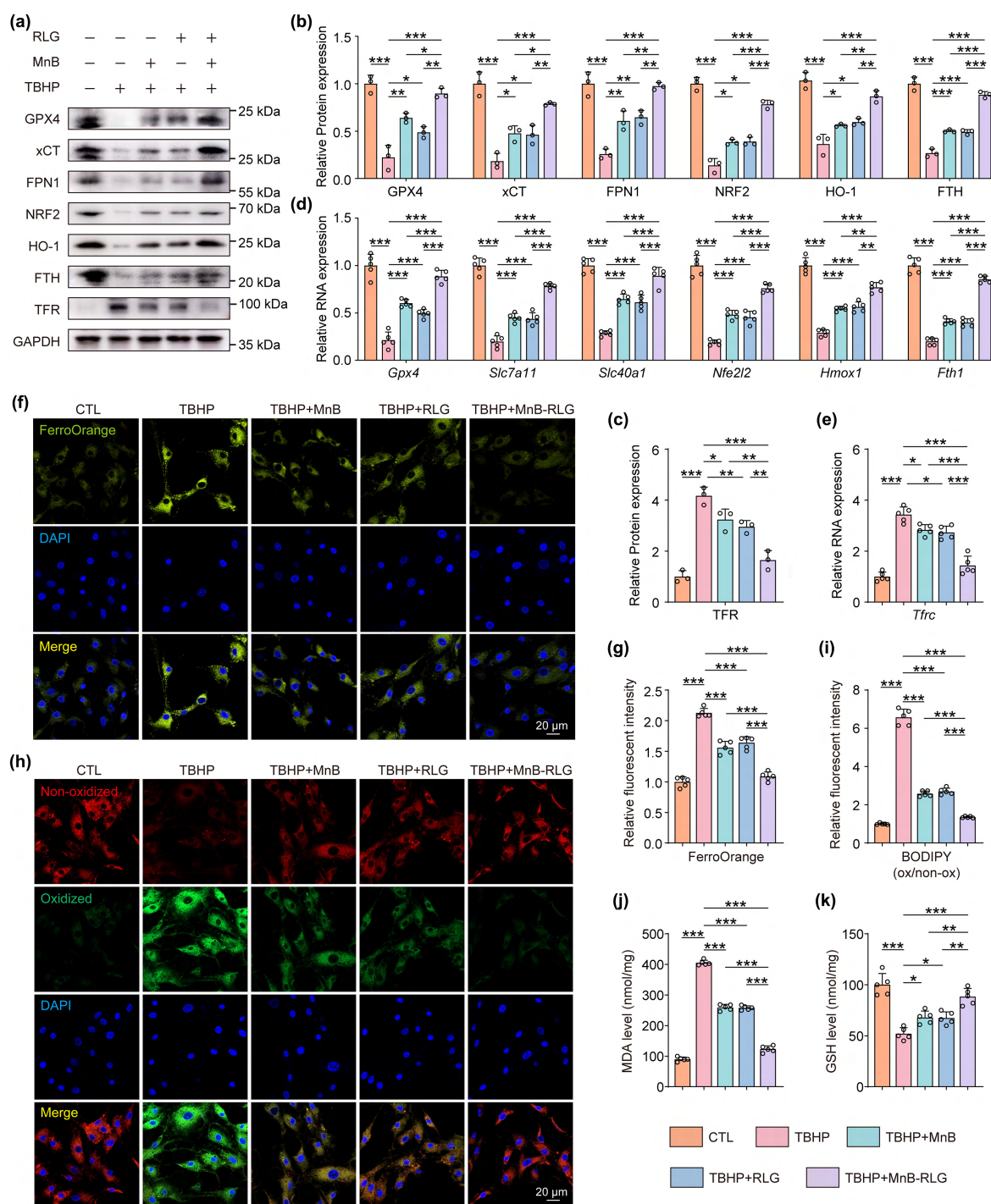


Figure 5 MnB-RLG attenuates ferroptosis and lipid peroxidation in NPCs. (a) Representative blots and quantification of (b) GPX4, xCT, FPN1, NRF2, HO-1, FTH, and (c) TFR protein expressions in NPCs ($n = 3$). Quantitative RT-PCR of (d) *Gpx4*, *Slc7a11*, *Slc40a1*, *Nfe2l2*, *Hmox1*, *Fth1*, and (e) *Tfrc* mRNA expressions in NPCs ($n = 5$). (f) Representative images and (g) quantification of FerroOrange staining in NPCs ($n = 5$). (h) Representative images and (i) quantification of BODIPY 581/591 C11 staining for lipid peroxidation examination in NPCs ($n = 5$). ELISA analysis of (j) MDA and (k) GSH levels ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups (panels (b)–(e), (g), and (i)–(k)).

and 5(i)). Treatment with MnB or RLG partially alleviated lipid peroxidation, whereas MnB-RLG treatment nearly restored lipid redox status to a level comparable to control group (Figs. 5(h) and 5(i)). Furthermore, MnB-RLG treatment most effectively reduced MDA accumulation and replenished GSH levels under TBHP stimulation (Figs. 5(j) and 5(k)), reinforcing its superior capacity to maintain intracellular redox balance.

Collectively, these changes in GPX4-related lipid peroxide detoxification, iron metabolism, and lipid peroxidation indicate that MnB-RLG restrains ferroptosis-associated cellular damage in NPCs.

3.8 MnB-RLG maintains mitochondrial structure and function in NPCs

Mitochondrial dysfunction is a hallmark of IDD and serves as both

a major source and a critical target of oxidative stress [15, 54]. Damaged mitochondria generate excessive ROS, which further exacerbates mitochondrial injury and forms a vicious cycle of oxidative damage [55, 56]. Moreover, impaired mitochondrial function leads to dysregulated