Knockdown of triggering receptor expressed on myeloid cells 1 (TREM1) inhibits endoplasmic reticulum stress and reduces extracellular matrix degradation and the apoptosis of human nucleus pulposus cells

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Abstract. According to the linear model of microarray data analysis, triggering receptor expressed on myeloid cells 1 (TREM1) has been shown to have a significantly different expression profile between intervertebral disc degeneration (IDD) samples and associated control samples. The purpose of the present study was to explore the probable role and underlying mechanism of TREM1 in IDD. To accomplish this, an *in vitro* model of IDD was established by using IL-1β to stimulate human nucleus pulposus cells (NPCs). After the level of TREM1 had been determined, its functions in terms of the viability of the NPCs, extracellular matrix (ECM) degradation, inflammation, apoptosis and endoplasmic reticulum stress (ERS) were assessed. The downstream target of TREM1 was predicted to be Toll-like receptor-4 (TLR-4) and its roles were then studied, incorporating experiments featuring an ERS agonist. IL-1β was found to elevate the level of TREM1 in NPCs. TREM1 knockdown reversed the observed effects of IL-1β on cell viability, ECM degradation, inflammation, apoptosis of NPCs, ERS and TLR4/NF-κB signaling. Subsequently, the TLR4 and ERS agonists were found to reverse the effect of TREM1 knockdown on NPCs, indicating that the TLR4/NF-κB signaling pathway and ERS were responsible for mediating the regulation of TREM1. In conclusion, the present study showed that TREM1 knockdown blocked the TLR4/NF-κB signaling pathway, inhibited ERS and reduced the levels of ECM degradation and apoptosis of NPCs induced by IL-1β.

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Introduction

The intervertebral disc provides the soft connection between the vertebral bodies, and is an important weight-bearing organ (1). Intervertebral disc degeneration (IDD) is considered to be the initiating factor of a series of spinal disorders. In clinical practice, the diseases triggered by IDD include lumbar disc herniation, spinal stenosis, degenerative spondylolisthesis and degenerative scoliosis (2). The incidence of IDD is closely correlated with increasing age and, given the intensification of social aging, its incidence rate is gradually increasing on annually (3). In addition to aging, several other factors, including spinal injury, environment and genetics, also contribute towards the morbidity associated with IDD (4). Furthermore, the occurrence and progression of IDD involve various alterations in the morphology and physiology of the intervertebral disc, including disruption of the intervertebral disc structure and fibrous annulus tissue, the inflammatory response, extracellular matrix (ECM) degradation and proteoglycan loss in the nucleus pulposus (5,6).

Normal intervertebral disc tissue consists of the upper and lower cartilage endplates, the surrounding fibrous annulus, and the jelly-like nucleus pulposus tissue wrapped in the center (7,8). The intervertebral disc is the largest hypovascular tissue in the human body, and it exchanges substances with the outside, mainly through penetration of the cartilage endplate (9). However, a previous study demonstrates that this form of material exchange is insufficient, and the nucleus pulposus tissue located in the center is in a harsh microenvironment comprising low levels of oxygen, sugar and growth factors (10). Therefore, transformation of nucleus pulposus tissue is the most obvious indicator in IDD. As the numbers of apoptotic nucleus pulposus cells (NPCs) increase, the quality of secreted ECM and hydration capacity declines, both causing dysfunction of the intervertebral discs and reducing their biomechanical properties (11). In terms of an overall strategy, inhibiting cell apoptosis in degenerative nucleus pulposus tissue (6) and promoting the expression of ECM (12) are expected to become mainstays in the treatment of IDD. In addition, inflammation plays a vital role in the pathogenesis and progression of IDD. Inflammatory factors are highly expressed in IDD tissues and are involved in inflammatory response, matrix destruction and

apoptosis. Reducing inflammation is considered to be useful in the IDD treatment (13).

Triggering receptor expressed on myeloid cells 1 (TREM1) is a receptor mainly expressed on the surface of peripheral blood mononuclear cells and neutrophils (14). A previous study indicates that TREM1 loss-of-function led to a reduction in acute intestinal dysfunction caused by lipopolysaccharide and TREM1 was revealed to be relevant for intestinal cell apoptosis and inflammation (15). In addition, another study describes how inhibition of TREM1 expression leads to a marked improvement in the prognosis of spinal cord injury, probably via reducing heme oxygenase-1-mediated inflammation and oxidative stress (16). Notably, according to the linear model of the microarray data analysis, a significant difference in the expression of TREM1 has been identified between IDD samples and the associated control samples (17). However, that study did not discuss the specific role of TREM1 in IDD.

Therefore, the present study aimed to explore the role of TREM1 in IDD in greater detail. To meet this aim, an *in vitro* model of IDD was established by using IL-1β to stimulate human NPCs. The purpose was to knock down the expression of TREM1 in order to explore its role in the degradation of ECM and in cell apoptosis, as well as to investigate the underlying mechanism of action.

Materials and methods

Materials. Human NPCs were purchased from Procell Life Science and Technology Co., Ltd. Recombinant human IL-1β was purchased from Beijing T&L Biotechnology Co., Ltd. and NPCs were stimulated with a concentration of IL-1β of 10 ng/ml, as previously described (18). Monophosphoryl lipid A (MPLA), a synthetic Toll-like receptor (TLR-4) agonist, was purchased from Carbosynth China Ltd. and NPCs were treated with MPLA at a concentration at 1 μg/ml (19). Tunicamycin, an endoplasmic reticulum stress (ERS) agonist, was purchased from Glpbio Technology, Inc. and NPCs were treated with tunicamycin at a concentration at 5 μg/ml (20). The primary and secondary antibodies that were used in the present study are listed in Table I.

Bioinformatics analysis. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; cn.string-db.org) is a database that provides online searching for known protein interactions (21). The STRING version 11.0 database contains over 20 million proteins, and this was used to search for proteins that interact with TREM1. 'TREM1' was entered as the protein name, '*Homo sapiens*' was selected as the organism on the webpage, and the results were displayed in the form of a network.

Cell culture. NPCs were cultured in NPC medium supplemented with 2% fetal bovine serum (FBS), 1% NPC growth supplement and 1% penicillin/streptomycin solution (All from ScienCell Research Laboratories, Inc.). NPCs were maintained at 37˚C in an atmosphere comprising 5% CO_2, and subcultured at a 1:3 ratio until the NPCs reached ~80% confluence.

