

UCHL1 enhances *TSC1* transcription by stabilizing FOXO1 through deubiquitination in knee osteoarthritis

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Summary. Ferroptosis has been shown to play a significant role in the pathophysiological progression of knee osteoarthritis (KOA). In this study, we sought to investigate the biological role of Ubiquitin C-terminal hydrolase 1 (UCHL1) in KOA and elucidate its underlying molecular mechanisms. An *in vitro* KOA cell model was established by stimulating C28/I2 chondrocytes with IL-1 β , and UCHL1 expression was decreased in IL-1 β -treated chondrocytes. Notably, overexpression of UCHL1 significantly alleviated IL-1 β -induced ferroptosis and extracellular matrix (ECM) degradation. Mechanistically, UCHL1 facilitated the deubiquitination and stabilization of FOXO1. Knockdown of FOXO1 partially reversed the inhibitory effects of UCHL1 on ferroptosis and ECM degradation. Furthermore, FOXO1 was found to bind to the Tuberous Sclerosis Complex 1 (*TSC1*) promoter, enhancing *TSC1* transcription. Intriguingly, knockdown of FOXO1 counteracted the inhibitory effects of UCHL1 overexpression on ferroptosis and ECM degradation, while these effects were rescued by *TSC1* overexpression. *In vivo* experiments demonstrated that UCHL1 alleviated cartilage damage in KOA rats by inhibiting ferroptosis and ECM degradation through the FOXO1/*TSC1* axis. These findings demonstrate the pivotal role of UCHL1 in regulating ferroptosis and maintaining ECM homeostasis, offering novel insights into the molecular mechanisms driving KOA progression.

Key words: UCHL1, FOXO1, *TSC1*, Deubiquitination, Knee osteoarthritis

Introduction

Knee osteoarthritis (KOA) is a common degenerative joint condition in middle-aged individuals (Ye and Liu, 2023). The incidence of KOA continues to rise annually, driven by an aging population and increasing obesity rates (Bijlsma et al., 2011). KOA affects up to 40% of patients over 70, with its high disability rate placing a substantial economic burden on health systems (Li et al., 2025). Symptomatically, KOA mainly manifests as restricted joint motion accompanied by erythema, swelling, heat, and pain during movement. Key pathological features include synovitis, articular cartilage damage, subchondral bone degeneration, and osteophyte formation (Mochizuki et al., 2024). There is currently no effective treatment for KOA, aside from total knee replacement for advanced cases (Lino et al., 2024). Early treatment of KOA is mainly limited to reducing joint wear and pain relief (Gu et al., 2020). Therefore, clarifying the pathogenesis of KOA and identifying novel target molecules involved in its pathophysiological processes are of great significance for the prevention and treatment of KOA.

Ferroptosis is a unique form of cellular death marked by iron-catalyzed aberrations in lipid peroxide metabolism (Stockwell et al., 2017). This process increases the production of reactive oxygen species (ROS), triggers lipid peroxidation, and ultimately leads to cell death (Tang et al., 2021). Ferroptosis was first expounded by Dixon et al. (2012) and is closely associated with various diseases. Moreover, several studies have demonstrated that ferroptosis occurs in the damaged synovium of KOA patients, who exhibit significantly higher levels of lipid peroxidation compared with healthy controls (Zhang et al., 2022). In MIA-induced rats, Lipoxin A4 remitted KOA progression through antagonizing ferroptosis (Hu et al., 2024). This study confirmed that ferroptosis is implicated in the development of KOA. However, the regulation of chondrocyte ferroptosis in KOA remains largely unknown.

Deubiquitinating enzymes (DUBs) are proteases that

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modulate various cellular processes and pathways by reversing the ubiquitination of key substrates and then maintaining their steady-state levels (Haq et al., 2018). Ubiquitin C-terminal hydrolase 1 (UCHL1) is a DUB responsible for dislodging ubiquitin or polyubiquitin chains from target proteins (Shi et al., 2023). It has been documented that UCHL1 influences skeletal muscle growth and function (Gao et al., 2019, 2020). Shim et al. demonstrated that UCHL1 depletion leads to significant reductions in bone mass and impaired bone mineralization (Shim et al., 2008). Feng et al. unveiled that depletion of UCHL1 deteriorates OVX-caused bone loss *in vivo* and hyperactivates osteoclasts *in vitro* (Feng et al., 2023). These findings suggest a role for UCHL1 in bone-related diseases. However, whether UCHL1 regulates KOA progression and the underlying mechanisms remain unknown.

The Forkhead box O (FOXO) family plays a momentous role in multiple physiological processes, including the modulation of bone resorption, formation, and cartilage homeostasis (Liu et al., 2021). Among these, FOXO1 has been specifically implicated in meniscal development and tissue maintenance, with emerging evidence highlighting its dysregulation during aging and osteoarthritis pathogenesis (Lee et al., 2020). Additionally, FOXO1 regulated chondrogenic differentiation through TGF β 1, highlighting its essential role in OA (Kurakazu et al., 2019). Therefore, we sought to explore whether UCHL1 affects KOA progression by regulating FOXO1. Moreover, as a transcription factor, FOXO1 regulated the level of Prg4 and maintained the integrity of the cartilage surface zone (Matsuzaki et al., 2018). ChIP-Atlas predicts that Tuberous Sclerosis Complex 1 (*TSC1*) is a gene that binds the transcription factor FOXO1. However, the transcriptional regulation of *TSC1* by FOXO1 and its functional relevance in KOA pathogenesis remain to be elucidated.

In this study, we conjectured that the UCHL1/FOXO1/TSC1 axis has a regulatory effect on KOA. This study expands the understanding of the mechanism by which UCHL1 regulates KOA and provides a new perspective for its treatment.

Materials and methods

Cell culture and treatment

Human C28/I2 chondrocytes were obtained from the BLUEFIBIO Shanghai Cell Bank (Shanghai, China) and maintained in Dulbecco's Modified Eagle Medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS). The cells were cultured at 37°C in a humidified atmosphere containing 5% CO₂. To establish an *in vitro* model of KOA, C28/I2 cells were stimulated with interleukin-1 β (IL-1 β ; 10 ng/mL) for 24 h. For additional experimental interventions, the cells were treated with 50 μ g/mL cycloheximide (CHX) for 30, 60, or 90 min to assess protein degradation, or with MG132 (5 μ M) for 24h to inhibit proteasomal activity.

Cell transfection

The overexpression plasmids for pcDNA3.1-UCHL1 (oe-UCHL1), pcDNA3.1-FOXO1 (oe-FOXO1), and TSC1 (oe-TSC1), along with pcDNA3.1 plasmid (oe-NC), as well as short hairpin (sh) RNA targeting UCHL1 (sh-UCHL1), FOXO1 (sh-FOXO1), TSC1 (sh-TSC1), and a negative control (sh-NC), were all purchased from GenePharma (Shanghai, China). For transfection, C28/I2 cells were seeded in six-well plates and cultured until they reached 50-70% confluence. The cells were then transfected with the respective plasmids (5 μ g) or shRNAs (100 pmol) using Lipofectamine 2000 (Invitrogen) in Opti-MEM for 6 h and then the medium was changed with DMEM containing 10% FBS. Subsequent experiments were performed 48h post-transfection.

RT-qPCR

Total RNA was extracted from chondrocytes using the TRIzol reagent according to the manufacturer's instructions. Subsequently, the extracted RNA was reverse-transcribed into complementary DNA (cDNA) using the PrimeScript RT reagent kit (Takara, Japan). Quantitative PCR (qPCR) was performed using the SYBR Green-based real-time PCR Master Mix (Takara, Japan) in an ABI StepOne Plus real-time PCR system (Thermo Fisher Scientific, USA). The relative expression levels of *UCHL1*, *FOXO1*, and *TSC1* were normalized to the housekeeping gene *GAPDH* and calculated using the 2^{- $\Delta\Delta C_t$} method. The primer sequences for RT-qPCR were: UCHL1 F: 5'-GGCC AATGTCGGGTAGATGA-3', R: 5'-CACCGGAAA AGGCATTCGTC-3'; FOXO1 F: 5'-TGGAGATCGA CCCGGACTTC-3', R: 5'-CCGAGTTGGACTGGC TAAACT-3'; TSC1 F: 5'-CCCTCTACCTCCCCA ATGGA-3', R: 5'-TGAAGGAGGAGAGCCTCCAA-3'; GAPDH F: 5'-AGGCCGGATGTGTTTCGC-3', R: 5'-CAAATCCGTTGACTCCGACC-3'.