cellular metabolism and decreased ATP synthesis, ultimately compromising the viability and function of NPCs [14, 55]. Thus, we further investigate whether MnB-RLG preserves mitochondrial homeostasis under oxidative stress in NPCs. TEM analysis showed that TBHP induced severe mitochondrial fragmentation and structural damage, whereas MnB-RLG treatment restored relatively intact mitochondrial morphology, which was consistent with the results of MitoTracker staining (Figs. 6(a)–6(d)). Moreover, MnB-RLG treatment obviously restored mitochondrial membrane potential after TBHP stimulation (Figs. 6(e) and 6(f)), and markedly reduced both mitochondrial and intracellular ROS levels (Figs. 6(g)–6(j)). Collectively, these findings demonstrate that MnB-RLG exerts a protective effect on mitochondrial structure and function.

Mitochondrial metabolism provides the fundamental material and energetic basis for mitochondrial function, whereas intact mitochondrial function offers the structural and regulatory prerequisite for sustaining normal mitochondrial metabolic activity [57]. Thus, we subsequently evaluated mitochondrial respiratory function using Seahorse metabolic flux analysis. TBHP treatment significantly decreased basal oxygen consumption rate, maximal respiratory capacity, ATP production, and spare respiratory capacity (Figs. 7(i) and 7(j)), indicating impaired mitochondrial bioenergetics. Notably, MnB-RLG treatment significantly restored mitochondrial respiration, particularly in maximal respiratory capacity and ATP production. Furthermore, MnB-RLG markedly upregulated the expression of mitochondrial oxidative phosphorylation-related genes, including *Atp5f1*, *Sdhb*, and *Uqcrc1* (Figs. 7(f)–7(h)), suggesting enhanced mitochondrial metabolic function.