Cell transfection. NPCs (10^6 cells/well) were planted into 6-well plates until they reached 70-80% confluence. A total of 5 μg small interfering (si)RNA-TREM1 and scrambled siRNA [as a negative control (NC); Shanghai GenePharma Co., Ltd.] were transfected into NPCs with Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's instructions. Following transfection at 37˚C for 48 h, these cells were used for analysis. Target sequence (5'-3') for siRNA-TREM1-1: GACCCTGGATGTGAAATG TGA, siRNA-TREM1-2: GCACAGAGAGGCCTTCAAAGA, siRNA-NC: GTGCGGCTTGCGATAGAAATA.

Reverse transcription-quantitative (RT-q)PCR. Total RNA from NPCs (10^6 cells/well) in a 6-well plate was isolated using TRIzol® reagent (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols. Complementary DNA was primed using a Sensiscript RT kit (Takara Biotechnology Co., Ltd.), and subsequently a QuantiTect SYBR Green PCR kit (Qiagen Sciences, Inc.) was used for RT-qPCR according to the manufacturer's protocols. The qPCR cycling conditions were as follows: 95˚C for 30 sec, 40 cycles of 95˚C for 5 sec, 60˚C for 30 sec, and 72˚C for 10 sec. The $2^{-\Delta\Delta Cq}$ method (22) was used to analyze the relative gene expression levels, and those of the genes of interest were normalized against those of β-actin. This experiment was repeated three times. The sequences of the primers were: For TREM1, forward, 5'-CAACTGCCGATG TCTCCACT-3' and reverse, 5'-AAGGGCTCAGTGTCCAAA CC-3'; and for β-actin, forward, 5'-CTTCGCGGGCGACGA T-3', and reverse, 5'-CCACATAGGAATCCTTCTGACC-3'.

Western blotting. Total protein was extracted from NPCs and homogenized in RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd.). Total protein was quantified using the BCA method (cat. no. P0012; Beyotime Institute of Biotechnology). Protein samples (30 μg) were then separated using 10% SDS-PAGE and transferred onto PVDF membranes (Beijing Solarbio Science & Technology Co., Ltd.). The membranes were first blocked with 5% non-fat milk at room temperature for 1 h and then washed with TBS-0.01% Tween-20. The blots were subsequently incubated with the respective antibodies (see Table I) at 4˚C overnight. They were then cut into strips, which were subsequently incubated with an HRP-conjugated secondary antibody at room temperature for 1 h. To visualize the proteins, a BeyoECL Plus kit (Beyotime Institute of Biotechnology) was used, following the manufacturer's instructions. Finally, ImageJ v.1.52 software (National Institutes of Health) was used for semi-quantitative analysis of the blots, and to measure the gray values.

MTT assay. NPCs ($5x10^3$ cells/well) were seeded into 96-well plates and cultured in an incubator with 5% CO_2 at 37˚C. Cells were treated with IL-1β at 37˚C for 24 h. MTT solution (200 μl) was then added to each well, and the incubation was allowed to continue at 37˚C for 4 h, after which the medium was removed and DMSO was added. The optical density was measured at a wavelength of 490 nm using a microplate reader (Thermo Fisher Scientific, Inc.).

Immunofluorescence assay. NPCs ($1x10^6$ cells/well) were seeded in 6-well plates and incubated until they reached ~90% confluence. Subsequently, the cells were subjected to treatment with 4% formaldehyde at room temperature for 30 min

Table I. Antibodies used for western blotting and immunofluorescence.

Antibody	Catalog number	Host	Dilution ratio	Company
Aggrecan	sc-33695	mouse	1:1,000	Santa Cruz Biotechnology, Inc.
MMP3	sc-21732	mouse	1:1,000	Santa Cruz Biotechnology, Inc.
MMP9	sc-393859	mouse	1:1,000	Santa Cruz Biotechnology, Inc.
MMP13	sc-515284	mouse	1:1,000	Santa Cruz Biotechnology, Inc.
ADAMTS4	ab185722	Rabbit	1:500	Abcam
ADAMTS5	ab41037	Rabbit	1:250	Abcam
Bcl-2	AB112	Rabbit	1:1,000	Beyotime Institute of Biotechnology
Bax	AF1270	Rabbit	1:2,000	Beyotime Institute of Biotechnology
cleaved-caspase3	ab32042	Rabbit	1:500	Abcam
cleaved-PARP	ab32064	Rabbit	1:5,000	Abcam
TLR4	ab13556	Rabbit	1:500	Abcam
MyD88	ab133739	Rabbit	1:5,000	Abcam
p-IKK	ab194528	Rabbit	1:1,000	Abcam
IKK	ab97406	Rabbit	1:1,000	Abcam
p-p65	AF5875	Rabbit	1:1,000	Beyotime Institute of Biotechnology
p65	AF0246	Rabbit	1:1,000	Beyotime Institute of Biotechnology
CHOP	AC532	mouse	1:1,000	Beyotime Institute of Biotechnology
ATF6	AF6243	Rabbit	1:1,000	Beyotime Institute of Biotechnology
GRP78	AF0171	Rabbit	1:1,000	Beyotime Institute of Biotechnology
β-actin	orb86987	Rabbit	1:1,000	Biorbyt
anti-rabbit lgG (HRP)	A0208	goat	1:1,000	Beyotime Institute of Biotechnology
anti-mouse lgG (HRP)	A0216	goat	1:1,000	Beyotime Institute of Biotechnology
Collagen II	PA1-26206	Rabbit	1:50	Invitrogen
anti-rabbit lgG (FITC)	P0186	goat	1:1,000	Beyotime Institute of Biotechnology

ADAMTS, ADAM metallopeptidase with thrombospondin type 1 motif; PARP, cleaved poly(ADP-ribose) polymerase; TLR4, Toll-like receptor-4; p-, phosphorylated; CHOP, C/EBP homologous protein; ATF6, activating transcription factor 6; GRP78, glucose-regulated protein 78.

to immobilize them. Triton X-100 (0.1%) was then added to the wells, and the NPCs were permeabilized for 15 min. The NPCs were blocked with 5% BSA (Merck KGaA) at room temperature for 30 min, and collagen II antibody (Table I) was added for overnight incubation at 4˚C, followed by incubation with FITC-labeled anti-rabbit antibody (Table I) for 1 h. Counterstaining with DAPI was continued for 5 min to visualize the nuclei, and images were obtained under a fluorescence microscope (Nikon Corporation).