Western blot

Total protein was extracted from C28/I2 cells and rat knee cartilage tissues using RIPA lysis buffer (Boster, Wuhan, China). Protein concentrations were quantified using a BCA assay kit (Boster, Wuhan, China). For western blot analysis, 10 μ g of protein per sample was separated by SDS-PAGE and subsequently transferred onto PVDF membranes (Millipore, Sigma, USA). The membranes were blocked with 5% non-fat milk for 1 hour at room temperature, followed by incubation overnight at 4°C with primary antibodies against UCHL1 (#13179, 1:1000, Cell Signaling), FOXO1 (ab179450, 1:1000, Abcam), TSC1 (#6935, 1:1000, Cell Signaling), SLC7A11 (ab307601, 1:1000, Abcam), GPX4 (#ER1803-15, 1:1000, HUABIO), Collagen II (#ER1906-48, 1:1000, HUABIO), MMP-13 (ab39012, 1:3000, Abcam), ADAMTS5 (ab41037, 1:250, Abcam),

and GAPDH (ab8245, 1:1000, Abcam). After incubation, the membranes were washed three times with TBST (Tris-buffered saline with 0.1% Tween-20) for 10 minutes each to remove unbound primary antibodies. The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 1h at room temperature. Subsequently, an enhanced chemiluminescence (ECL) kit was used in a chemiluminescence detection system and protein expression was quantified using ImageJ software.

Ferrous iron detection

Intracellular ferrous iron was detected using the FerroOrange fluorescent probe (Dojindo, Beijing, China). To prepare a 1 mmol/L FerroOrange solution, 35 μ L of dimethyl sulfoxide (DMSO; Beyotime Biotechnology, Shanghai, China) was added to a tube containing 24 μ g FerroOrange. This was then diluted with Hank's Balanced Salt Solution (HBSS; Servicebio Technology) to generate a 1 μ mol/L working solution. The cells were treated with a working solution of FerroOrange and incubated at 37°C for 30 min. After routine washing and DAPI staining, the plates were observed under an inverted microscope.

Measurement of Malondialdehyde (MDA) and Glutathione (GSH)

The levels of glutathione (GSH) and malondialdehyde (MDA) were measured using a GSH Detection Kit (Catalog No. A006-2-1, Nanjing Jiancheng Bioengineering Institute, China) and an MDA Assay Kit (Catalog No. A003-1-2, Nanjing Jiancheng Bioengineering Institute, China), respectively, following the manufacturer's protocols.

Dichlorofluorescein diacetate assay

Reactive oxygen species (ROS) generation was detected using a 6-carboxy-2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) ROS assay kit (Beyotime, Shanghai, China). C28/I2 cells were incubated with DCFH-DA (10 μ mol/L), washed with PBS three times, and stained with DAPI (10 μ g/mL). Afterward, C28/I2 cells were observed under a fluorescence microscope.

Flow cytometry assay

To determine the proportion of chondrocytes undergoing apoptosis, we employed the FITC Annexin V apoptosis detection kit (BD Pharmingen, San Diego, CA, USA) following the manufacturer's protocol, as previously described (Yuan et al., 2024). Briefly, harvested chondrocytes were washed twice with ice-cold PBS and resuspended in 100 μ L of binding buffer. Subsequently, 5 μ L of FITC Annexin V and 5 μ L of propidium iodide (PI) were added to the cell suspension.

Following a 15-min incubation at room temperature in the dark, 400 μ L of binding buffer was added to each sample. Cell analysis was then performed using a FACScan flow cytometer (BD Biosciences, San Jose, CA, USA).

Dual-luciferase reporter assay

The binding site of FOXO1 on the *TSC1* promoter region was obtained from the Jaspas Website. The wild-type (WT-TSC1; 5'-GCTTGTTTTTG-3') and mutant (Mut-TSC1; 5'-GGTACACTGCG-3') sequences were synthesized and inserted into the luciferase reporter gene vectors (pGL3-Basic). C28/I2 cells were co-transfected with oe-NC or oe-FOXO1, reporter plasmid, and pRL-TK plasmid using Lipofectamine 2000. Luciferase activity was measured 48h after transfection using the Dual-Luciferase Reporter Assay System (Promega).

Chromatin immunoprecipitation (ChIP) assay

Chromatin was extracted from cellular lysates after the introduction of formaldehyde to induce cross-linking between endogenous proteins and DNA, followed by fragmentation. Sonicated chromatin was immunoprecipitated using anti-FOXO1 antibody or IgG with Protein A/G agarose beads overnight. The purified DNA was detected using qPCR with primers targeting the TSC1 binding sites.

Co-immunoprecipitation (Co-IP)

Cells were lysed in RIPA lysis buffer (Beyotime) supplemented with a protease inhibitor cocktail and 1 mM PMSF on ice for 15 minutes. The lysates were centrifuged at 12,000 $\times$ g for 15 minutes at 4°C to remove cellular debris. Afterward, the supernatant was incubated overnight at 4°C with 2 μ g of UCHL1 antibody (14730-1-AP, Proteintech) or rabbit IgG (30000-0-AP, Proteintech). Following this, 40 μ L of protein A/G magnetic beads (Beyotime) were added to the mixture, which was then incubated for 1 hour at room temperature with gentle rotation. The beads were washed three times with cold lysis buffer to remove non-specific binding. After washing, the bound proteins were eluted by adding SDS loading buffer (Beyotime) and boiling the samples at 95°C for 5 min. The precipitated proteins were then analyzed using western blot.

Ubiquitination assay

To assess the ubiquitination levels of FOXO1, C28/I2 cells were treated with 5 μ M MG132 (Sigma) for 8 hours to inhibit proteasome-mediated degradation. The cells were then lysed using RIPA buffer, and the lysates were subjected to immunoprecipitation with an anti-FOXO1 antibody (ab179450, Abcam). The immunoprecipitated proteins were analyzed by western blotting using an anti-ubiquitin antibody (ab134953, Abcam).