Mitophagy plays a pivotal role in maintaining mitochondrial quality control by selectively eliminating damaged mitochondria

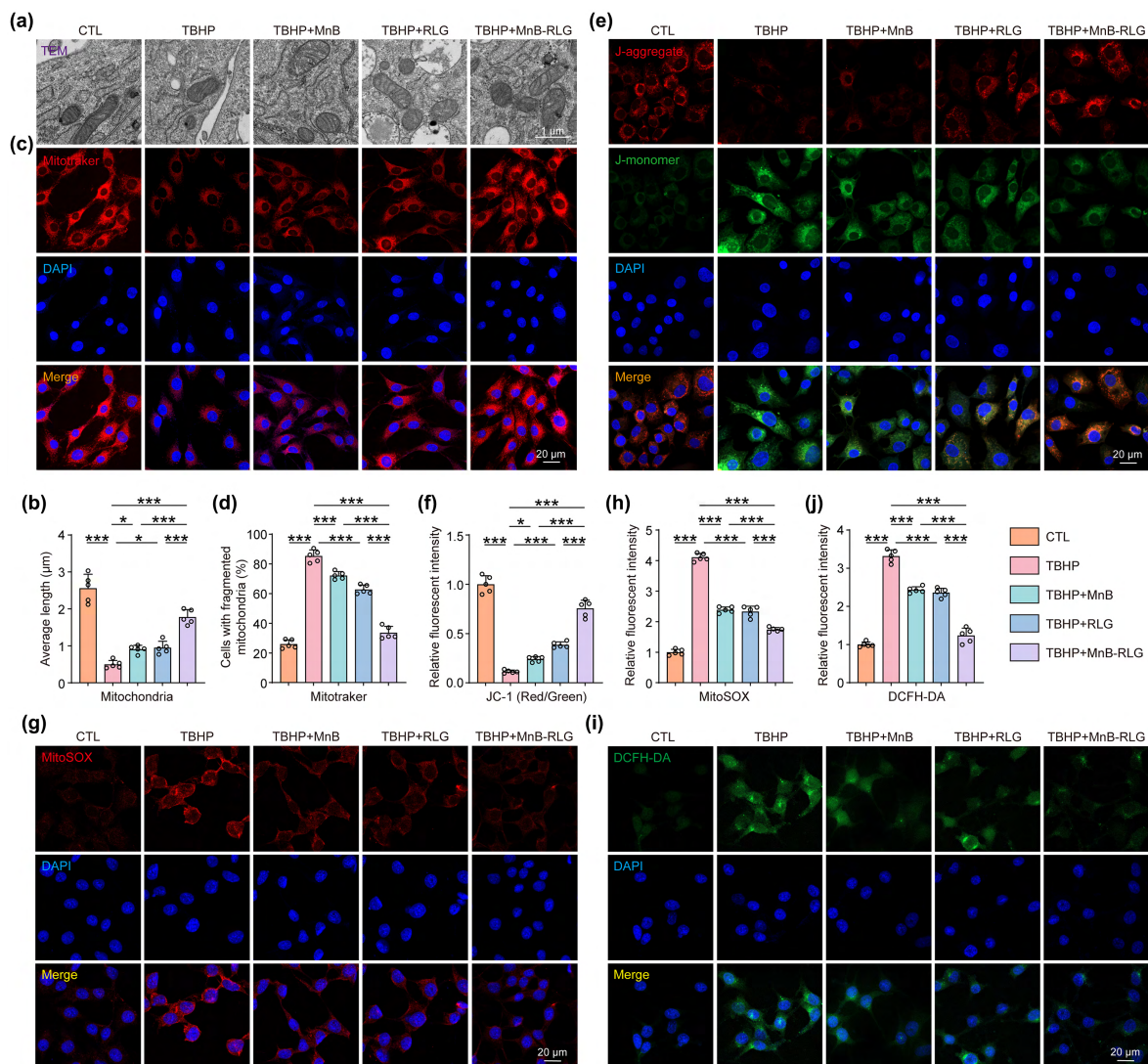


Figure 6 MnB-RLG maintains mitochondrial structure and function in NPCs. (a) Representative TEM images and (b) quantification of mitochondrial length in NPCs ($n = 5$). (c) Representative images and (d) quantification of MitoTracker staining in NPCs ($n = 5$). (e) Representative images and (f) quantification of JC-1 staining in NPCs ($n = 5$). (g) Representative images and (h) quantification of MitoSOX staining in NPCs ($n = 5$). (i) Representative images and (j) quantification of DCFH-DA staining in NPCs ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups (panels (b), (d), (f), (h), and (j)).