Enzyme-linked immunosorbent assay (ELISA). The supernatant of the NPCs was centrifuged at 500 x g at 4˚C for 5 min, and then collected. The expression levels of TNF-α and IL-6 were measured using the corresponding ELISA kits (cat. no. H052-1 for TNF-α and cat. no. H007-1 for IL-6; Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's recommendations. Finally, the absorbance was determined at 450 nm using a microplate reader (BioTek Instruments, Inc.).

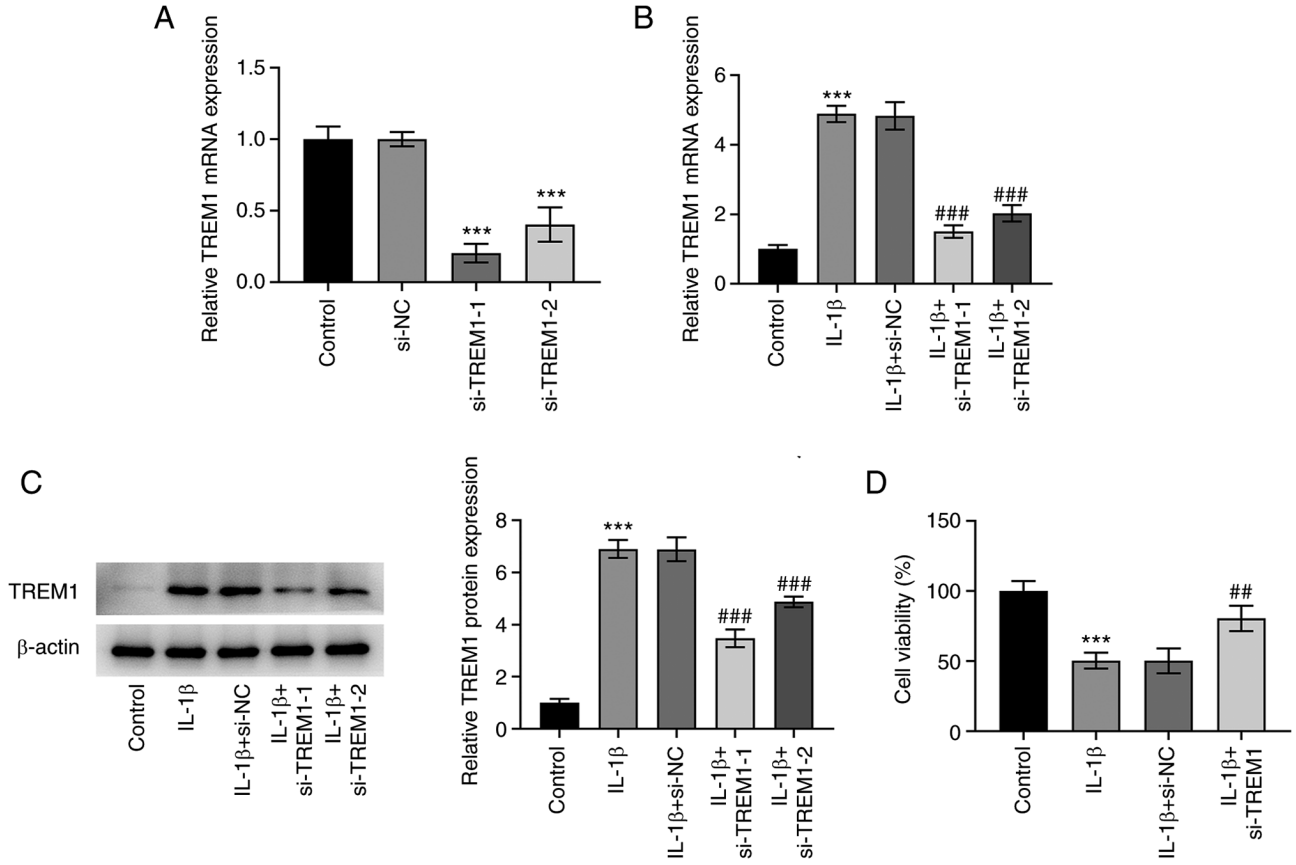

Figure 1. TREM1 knockdown increases the viability of NPCs. (A) The expression level of TREM1 in the transfected NPCs was assessed using RT-qPCR. The effect of IL-1β stimulation on the expression level of TREM1 in the NPCs was assessed using (B) RT-qPCR and (C) western blot analyses. (D) The viability of each treatment group of NPCs was evaluated using an MTT assay. ***P<0.001 vs. si-NC or control; $^{##}$P<0.01, $^{###}$P<0.001 vs. IL-1β + si-NC. NC, negative control; TREM1, triggering receptor expressed on myeloid cells 1; NPC, nucleus pulposus cell; RT-qPCR, reverse transcription-quantitative PCR.

TUNEL assay. NPCs ($2x10^4$ cells/well) were seeded into a 24-well plate, and TUNEL assays were performed using a TUNEL kit (cat. no. C1086; Beyotime Institute of Biotechnology), according to the manufacturer's instructions. Briefly, cells were fixed with 4% paraformaldehyde for 30 min at room temperature, and subsequently incubated with PBS containing 0.3% Triton X-100 for 5 min at room temperature. Following the addition of TUNEL working solution, cells were incubated for a further 1 h at 37˚C in the dark. The nuclei were subsequently counterstained with DAPI for 10 min at room temperature. The images were captured at five random fields of view using a fluorescence microscope (magnification, x200; Olympus Corporation).

Statistical analysis. Experimental data were analyzed using GraphPad 7.0 software (GraphPad Software, Inc.), and the data are shown as the mean ± SD of three replicates. Student's t-test (for comparisons between two groups) or one-way ANOVA followed by Tukey's post-hoc test (for comparisons among multiple groups) were used to evaluate statistical differences. P<0.05 was considered to indicate a statistically significant difference.