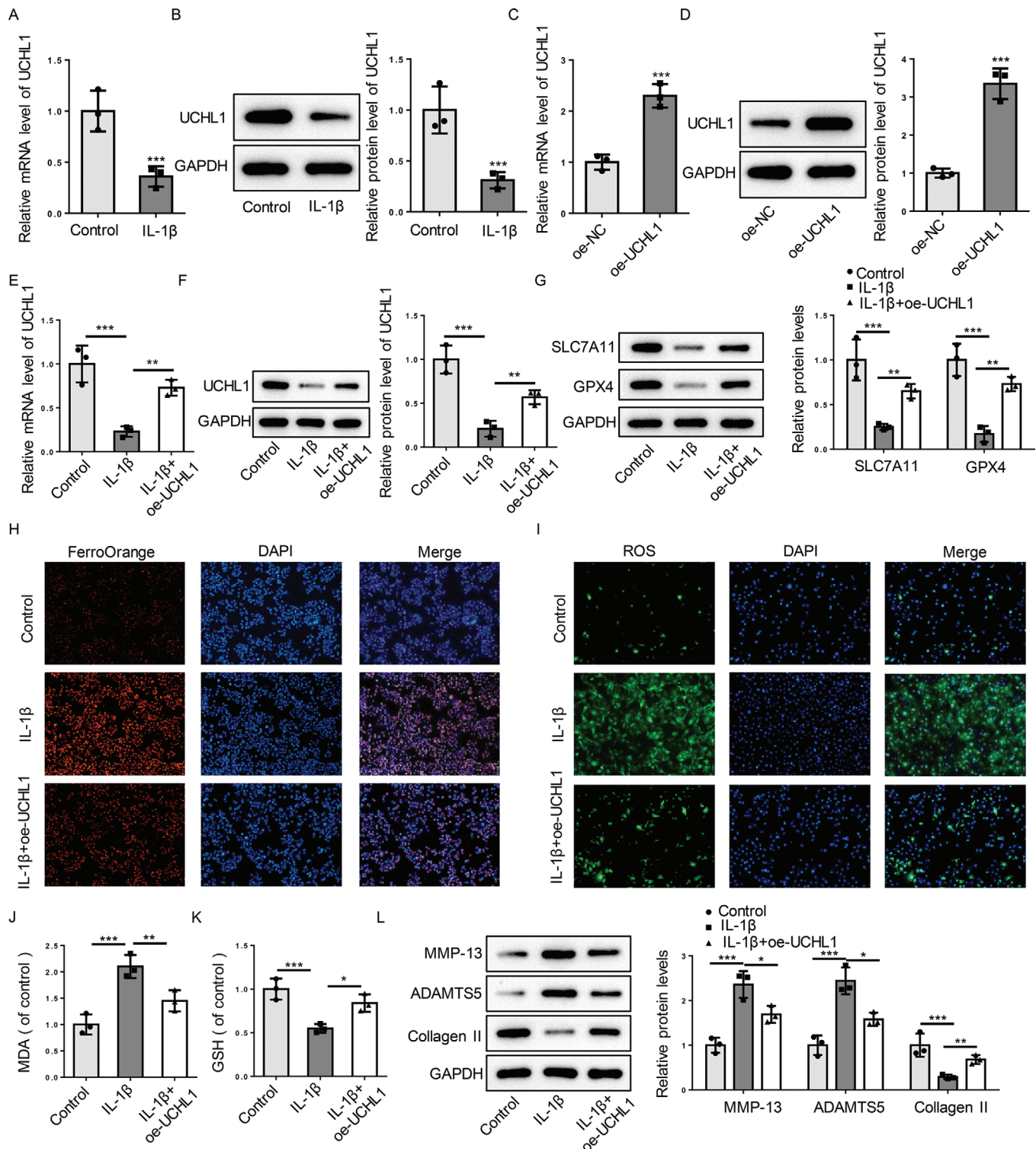


Fig. 1. UCHL1 represses IL-1 β -triggered ferroptosis and ECM degradation in chondrocytes. **A, B.** RT-qPCR and western blot assays were used to analyze the mRNA and protein levels of UCHL1 in chondrocytes after IL-1 β treatment. **C, D.** Validation of overexpression efficiency of UCHL1 in chondrocytes. Chondrocytes were transfected with oe-UCHL1, and then subjected to IL-1 β treatment. **E, F.** UCHL1 mRNA and protein levels were evaluated by RT-qPCR and western blot assays (**G**) SLC7A11 and GPX4 protein levels were measured in chondrocytes. Semiquantitative analysis of the fluorescence intensity of intracellular Fe²⁺ (**H**) and ROS (**I**). **J.** MDA levels in chondrocytes were quantitatively determined using an MDA assay kit. **K.** GSH levels in chondrocytes were quantitatively determined using a GSH assay kit. **L.** MMP-13, ADAMTS5, and Collagen II protein levels were detected by western blot. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Establishment of the KOA rat model and grouping

The animals were randomized and assigned to three groups (n=6 per group): Sham, KOA model, and KOA+oe-UCHL1. The rats in the KOA model and KOA+oe-UCHL1 groups received an intra-articular (IA) injection of monosodium iodoacetate (MIA) (2 mg/50 μ L) through the patellar ligament into the intra-articular space of the right knee. One week after MIA injection, rats in the KOA+oe-UCHL1 group were additionally treated with IA injections of AAV-oe-UCHL1 (1×10^{12} vg/50ul). The vector was an AAV9 serotype carrying the UCHL1-overexpression construct under the control of a CMV promoter. The sham group received an IA injection of 50 μ L sterile saline as a control. After six weeks, all rats were euthanized via cervical dislocation. All animal experiments were conducted in accordance with ethical guidelines and were approved by the Institutional Animal Care and Use Committee of Changzhou Hospital of Traditional Chinese Medicine.

Evaluation of pain behavior

Pain behavior was evaluated by measuring the mechanical paw withdrawal threshold (PWT) every week before and after KOA induction. Briefly, the rats were placed in a Plexiglas box with a screen platform and allowed to acclimate to the environment for 10 min. A series of calibrated von Frey monofilaments of various bending forces were applied in ascending order of bending force to the plantar surface of the hind paw and held for approximately 6-8 s. A positive response was

defined as paw withdrawal occurring twice in the 10 applications. When negative responses occurred, a larger force monofilament was applied.

Histological analysis

Knee joint tissues were fixed, decalcified for four weeks, embedded in paraffin, and sectioned into 5- μ m slices. After dewaxing and dehydration, sections were stained with hematoxylin and eosin (H&E) using a commercial kit (G1003, Servicebio) or Safranin O-Fast Green (G1371, Solarbio) following manufacturers' protocols. Stained sections were visualized under an optical microscope (BX53, OLYMPUS), and cartilage degeneration was assessed using the Osteoarthritis Research Society International (OARSI) scoring system.

Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Measurement data are presented as the mean $\pm$ standard deviation (SD). For comparisons between two groups, the Student's t-test was used, while one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test was applied for comparisons involving three or more groups. Normality and homogeneity of variance were assessed using the Shapiro-Wilk and Levene's tests, respectively. For data that did not meet the assumptions of parametric tests, non-parametric alternatives such as the Mann-Whitney U test (for two groups) or Kruskal-Wallis test (for multiple groups) were employed. A *p*-

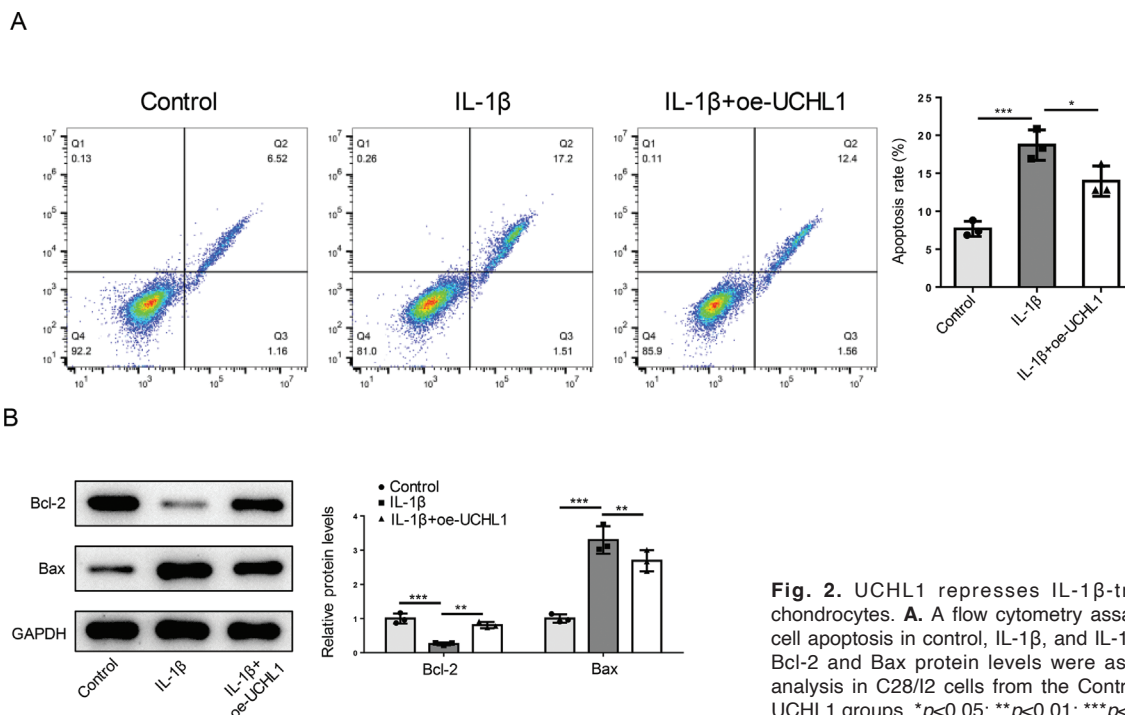


Fig. 2. UCHL1 represses IL-1 β -triggered apoptosis in chondrocytes. **A.** A flow cytometry assay was used to measure cell apoptosis in control, IL-1 β , and IL-1 β +oe-UCHL1 groups. **B.** Bcl-2 and Bax protein levels were assessed by western blot analysis in C28/I2 cells from the Control, IL-1 β , and IL-1 β +oe-UCHL1 groups. **p*<0.05; ***p*<0.01; ****p*<0.001.

value of <0.05 was considered statistically significant. All statistical tests were two-tailed, and adjustments for multiple comparisons were made where applicable.