[14, 18, 19]. During this process, dysfunctional mitochondria are recognized and engulfed by autophagosomes, which subsequently fuse with lysosomes for degradation. The PINK1/Parkin pathway is one of the most extensively studied mitophagy signaling pathways, which mediates the recognition and clearance of dysfunctional mitochondria, and restricts excessive ROS generation [20, 58]. SIRT1, a NAD⁺-dependent deacetylase, has been widely reported to regulate mitophagy activation, mitochondrial function, and oxidative stress responses [23, 25]. Given the presence of resveratrol in RLG and the established role of SIRT1 in mitochondrial quality control, we next examined changes in SIRT1/PINK1/Parkin-related mitophagy markers after MnB-RLG treatment. Western blot analysis showed that RLG and MnB-RLG significantly upregulated the expression of SIRT1, which was consistent with the presence of resveratrol in the RLG component. In addition, TBHP exposure markedly suppressed the expression of PINK1 and PARKIN,

decreased the LC3-II/LC3-I ratio, and significantly increased p62 accumulation (Figs. 7(a)–7(c)), whereas MnB-RLG treatment partially reversed these effects. Consistently, Mitophagy Dye staining showed increased mitophagy-associated fluorescence after MnB-RLG treatment (Figs. 7(d) and 7(e)).

Collectively, these results demonstrate that MnB-RLG significantly promotes PINK1/Parkin-associated mitophagy, restores mitochondrial function and respiratory capacity, and suppresses oxidative stress in NPCs. Through coordinated regulation of mitochondrial quality control and bioenergetic metabolism, MnB-RLG maintains mitochondrial homeostasis under oxidative stress conditions.

3.9 MnB-RLG ameliorates IDD progression *in vivo*

To evaluate the therapeutic efficacy of MnB-RLG *in vivo*, a puncture-induced rat IDD model was established, and therapeutic