Results

TREM1 knockdown increases the viability of NPCs and reduces ECM degradation. After confirming that TREM1 was knocked down in the transfected NPCs (Fig. 1A), the effect of IL-1β stimulation on TREM1 expression levels was assessed using RT-qPCR and western blot analyses (Fig. 1B and C). The level of TREM1 was significantly increased in the IL-1β group compared with the control group. Furthermore, the levels of TREM1 in the NPCs transfected with si-TREM1 were also determined. The expression level of TREM1 was lower in the si-TREM1-1 group compared with the si-TREM1-2 group, and therefore the si-TREM1-1 group of NPCs was used for the subsequent assays. Next, the viability of each group of NPCs was evaluated using an MTT assay (Fig. 1D). The cell viability of the IL-1β group was found to sharply decline, whereas TREM1 knockdown partially increased the viability. The expression level of collagen II in each group was subsequently determined using an immunofluorescence assay (Fig. 2A). The results obtained indicated that the fluorescence of the IL-1β group was clearly weaker compared with that of the control group, whereas the fluorescence of the si-TREM1 group was strengthened. In addition, the protein expression levels of aggrecan, MMP3, MMP9, MMP13, ADAM metallopeptidase with thrombospondin type 1 motif (ADAMTS)-4 and ADAMTS-5 were determined by western blot analysis (Fig. 2B and C). The level of aggrecan was found to be reduced in the IL-1β group, whereas TREM1 knockdown caused a marked elevation in the level of aggrecan; furthermore, the trends of changes in the levels of the other proteins were found to be the opposite of those of aggrecan.

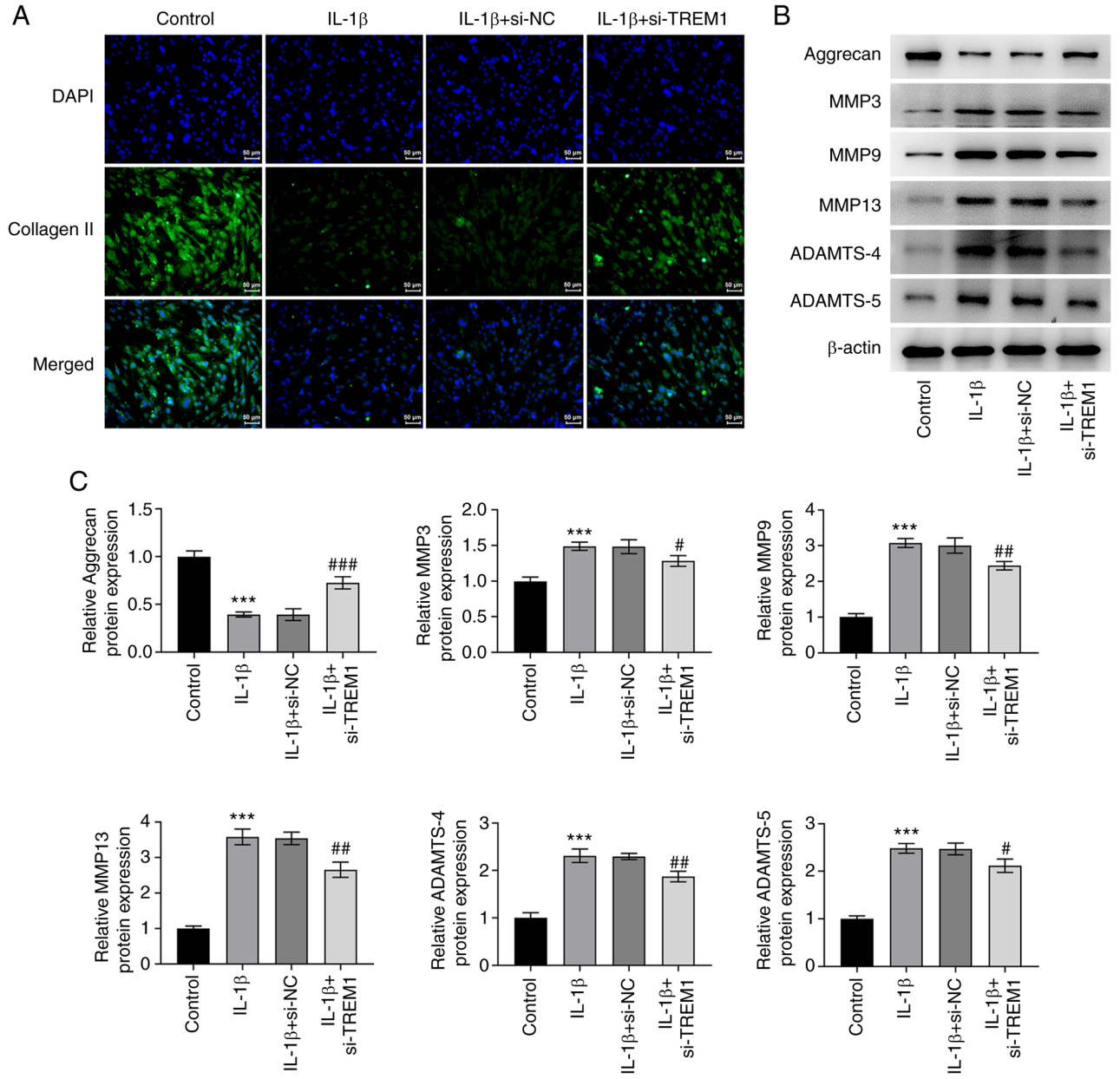

Figure 2. TREM1 knockdown reduces ECM degradation. (A) The expression level of collagen II in each group was determined using an immunofluorescence assay. (B and C) The protein expression levels of aggrecan, MMP3, MMP9, MMP13, ADAMTS-4 and ADAMTS-5 were determined using western blotting. ***P<0.001 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β + si-NC. TREM1, triggering receptor expressed on myeloid cells 1; ECM, extracellular matrix; ADAMTS; ADAM metallopeptidase with thrombospondin type 1 motif; si, short interfering; NC, negative control.