Results

UCHL1 inhibits IL-1 β -induced apoptosis, ferroptosis, and ECM degradation in chondrocytes

To establish an *in vitro* model of KOA, chondrocytes

were treated with 10 ng/mL IL-1 β . The mRNA and protein levels of UCHL1 were significantly reduced in IL-1 β -treated chondrocytes (Fig. 1A,B). Then, UCHL1 was overexpressed in chondrocytes, and the overexpression efficiency was confirmed by RT-qPCR and western blot assays (Fig. 1C,D). Overexpression of UCHL1 reversed the downregulation of UCHL1 induced by IL-1 β in chondrocytes (Fig. 1E,F). IL-1 β increased the apoptosis rate of C28/I2 cells, which was mitigated by UCHL1 overexpression (Fig. 2A). Besides, in IL-1 β -

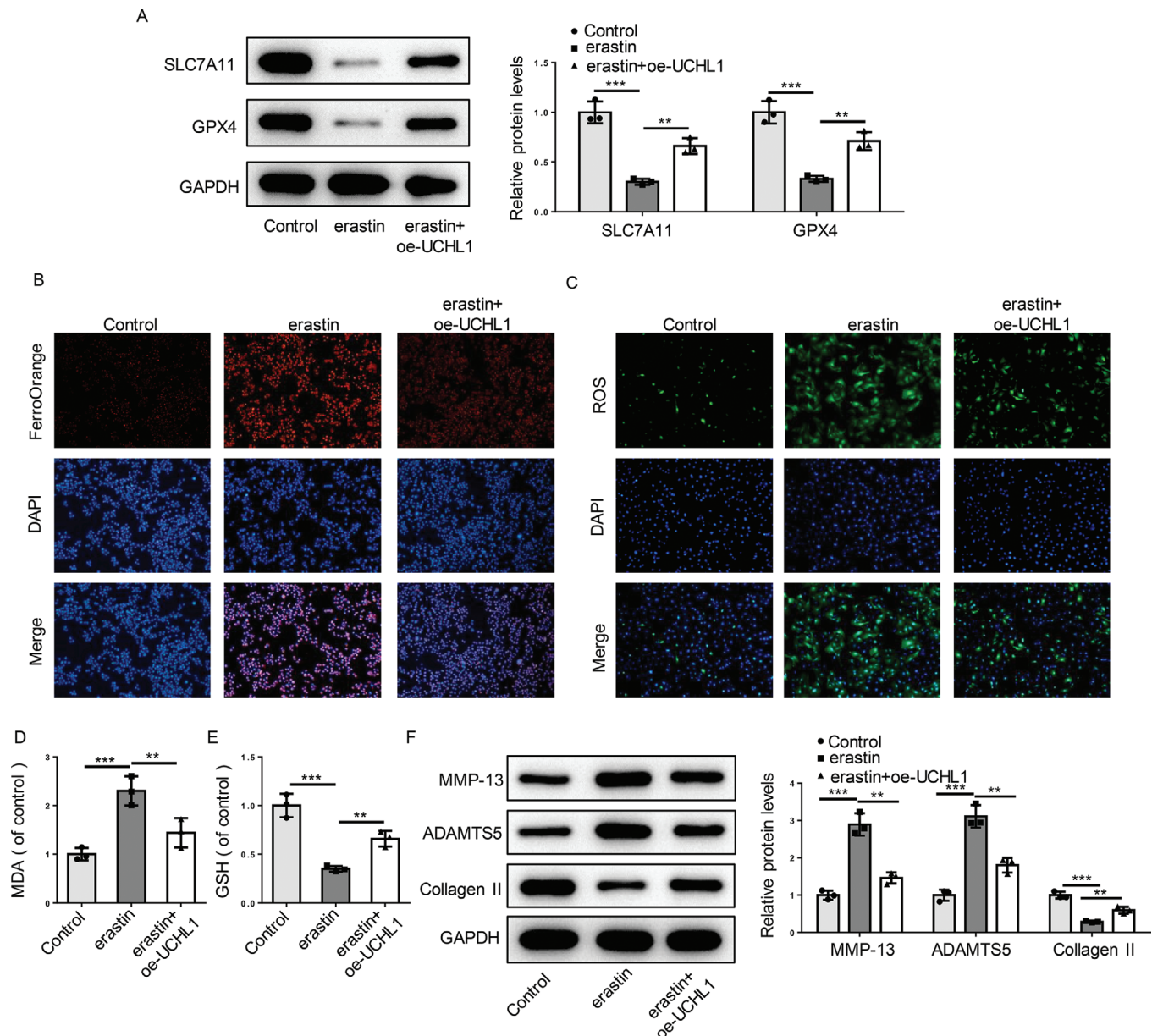


Fig. 3. Erastin mitigates the protective effect of UCHL1 on KOA chondrocytes. The cells were divided into control, erastin, and erastin+oe-UCHL1 groups. **A.** SLC7A11 and GPX4 protein levels were measured in chondrocytes. Intracellular Fe²⁺ levels (**B**) and ROS levels (**C**) were measured in the control, erastin, and erastin+oe-UCHL1 groups. **D.** MDA levels in chondrocytes were quantitatively determined using an MDA assay kit. **E.** GSH levels in chondrocytes were quantitatively determined using an GSH assay kit. **F.** MMP-13, ADAMTS5, and Collagen II protein levels were detected by western blot. * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

stimulated C28/I2 cells, the Bcl-2 protein level was reduced while the Bax protein level was increased, and these changes were reversed by UCHL1 overexpression (Fig. 2B). Furthermore, UCHL1 counteracted IL-1 β -induced ferroptosis by upregulating SLC7A11 and GPX4 protein levels (Fig. 1G) and reducing ferrous ion accumulation (Fig. 1H). UCHL1 also decreased ROS and MDA levels while increasing GSH levels in IL-1 β -treated chondrocytes (Fig. 1I-K). In terms of ECM degradation, IL-1 β increased MMP-13 and ADAMTS5 levels and decreased Collagen II expression, all of which were reversed by UCHL1 overexpression (Fig. 1L). These findings demonstrate that UCHL1 suppresses IL-1 β -induced apoptosis, ferroptosis, and ECM degradation in chondrocytes.

Erastin mitigates the protective effect of UCHL1 on KOA chondrocytes

Next, we explored whether UCHL1 alleviates ECM degradation in KOA chondrocytes by regulating chondrocyte ferroptosis. The cells were divided into control, erastin, and erastin+oe-UCHL1 groups. As shown in Fig. 3A, the protein levels of SLC7A11 and GPX4 were reduced in erastin-treated chondrocytes, and this effect was reversed by UCHL1. Ferrous ions (Fig. 3B), ROS (Fig. 3C), and MDA (Fig. 3D) levels were enhanced and the GSH (Fig. 2E) level was decreased in erastin-treated chondrocytes, which were counteracted by UCHL1. Besides, erastin treatment promoted ECM degradation in chondrocytes by increasing the protein