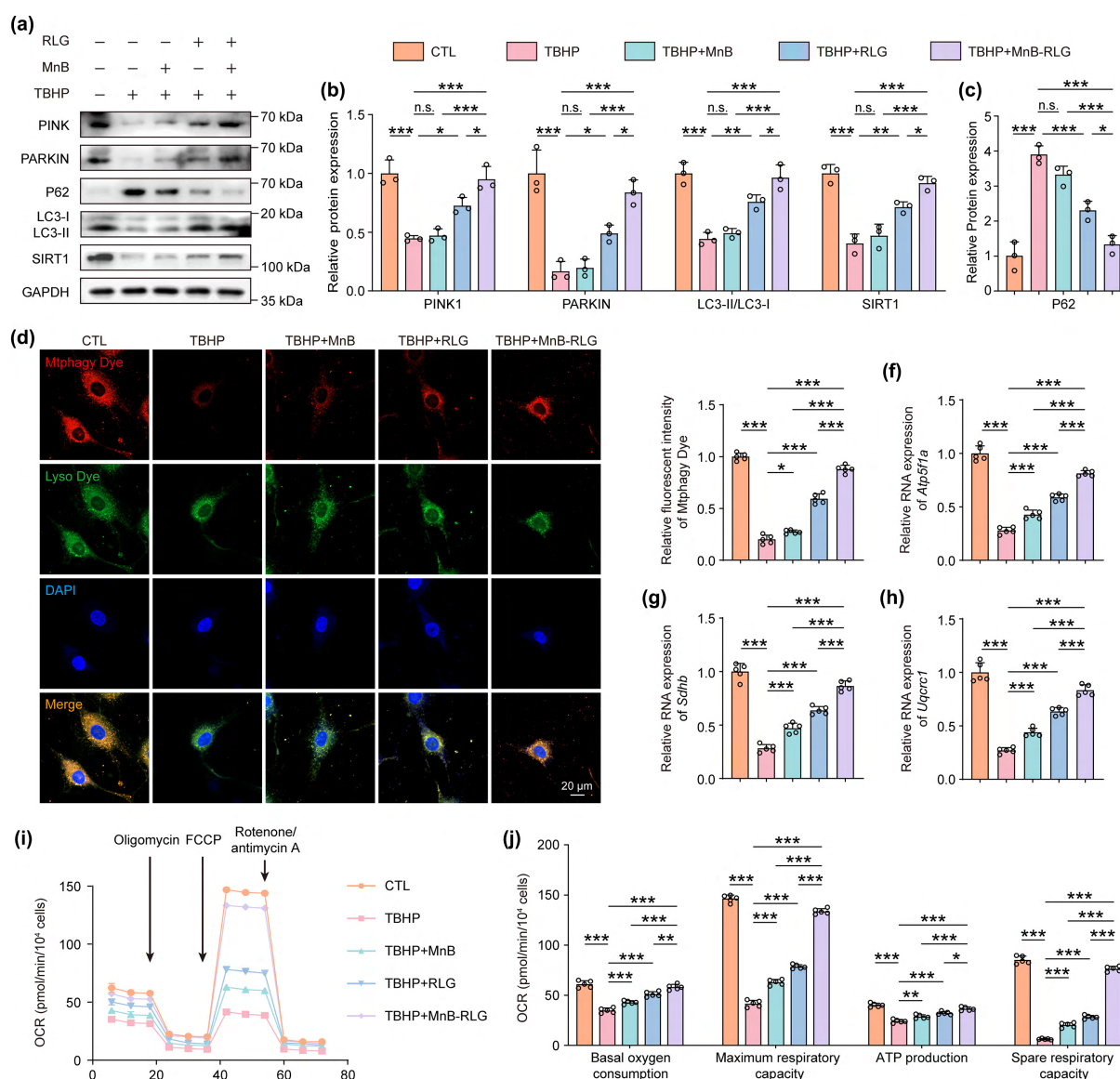


Figure 7 MnB-RLG promotes mitophagy activation and improves mitochondrial metabolic function in NPCs. (a) Representative blots and quantification of (b) PINK1, PARKIN, LC3-I/II, SIRT1, and (c) p62 protein expressions ($n = 3$). (d) Representative images and (e) quantification of Mitophagy Dye staining in NPCs ($n = 5$). Quantitative RT-PCR of (f) *Atp5f1*, (g) *Sdhb*, and (h) *Uqcrc1* mRNA expressions in NPCs ($n = 5$). (i) OCR assay of NPCs ($n = 5$). (j) Quantitative analysis of mitochondrial respiration profiles ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups (panels (b)–(c), (e)–(h), and (j)).

outcomes were assessed at 4th and 8th weeks post-treatment (Fig. 8(a)). Gross anatomical observation revealed that discs in the IDD group exhibited obvious collagen depletion and structural collapse, whereas MnB-RLG markedly ameliorated disc morphological integrity (Fig. 8(b)), indicative of effective preservation of disc structure. X-ray imaging demonstrated that MnB-RLG treatment effectively maintained disc height compared to IDD group (Figs. 8(c) and 8(d)). Furthermore, MR imaging showed that MnB-RLG-treated group exhibited stronger T_2 signals and higher collagen content compared with the IDD group (Fig. 8(e)), which was further verified by Pfirrmann grading analysis (Fig. 8(f)).

Histological evaluation further demonstrated that, compared with IDD group, MnB-RLG treatment significantly reversed structural disruption, and preserved extracellular matrix composition, including proteoglycan and glycosaminoglycans contents (Figs. 8(g)–8(i)). Quantitative histological scoring further

confirmed significantly lower degeneration scores in the MnB-RLG-treated group (Fig. 8(j)). Moreover, MnB-RLG treatment markedly upregulated the expression of COL II, and suppressed the expression of MMP13, indicating restored extracellular matrix homeostasis (Figs. S2(a), S2(b), S2(e), and S2(f) in the ESM). In addition, MnB-RLG treatment significantly downregulated the expression of key ferroptosis-associated enzyme ACSL4, and oxidative DNA damage marker 8-OHdG (Figs. S2(c)–S2(f) in the ESM), further supporting the inhibitory effects of MnB-RLG on ferroptotic activity and oxidative stress in degenerative discs.