TREM1 knockdown reduces the level of inflammation and apoptosis of the NPCs. The levels of the inflammatory factors TNF-α and IL-6 were measured with ELISA kits (Fig. 3A). The results revealed that the levels of TNF-α and IL-6 were both significantly increased in the IL-1β group, whereas TREM1 knockdown led to a partial downregulation of their levels. Subsequently, the influence of IL-1β and TREM1 knockdown on the level of apoptosis of the NPCs was assessed using TUNEL assay (Fig. 3B and C) and western blotting (Fig. 3D). The apoptotic cells in the IL-1β group were found to emit stronger fluorescence signals compared with the control group. In addition, the fluorescence in the si-TREM1 group was weaker compared with that in the si-NC group, indicating that the downregulation of TREM1 suppressed the apoptosis of NPCs. At the same time, the protein expression level of Bcl-2 was decreased in the IL-1β group, whereas the levels of Bax, cleaved caspase 3 and cleaved poly(ADP-ribose) polymerase were all increased. Following TREM1 knockdown, however, the aforementioned apoptosis-associated protein expression level changes were all reversed.

TREM1 knockdown inhibits ERS via blocking TLR4/NF-κB. According to the STRING database, TREM1 was predicted to be associated with TLR4. As a consequence, the expression levels of proteins associated with TLR4/NF-κB were detected using western blotting (Fig. 4A). The levels of TLR4, MyD88, phosphorylated (p)-IKK and p-p65 were increased as the result of IL-1β stimulation. TREM1 knockdown led to a partial reduction in the elevated levels, indicating that TREM1 knockdown had the effect of blocking TLR4/NF-κB signaling.

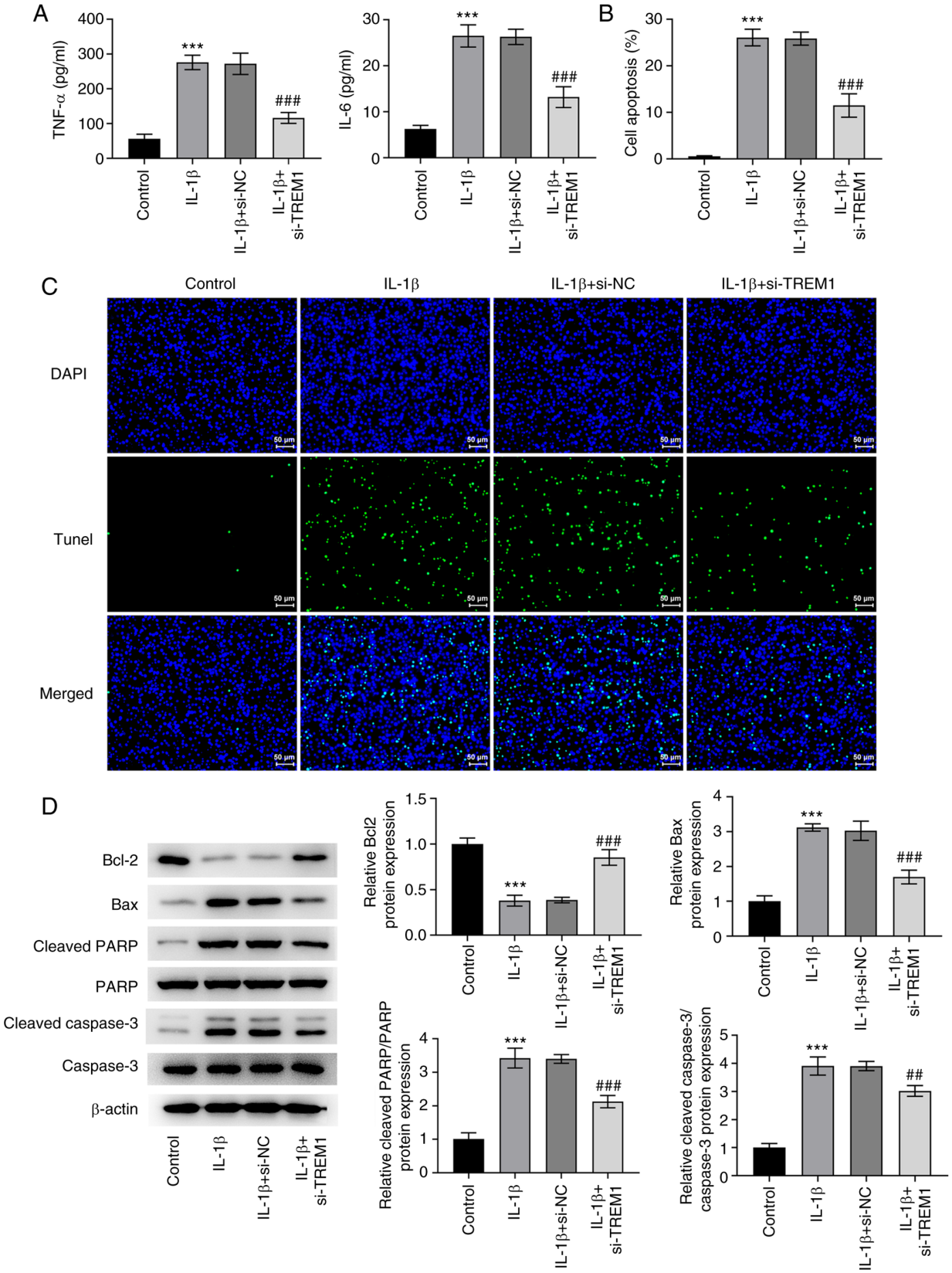

Figure 3. TREM1 knockdown reduces the inflammation and NPC apoptosis. (A) The levels of inflammatory factors were measured with ELISA kits. The influence of IL-1β and TREM1 knockdown on apoptosis was assessed using (B and C) TUNEL assay and (D) western blot analysis. ***P<0.001 vs. control; $^{##}$P<0.01, $^{###}$P<0.001 vs. IL-1β + si-NC. TREM1, triggering receptor expressed on myeloid cells 1; NPC, nucleus pulposus cell; si, short interfering; NC, negative control.