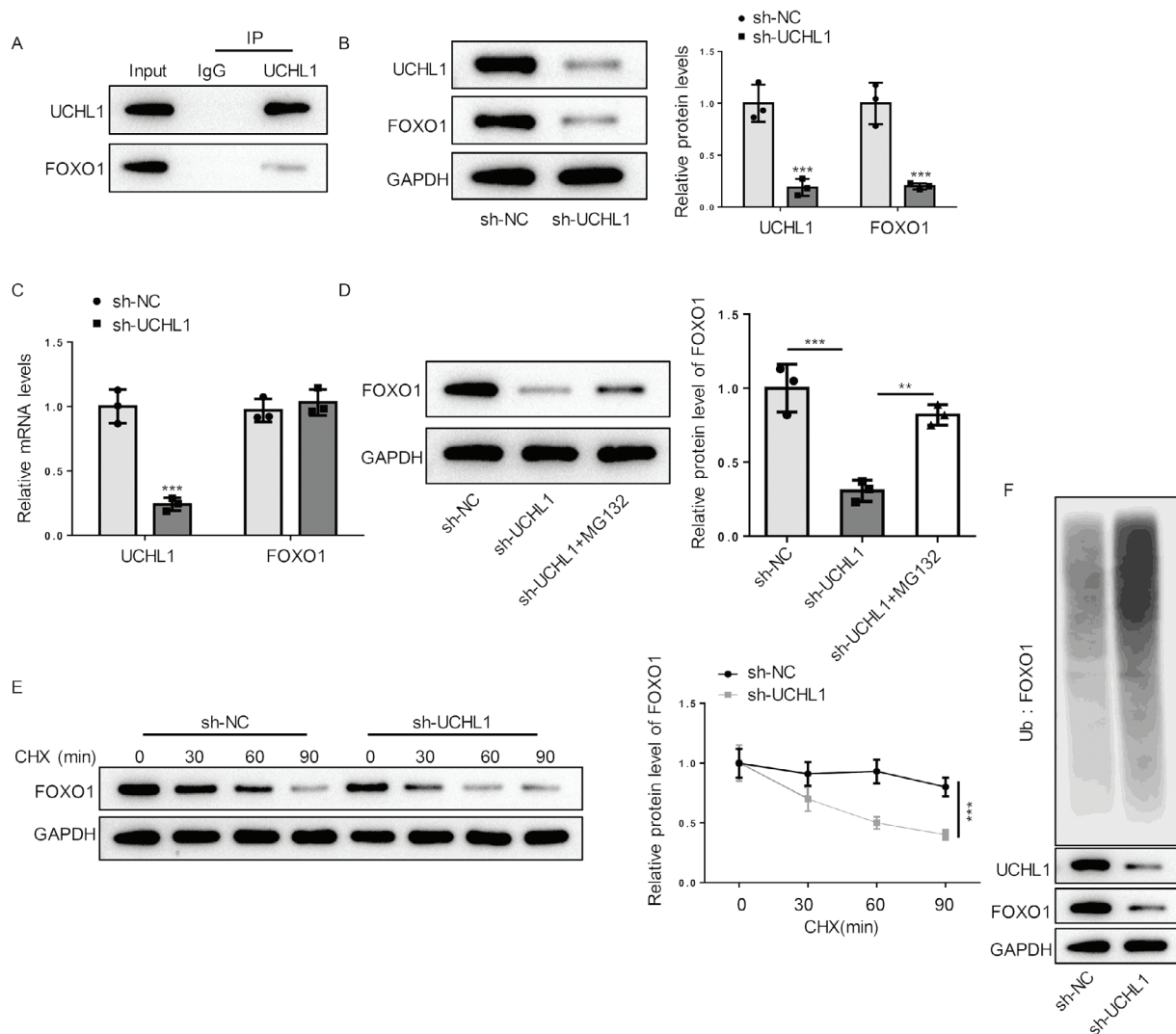


Fig. 4. UCHL1 facilitates the deubiquitination and stability of FOXO1 in chondrocytes. **A.** Co-IP assay demonstrated the interplay between the UCHL1 and FOXO1 proteins. **B, C.** The protein and mRNA levels of UCHL1 and FOXO1 were detected in chondrocytes transfected with sh-NC or sh-UCHL1. **D.** Chondrocytes were transfected with sh-NC, or sh-UCHL1, with or without MG132 treatment. The protein expression of FOXO1 was analyzed by western blot. **E.** The protein levels of UCHL1 and FOXO1 after treatment with CHX for 0, 30, 60, and 90 min were detected by western blotting. **F.** The ubiquitination level of FOXO1 after knockdown of UCHL1 was evaluated by a ubiquitination assay. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

UCHL1 in knee osteoarthritis

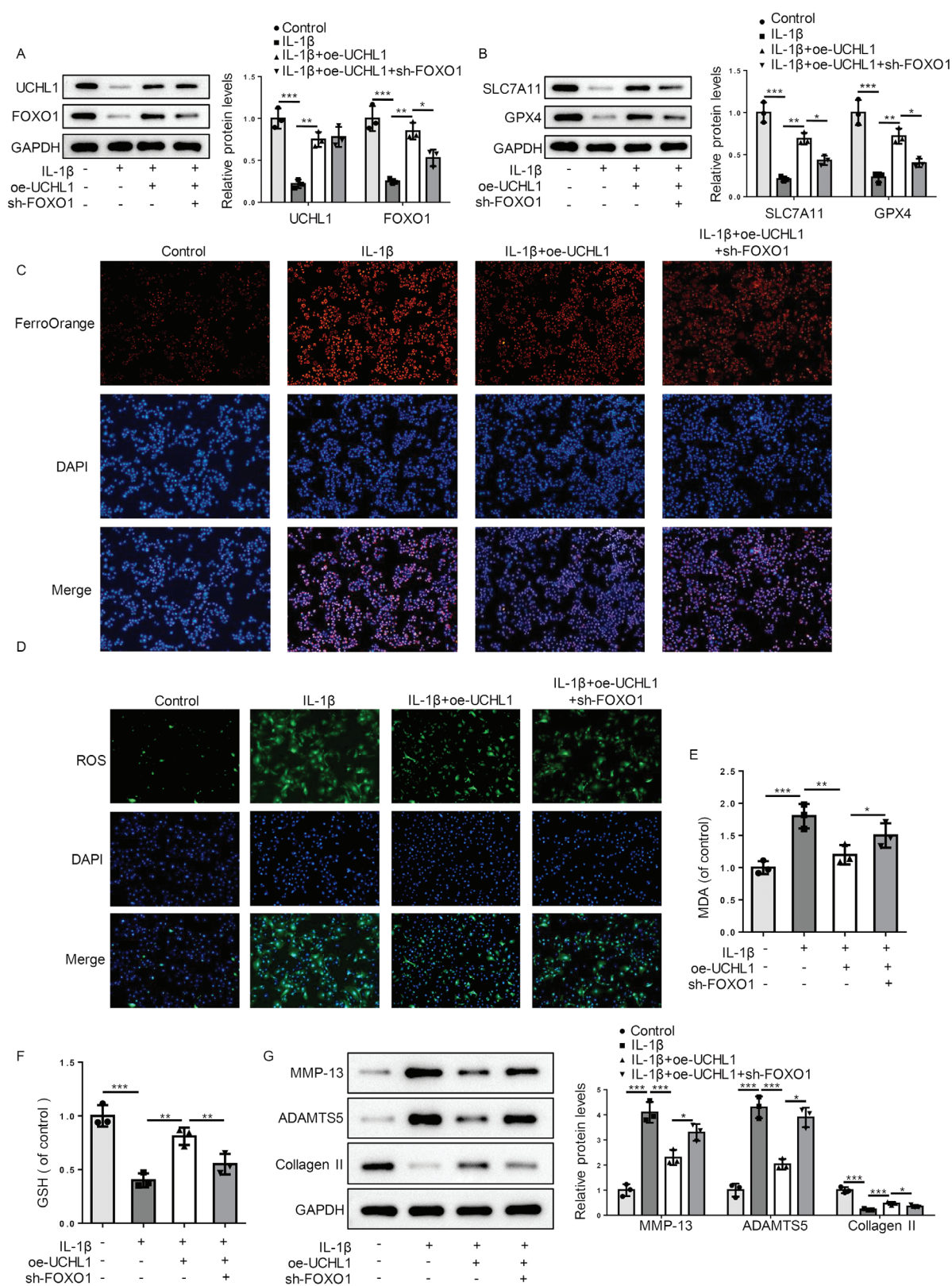


Fig. 5. Knockdown of FOXO1 antagonizes the effect of UCHL1 overexpression. The experiment was divided into the following groups: Control, IL-1 β , IL-1 β +oe-UCHL1, and IL-1 β +oe-UCHL1+sh-FOXO1. **A.** The protein levels of UCHL1 and FOXO1 were measured in chondrocytes after transfection of the above plasmid. **B.** SLC7A11 and GPX4 protein levels were measured by western blot. **C, D.** Results of incubation with the FerroOrange probe and DCFH-DA. **E, F.** MDA and GSH levels were measured using the MDA and GSH assay kits. **G.** MMP-13, ADAMTS5, and Collagen II protein levels were measured by western blot. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

levels of MMP-13 and ADAMTS5, while decreasing Collagen II expression. However, UCHL1 counteracted erastin-induced ECM degradation (Fig. 3F).

UCHL1 enhances the stability of FOXO1 in chondrocytes

Previous studies have shown that FOXO1 plays a protective role in osteoarthritis progression (Ohzono et al., 2023). Based on this, we hypothesized that UCHL1 might regulate KOA progression through FOXO1. Co-IP confirmed a direct interaction between UCHL1 and FOXO1 (Fig. 4A). Although UCHL1 deficiency

markedly reduced FOXO1 protein levels (Fig. 4B), it had no significant impact on FOXO1 mRNA expression (Fig. 4C). Next, we found that proteasome inhibitor MG132 reversed the decrease in FOXO1 protein levels caused by UCHL1 knockdown (Fig. 4D). Furthermore, UCHL1 depletion accelerated FOXO1 degradation in the presence of cycloheximide (CHX) (Fig. 4E). Ubiquitination assays revealed that *UCHL1* silencing significantly increased FOXO1 ubiquitination (Fig. 4F). Collectively, these findings demonstrate that UCHL1 interacts with FOXO1 and stabilizes it by inhibiting ubiquitination-mediated degradation.