In conclusion, these results demonstrate that MnB-RLG effectively attenuates disc degeneration *in vivo*.

Nevertheless, several limitations should be acknowledged. First, the current findings should be interpreted as evidence of mechanistic association rather than definitive proof of pathway dependence. Future studies using pathway-specific inhibitors, gene knockdown, or overexpression rescue experiments are needed to

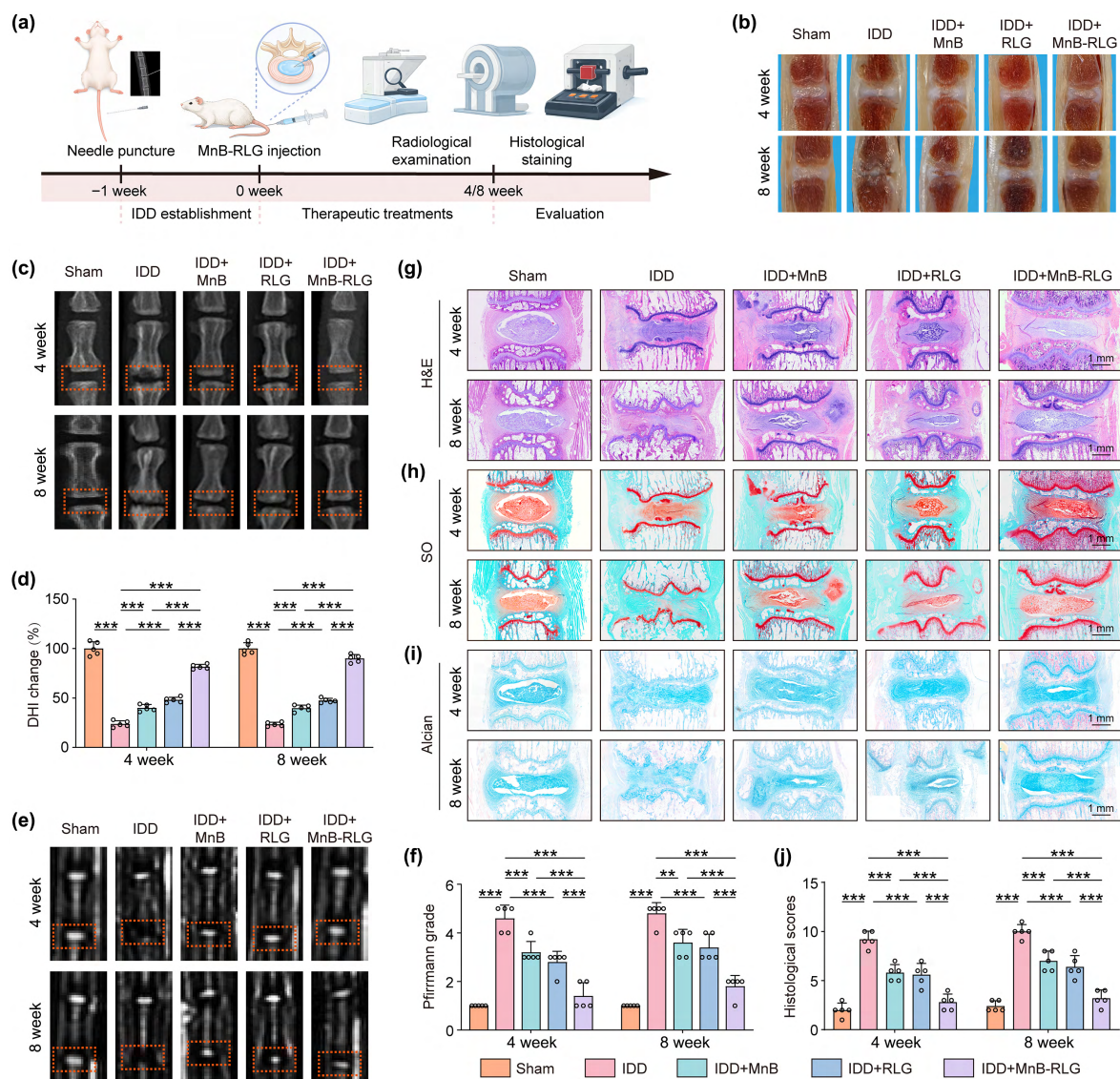


Figure 8 MnB-RLG ameliorates IDD progression *in vivo*. (a) Schematic illustration of the experimental manipulation *in vivo*. (b) Representative gross morphological images of discs at 4th and 8th weeks post-treatment. (c) Representative X-ray images and (d) quantification of DHI changes at 4th and 8th weeks post-treatment ($n = 5$). (e) Representative MR images and (f) quantification of Pfirrmann scores at 4 and 8 weeks post-treatment ($n = 5$). (g) H&E, (h) SO, and (i) Alcian Blue staining at 4th and 8th weeks post-treatment ($n = 5$). (j) Quantitative analysis of the histological scores at 4th and 8th weeks post-treatment ($n = 5$). Values are presented as means $\pm$ SD. n.s.: $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA followed by Bonferroni's *post hoc* tests among groups (panels (d), (f), and (j)).

further confirm the causal roles of SIRT1/PINK1/Parkin-related mitophagy and ferroptosis modulation in the protective effects of MnB-RLG. Second, although intradiscal injection enabled direct delivery of MnB-RLG into the degenerative disc, the retention, diffusion, and targeting efficiency of MnB-RLG within the intervertebral disc microenvironment still require further quantitative evaluation. Third, long-term tracking, biodistribution analysis, and large animal models are needed to further evaluate the biosafety, delivery efficiency, and translational potential of MnB-RLG.

4 Conclusions