Subsequently, MPLA (the TLR-4 agonist) was applied to the NPCs, and the levels of ERS-associated proteins were then detected using western blotting (Fig. 4B). The expression levels of C/EBP homologous protein, activating transcription factor 6 and glucose-regulated protein 78 were increased as a consequence of IL-1β induction, although they were decreased

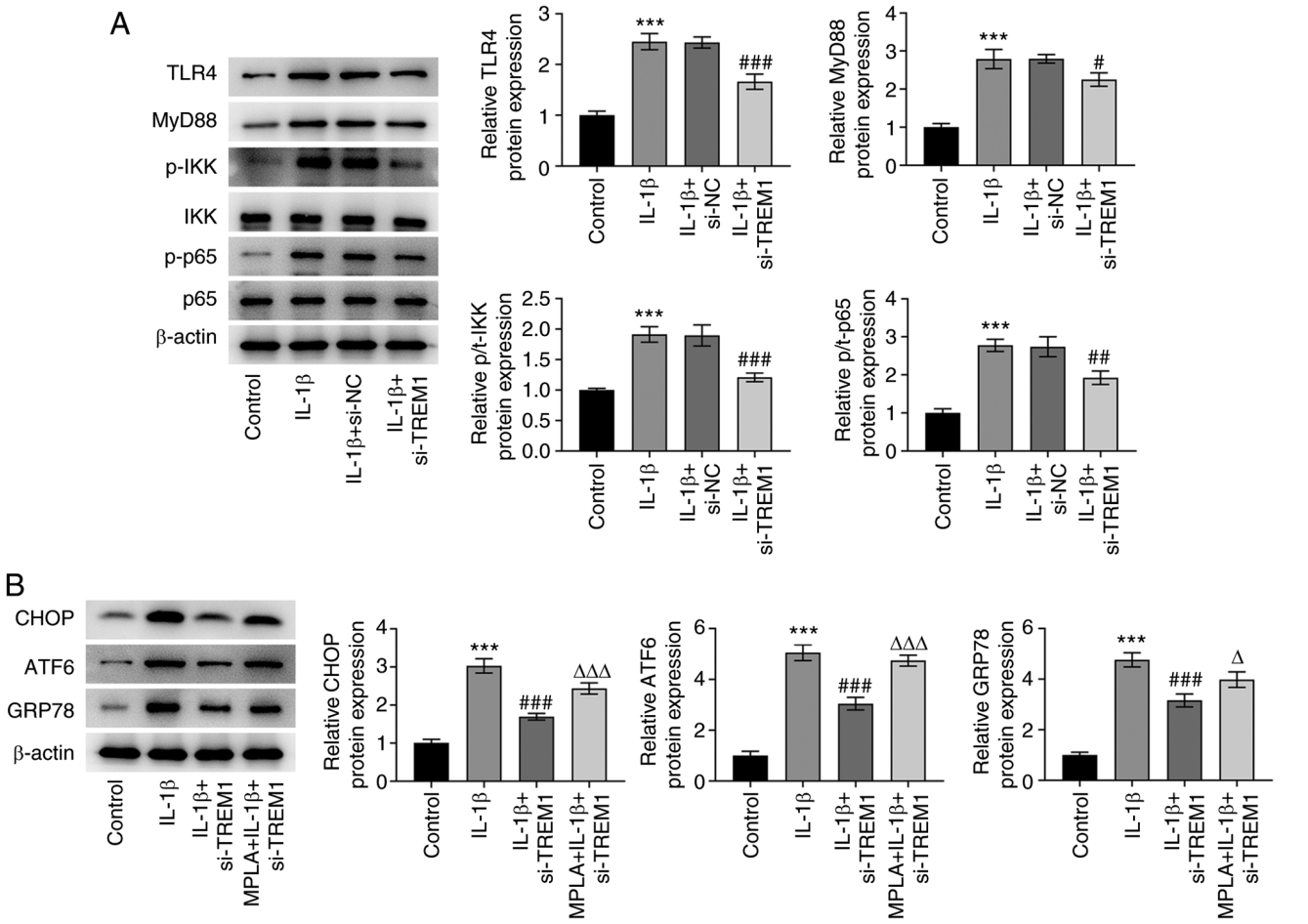

Figure 4. TREM1 knockdown inhibits ERS via blocking the TLR4/NF-κB pathway. (A) The expression levels of proteins associated with TLR4/NF-κB signaling were determined using western blotting. (B) The levels of ERS-associated proteins were detected using western blotting. ***P<0.001 vs. control; $^{\#}$P<0.05, $^{\#\#}$P<0.01, $^{\#\#\#}$P<0.001 vs. IL-1β + si-NC; $^{\Delta}$P<0.05, $^{\Delta\Delta\Delta}$P<0.001 vs. IL-1β + si-TREM1. TREM1, triggering receptor expressed on myeloid cells 1; ERS, endoplasmic reticulum stress; TLR4, Toll-like receptor 4; si, short interfering; NC, negative control; p-, phosphorylated.

due to TREM1 knockdown, and partly increased as a result of TLR4 activation.

TREM1 knockdown reduces both ECM degradation and the levels of inflammation and apoptosis of the NPCs via inhibiting ERS. Subsequently, the effects of tunicamycin (an ERS agonist) on NPCs were investigated. The effect of tunicamycin on the expression of collagen II was assessed first (Fig. 5A). It was found that MPLA and tunicamycin similarly caused a partial reversal of the increases in the collagen II level elicited by TREM1 knockdown. Additionally, the expression levels of aggrecan were reduced upon treatment with MPLA or tunicamycin, whereas those of MMP3, MMP9, MMP13, ADAMTS-4 and ADAMTS-5 were concomitantly increased (Fig. 5B). Moreover, treatment with either MPLA or tunicamycin also boosted the levels of TNF-α and IL-6, even though TREM1 expression was knocked down (Fig. 6A). Taken together, the results of the TUNEL assay and western blotting experiments indicated that MPLA or tunicamycin treatment could promote the apoptosis of NPCs (Fig. 6B-D).

Discussion

Various strategies are employed at present for the treatment of IDD, including conservative treatment, surgical removal, collagenase dissolution, ozone ablation and artificial disc replacement (23). Although surgery is relatively effective as a method, it has the disadvantage of being an invasive treatment (24) which has a huge effect on the patient's psychology and physiology. Moreover, the operation leads to large trauma, necessitates a long recovery period and is associated with high costs (25). Furthermore, surgery can also destroy the integrity of the spinal motor unit, easily causing secondary degeneration of adjacent segments (26). Hence, the long-term effects of surgical intervention remain undesirable. In light of the deepening of research in the field of IDD worldwide, considerable research efforts are being focused at the cellular therapeutic level. Through suppressing the apoptosis of intervertebral disc cells, promoting the secretion of cells and raising the levels of intercellular substances, the goals of postponing degeneration of the intervertebral disc or promoting its regeneration can be readily achieved (27-29).