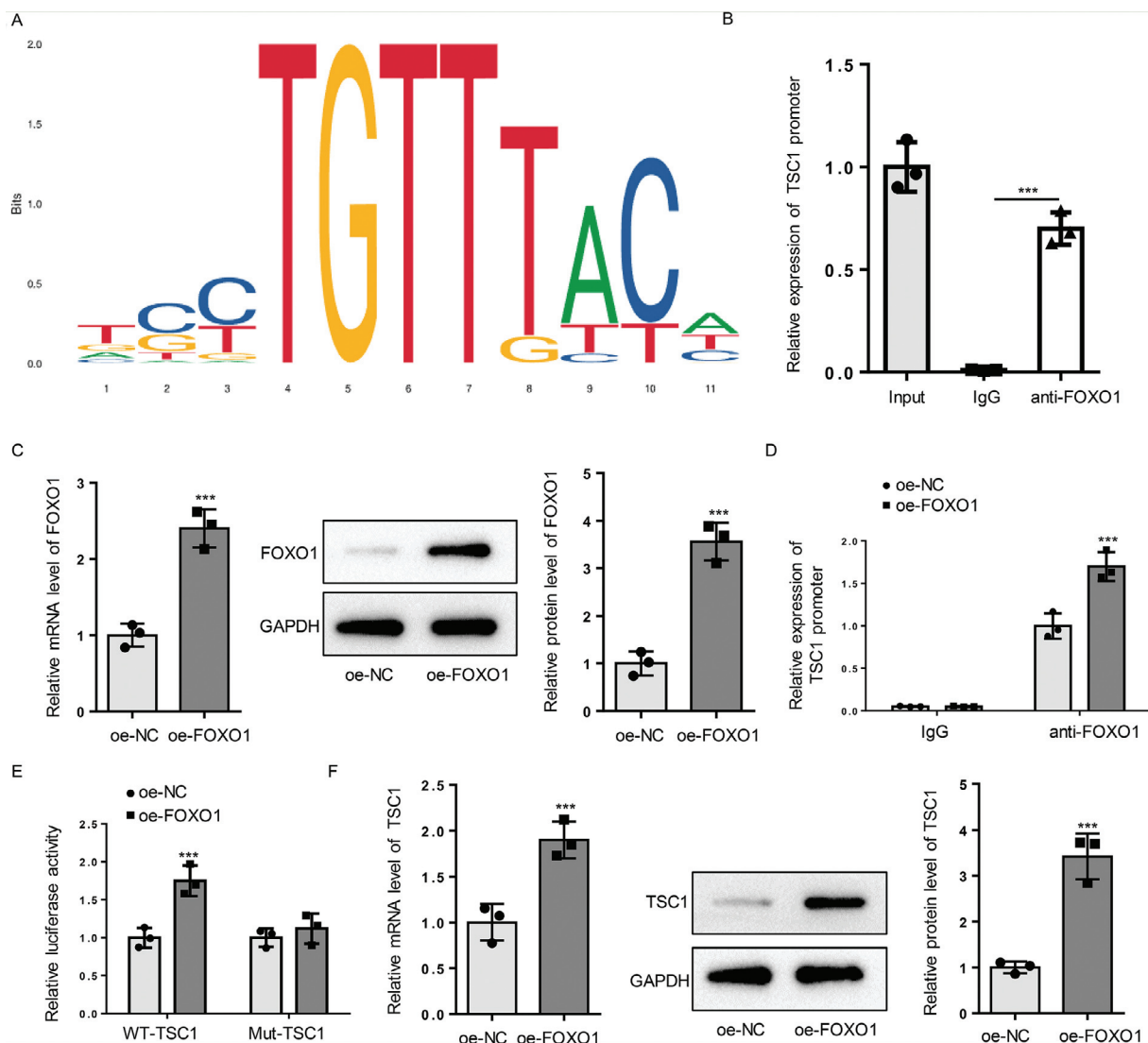


Fig. 6. The transcription factor FOXO1 binds to the *TSC1* promoter and accelerates *TSC1* transcriptional expression. **A.** The Jaspar database predicted the binding of FOXO1 to the *TSC1* promoter sequence. **B.** ChIP detection of the binding of FOXO1 to the *TSC1* promoter. **C.** RT-qPCR and western blot evaluation of FOXO1 mRNA and protein expression in chondrocytes transfected with oe-NC or oe-FOXO1. **D.** ChIP detection of enrichment of the *TSC1* promoter by FOXO1 antibodies in chondrocytes transfected with oe-NC or oe-FOXO1. **E.** Dual-luciferase reporter assay verification of the binding of FOXO1 to the *TSC1* promoter. **F.** RT-qPCR and western blot evaluation of *TSC1* expression in chondrocytes transfected with oe-NC or oe-FOXO1. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

UCHL1 in knee osteoarthritis

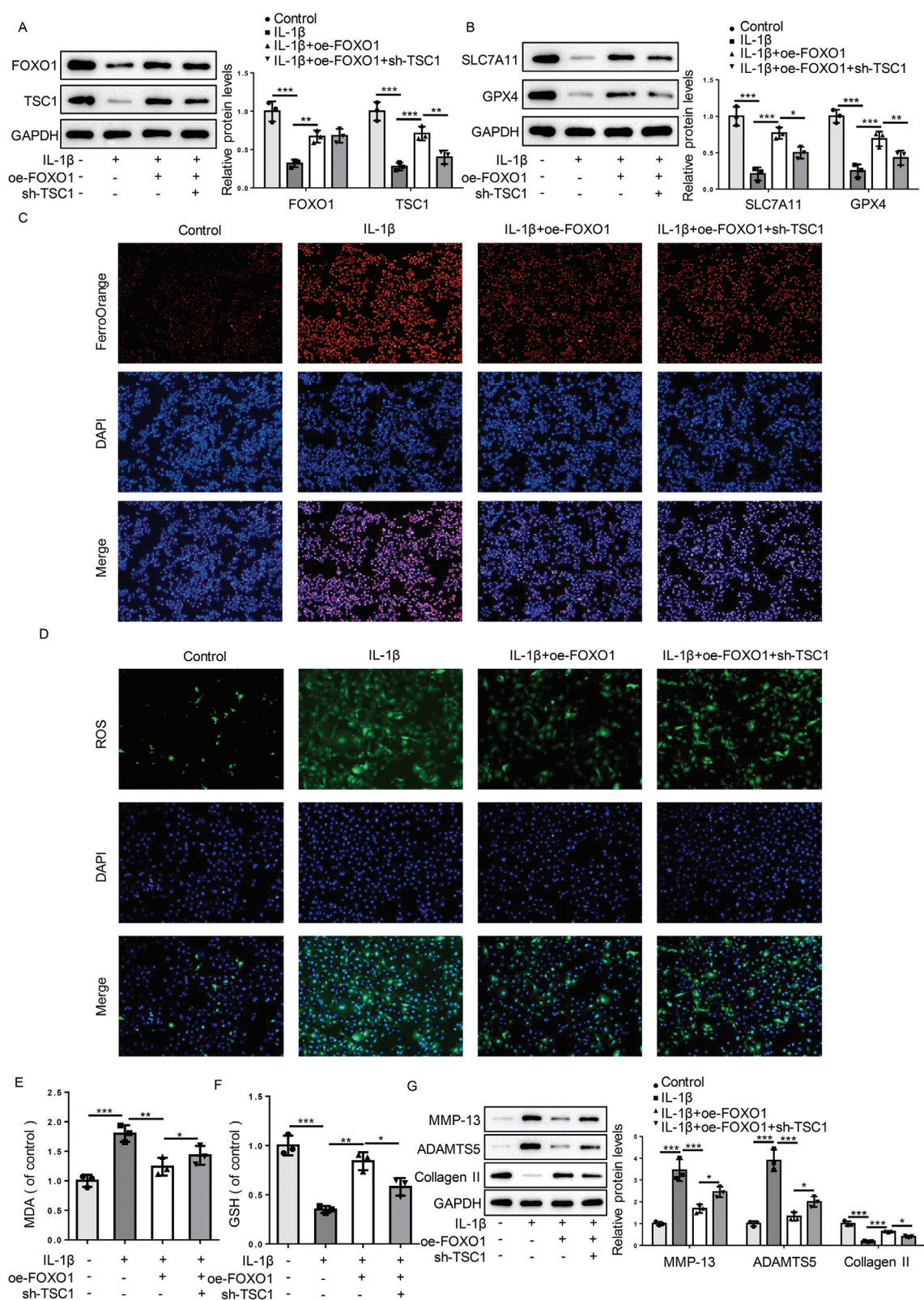


Fig. 7. TSC1 depletion partially neutralizes the suppression of ferroptosis and ECM degradation by FOXO1. The experiment was divided into the following groups: Control, IL-1 β , IL-1 β +oe-FOXO1, and IL-1 β +oe-FOXO1+sh-TSC1. **A.** FOXO1 and TSC1 protein levels were measured by western blot assay. **B.** SLC7A11 and GPX4 protein levels were measured by western blot. **C, D.** Results of incubation with the FerroOrange probe and DCFH-DA. **E, F.** MDA and GSH levels were assessed using MDA and GSH assay kits. **G.** MMP-13, ADAMTS5, and Collagen II protein levels were detected by western blot. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