In summary, we constructed a multifunctional MnB-RLG nanozyme for IDD treatment through interfacial assembly between MnB MBene nanosheets and RLG nanomicelles. MnB-RLG exhibits superior SOD-, CAT-, and GPx-like activities, enabling efficient ROS scavenging and robust oxidative stress suppression. Mechanistically, MnB-RLG markedly inhibits ferroptosis by restoring iron homeostasis and reducing lipid peroxidation. Meanwhile, it activates the SIRT1/PINK1/Parkin-related mitophagy to repair mitochondrial structure and function, thereby maintaining ECM homeostasis in NPCs. Notably, MnB-RLG significantly preserves disc height and structural integrity, and further alleviates IDD progression *in vivo*. Collectively, this study provides a metabolic-redox synergistic regulatory strategy and highlights MnB-RLG as a promising nanotherapeutic candidate for the clinical management of IDD.

Electronic Supplementary Material: Supplementary material (enzyme-mimicking antioxidant activity assays (Fig. S1) and *in vivo* immunohistochemical analyses of IDD progression (Fig. S2)) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94908983>.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Author contribution statement

F. T.: Methodology, conceptualization, data curation, writing. X. Y. W.: Methodology, writing. H. X. H.: Data curation, writing. Z. Y. F.: Methodology, resources. L. L. Z.: Methodology, Validation. K. T. Z.: Methodology. Y. Z.: Methodology. J. Q. B.: Methodology. J. Y. C.: Methodology. Y. Y. C.: Data curation. H. X. C.: Validation, review & editing. Z. H. H.: Language polishing, review & editing. C. J.:

Conceptualization, project administration, review & editing, funding acquisition.

Informed consent

Not applicable.

Ethics statement

The animal experimental procedures were approved by the Institutional Animal Care Committee of Taizhou Hospital (Approval No. TZYY-2026002).

Use of AI statement

None.

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