Previous studies have revealed that inflammatory factors serve important roles in the progression of IDD. The degenerated intervertebral discs are generally accompanied by high expression levels of inflammatory factors (13). Among them, IL-1β has been the most widely studied (3). IL-1β has been shown to be elevated in nucleus pulposus tissue (25) to promote apoptosis of NPCs and to increase the degradation of ECM (30).

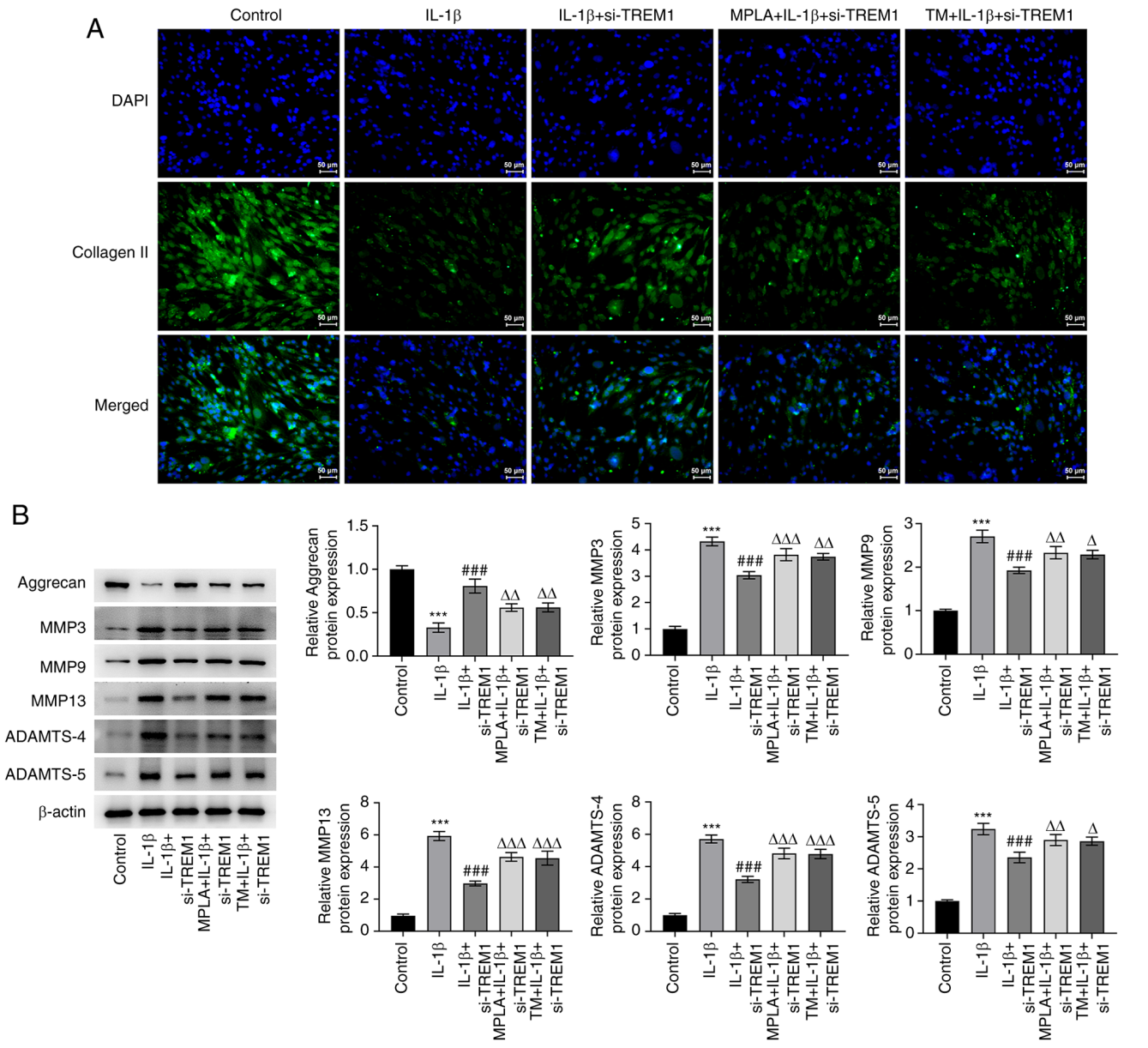

Figure 5. TREM1 knockdown reduces ECM degradation through inhibiting ERS. (A) The expression level of collagen II in each group was determined using an immunofluorescence assay. (B) The protein expression levels of aggrecan, MMP3, MMP9, MMP13, ADAMTS-4 and ADAMTS-5 were determined using western blot analysis. ***P<0.001 vs. control; $^{###}$P<0.001 vs. IL-1β; ^{Δ}P<0.05, ^{ΔΔ}P<0.01, ^{ΔΔΔ}P<0.001 vs. IL-1β + si-TREM1. TREM1, triggering receptor expressed on myeloid cells 1; ECM, extracellular matrix; ERS, endoplasmic reticulum stress; ADAMTS; ADAM metallopeptidase with thrombospondin type 1 motif; si, short interfering; MPLA, monophosphoryl lipid A.

Therefore, the present study made use of IL-1β to induce NPCs, and the results obtained showed that TREM1 knockdown was able to inhibit the ECM degradation and cell apoptosis that were induced by IL-1β. These results suggested that IDD may be alleviated via the therapeutic blocking of TREM1.