UCHL1 in knee osteoarthritis

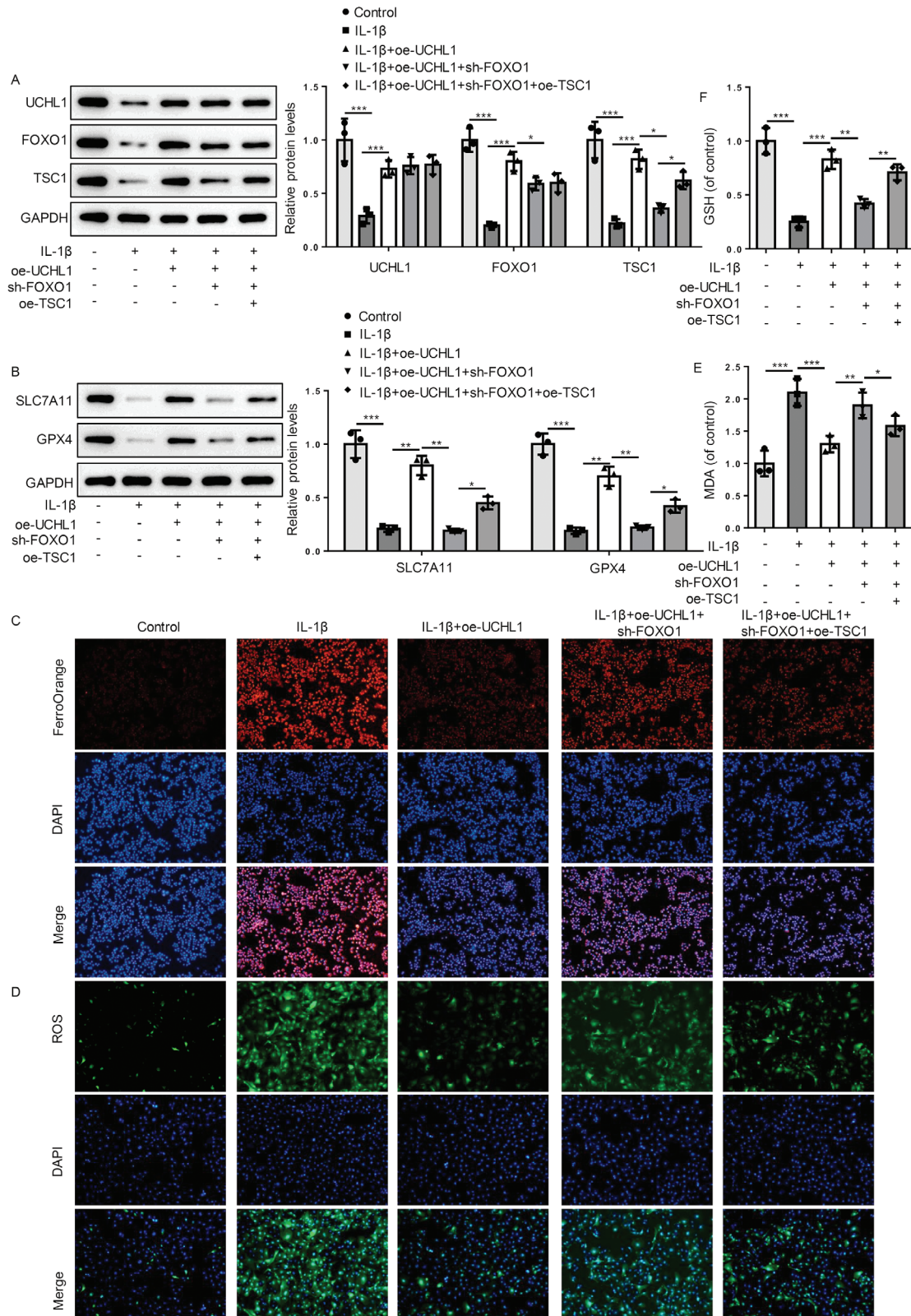


Fig. 8. UCHL1 inhibits ferroptosis and ECM degradation by regulating the FOXO1/TSC1 axis. The experiment was divided into the following groups: Control, IL-1β, IL-1β+oe-UCHL1, IL-1β+oe-UCHL1+sh-FOXO1, and IL-1β+oe-UCHL1+sh-FOXO1+oe-TSC1. **A.** UCHL1, FOXO1, and TSC1 protein levels were measured by western blot assay. **B.** SLC7A11 and GPX4 protein levels were assessed by western blot. **C, D.** Results of incubation with the FerroOrange probe and DCFH-DA. **E, F.** MDA and GSH levels were assessed using MDA and GSH assay kits. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

UCHL1 regulates KOA progression in vitro via FOXO1

To explore whether UCHL1 inhibits IL-1 β -induced ferroptosis and ECM degradation through FOXO1, IL-1 β -treated chondrocytes were transfected with oe-UCHL1 alone or in combination with sh-FOXO1. As shown in Fig. 5A,B, UCHL1 overexpression significantly upregulated FOXO1, SLC7A11, and GPX4 expression, which was markedly reversed by sh-FOXO1 transfection. Additionally, UCHL1 overexpression reduced ferrous ion levels (Fig. 5C), ROS (Fig. 5D), and MDA (Fig. 5E), while increasing GSH levels (Fig. 5F); however, these effects were partially counteracted by FOXO1 knockdown. Furthermore, FOXO1 knockdown reversed the inhibitory effect of UCHL1 on ECM degradation in IL-1 β -stimulated chondrocytes, as evidenced by increased MMP-13 and ADAMTS5 levels and decreased Collagen II expression (Fig. 5G). These results suggest that UCHL1 regulates KOA progression through FOXO1.

FOXO1 promotes the transcription activity of TSC1

Considering the transcriptional regulatory function of FOXO1, the potential FOXO1 binding sites within the TSC1 promoter region were predicted using the JASPAR database (Fig. 6A). ChIP assays indicated that FOXO1 was enriched in the promoter region of TSC1 (Fig. 6B). Chondrocytes were then transfected with oe-NC or oe-FOXO1 plasmids (Fig. 6C), and ChIP results showed a distinct increase in the TSC1 promoter pulled down by FOXO1 antibodies in the oe-FOXO1 group (Fig. 6D). As displayed in Figure 6E, FOXO1 overexpression significantly increased luciferase activity in the WT-TSC1 group, whereas no change in luciferase activity was observed in the Mut-TSC1 groups. In addition, RT-qPCR and western blot illustrated that TSC1 levels were strikingly enhanced in FOXO1-overexpressed chondrocytes (Fig. 6F). In conclusion, FOXO1 acts as a transcription factor of TSC1 and facilitates TSC1 transcription.

FOXO1 regulates ferroptosis and ECM degradation via TSC1

To examine whether FOXO1 modulates ferroptosis and ECM degradation by regulating TSC1, IL-1 β -induced chondrocytes were transfected with oe-FOXO1 with or without sh-TSC1 (Fig. 7A). Knockdown of TSC1 reversed the promotive effect of FOXO1 overexpression on SLC7A11 and GPX4 (Fig. 7B). Besides, FOXO1 decreased levels of ferrous ions (Fig. 7C), ROS (Fig. 7D), and MDA (Fig. 7E), and enhanced GSH levels (Fig. 7F), while TSC1 knockdown partially reversed these effects. In addition, the knockdown of TSC1 reversed FOXO1-induced repressive effect on ECM degradation in IL-1 β -irritated chondrocytes (Fig. 7G). The above results indicate that FOXO1 inhibits ferroptosis and ECM degradation via regulating TSC1 in IL-1 β -treated chondrocytes.