Preliminary research on the regulatory mechanism of TREM1 was also undertaken in the present study. TREM1 is associated with TLR4/MyD88/NF-κB-dependent signal transduction and it also aggravates lung damage in a mouse model (31). Furthermore, TREM1 regulates inflammation resulting from *Mycoplasma pneumoniae* infection via the NF-κB signaling pathway (32). These findings suggest that the classic inflammatory pathway involving NF-κB is likely to be the key mechanism through which TREM1 influences human NPCs. Therefore, the levels of NF-κB pathway-associated proteins were investigated in cells whose TREM1 expression had been knocked down, and these experiments showed that decreased levels of TREM1 expression could inhibit activation of the NF-κB pathway. TREM1 is able to amplify the inflammatory response mediated by TLRs and Nod-like receptors and TLRs are the upstream signaling components of the NF-κB pathway involved in the cellular stress response (33). Moreover, TREM1 has been identified to bind to TLR4 (34). The present study used the TLR4 agonist, MPLA, to treat cells to investigate whether TLR4 could mediate regulation of the NF-κB signaling pathway via TREM1. The experimental findings in the present study confirmed that TREM1 knockdown was able to block the TLR4/NF-κB cascade signaling pathway. In addition, given that the endoplasmic reticulum is the major site for the biosynthesis of ECM components (27), experiments

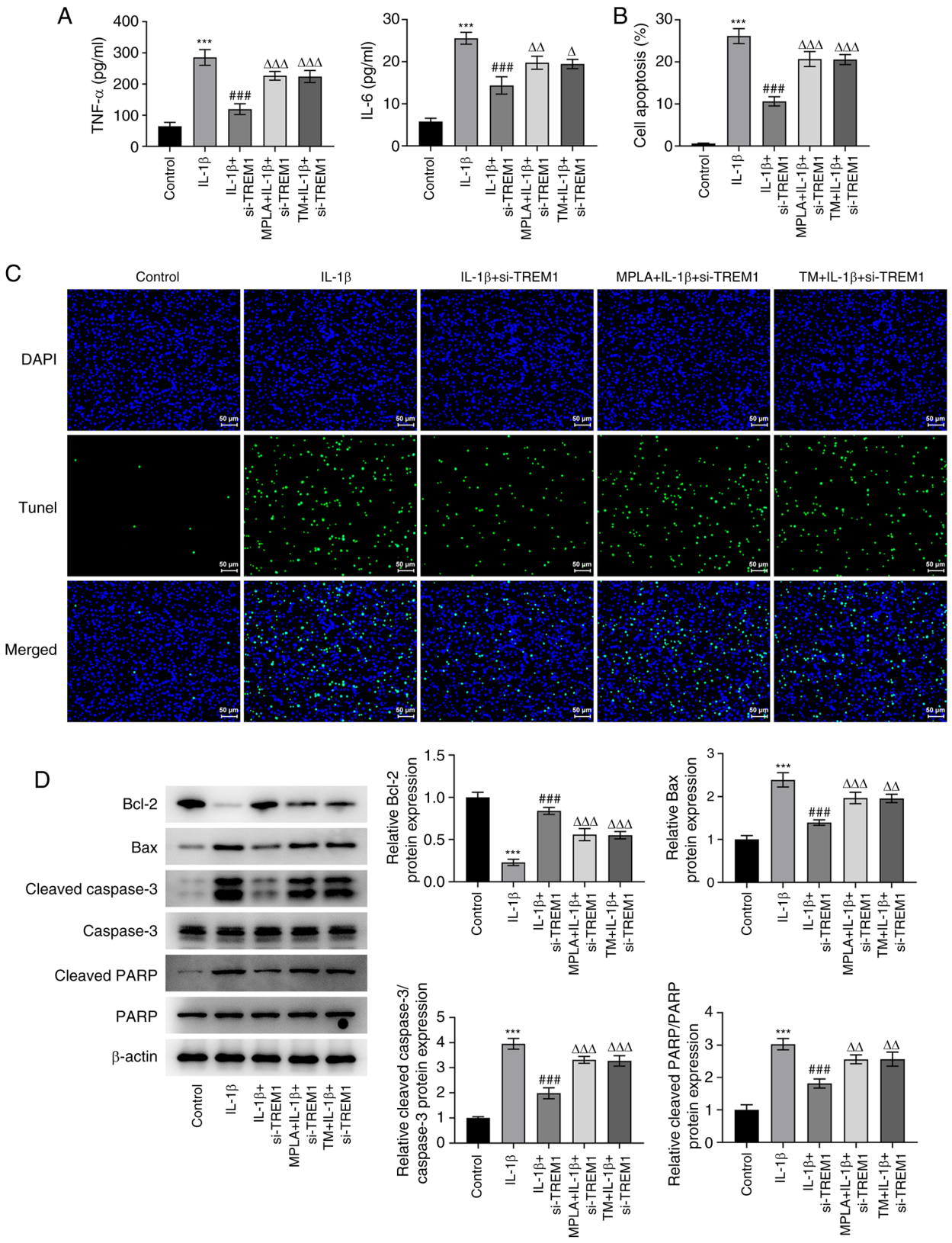

Figure 6. TREM1 knockdown reduces inflammation and apoptosis of NPCs through inhibiting ERS. (A) The levels of inflammatory factors were measured with ELISA kits. The influence of MPLA and tunicamycin on apoptosis of the NPCs was assessed using (B and C) TUNEL assay and (D) western blot analysis. ***P<0.001 vs. control; $^{###}$P<0.001 vs. IL-1β; ^{Δ}P<0.05, ^{ΔΔ}P<0.01, ^{ΔΔΔ}P<0.001 vs. IL-1β + si-TREM1. TREM1, triggering receptor expressed on myeloid cells 1; NPC, nucleus pulposus cell; ERS, endoplasmic reticulum stress; MPLA, monophosphoryl lipid A si, short interfering.

were subsequently designed featuring an ERS agonist to assess whether TREM1 knockdown influenced ERS. These experiments confirmed that TREM1 knockdown could inhibit the ERS via blocking the TLR4/NF-κB signaling pathway. Nevertheless, multiple subtypes of TLR have been discovered in human intervertebral disc cells; with the exception of TLR4,

it is still unknown whether other subtypes are able to mediate the regulation of TREM1. Furthermore, the present study was limited to *in vitro* experiments and further *in vivo* studies are required to verify the experimental findings.

In conclusion, the present study showed that TREM1 knockdown blocked the TLR4/NF-κB signaling pathway, inhibited ERS and reduced the ECM degradation and apoptosis of NPCs induced by IL-1β. Taken together, these findings suggested that blocking TREM1 may be a novel therapeutic approach as a means for better treatment of IDD.

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Availability of data and materials

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

Authors' contributions

JZ and LD contributed to design and experiments. HJ and ML contributed to experiments, analysis and drafting the manuscript. All authors read and approved the final manuscript. JZ and LD confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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