UCHL1 inhibits ferroptosis and ECM degradation via the FOXO1/TSC1 axis

To further verify whether UCHL1 relieved IL-1 β -induced chondrocyte injury by promoting TSC1 transcription through increasing FOXO1 stability, chondrocytes were divided into different groups. Consistent with the above results, UCHL1 overexpression significantly suppressed ferroptosis and ECM degradation. FOXO1 silencing partially reversed these protective effects, while TSC1 upregulation restored the protective effects of UCHL1 (Figs. 8A-F, 9). These data suggest that UCHL1 suppresses ferroptosis and ECM degradation via the FOXO1/TSC1 axis.

UCHL1 delays KOA progression in vivo

To further verify the effect of UCHL1 on KOA, a rat model of KOA was established. As demonstrated in Figure 10A, UCHL1, FOXO1, and TSC1 protein levels were decreased in the MIA-induced rat model, which were reversed by UCHL1. According to the results of

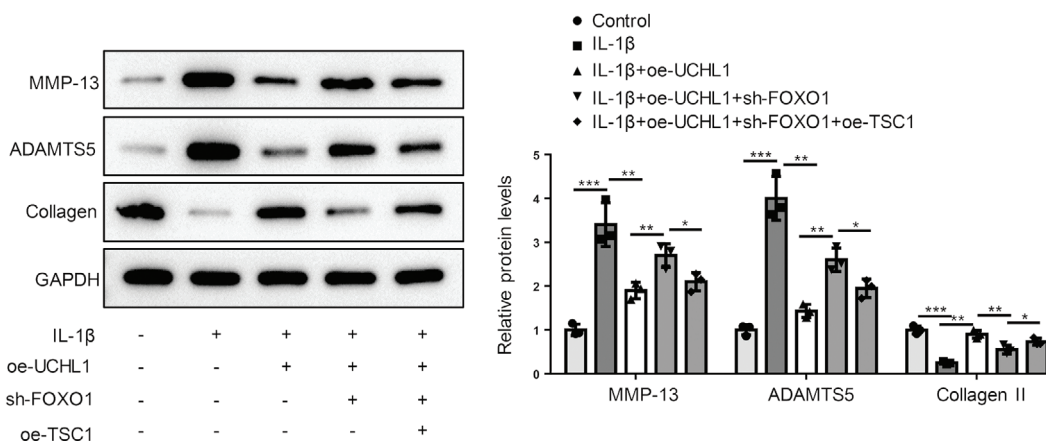


Fig. 9. UCHL1 inhibits ECM degradation by regulating the FOXO1/TSC1 axis. The experiment was divided into the following groups: Control, IL-1 β , IL-1 β +oe-UCHL1, IL-1 β +oe-UCHL1+sh-FOXO1, and IL-1 β +oe-UCHL1+sh-FOXO1+oe-TSC1. MMP-13, ADAMTS5, and Collagen II protein levels were detected by western blot. * p <0.05; ** p <0.01; *** p <0.001.

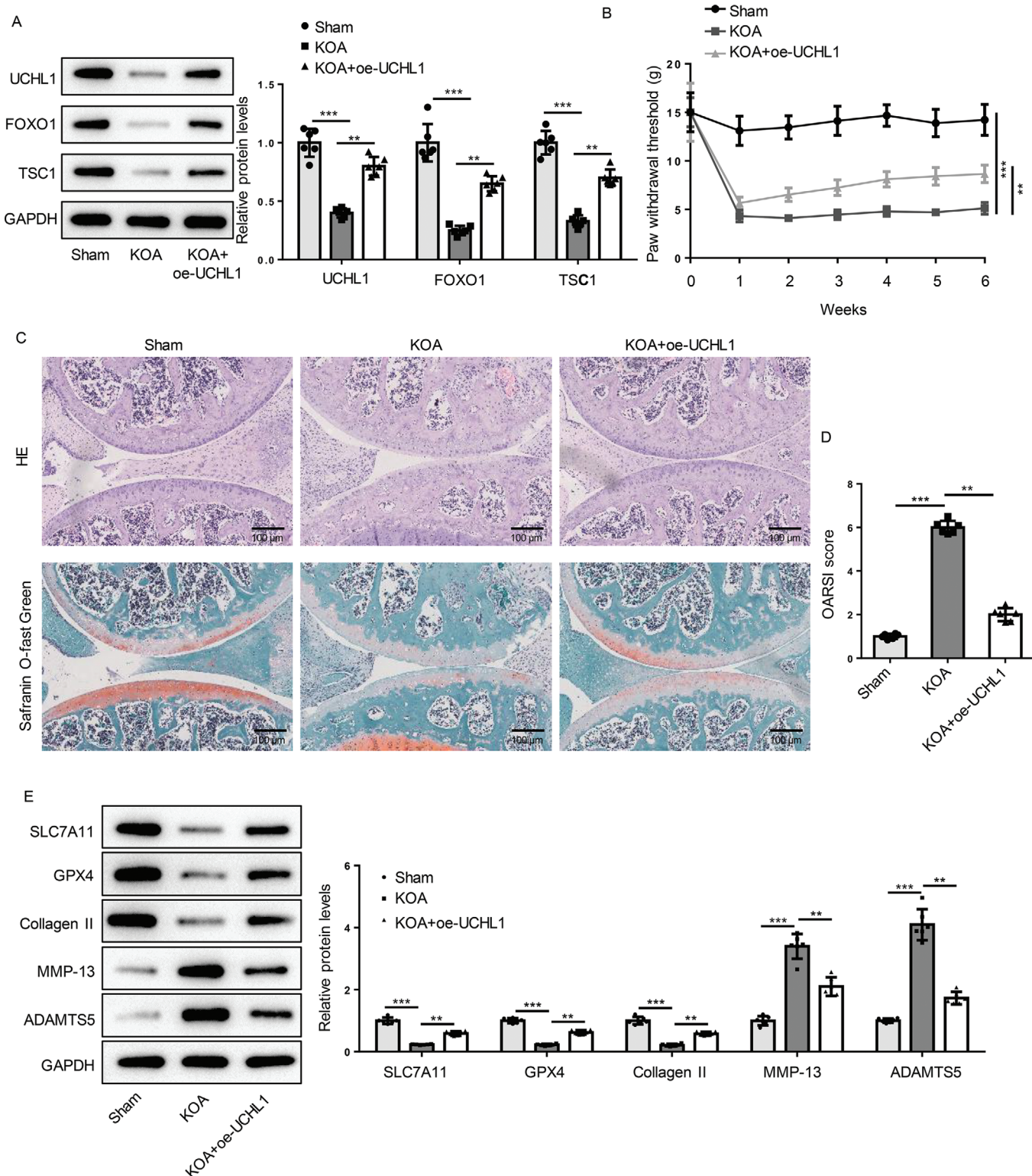


Fig. 10. UCHL1 delays KOA progression in rat KOA models. The experiment was divided into the following groups: Sham, KOA, KOA+oe-UCHL1. **A.** UCHL1, FOXO1, and TSC1 protein levels were measured by western blot assay. **B.** PWT of rats at different times. **C.** HE and Safranin O-Fast Green staining were utilized to observe pathological changes in the joint cartilage. Scale bar: 100 μ m. **D.** The OARSI score of cartilage tissues was analyzed. **E.** SLC7A11, GPX4, Collagen II, MMP-13, and ADAMTS5 protein levels were measured by western blot assay. (n=6). * p <0.05; ** p <0.01; *** p <0.001.

Figure 10B, the PWT was significantly improved in KOA rats following AAV-UCHL1 injection compared with the untreated KOA group. HE and Safranin O-Fast Green staining illustrated that the articular cartilage in control knees was smooth, with evenly distributed cartilage cells. In contrast, the KOA model group exhibited irregular contours, roughness, wear, and deterioration of the articular cartilage, which could be mitigated by UCHL1 overexpression (Fig. 10C). Additionally, the elevated OARSI score in KOA rats was significantly reduced by UCHL1 overexpression (Fig. 10D). Furthermore, in KOA rats, the protein levels of SLC7A11, GPX4, and Collagen II were decreased, while the levels of MMP-13 and ADAMTS5 were enhanced. These changes were reversed by UCHL1 overexpression (Fig. 10E). In sum, UCHL1 overexpression alleviates cartilage damage in KOA rats by inhibiting ferroptosis and ECM degradation through the FOXO1/TSC1 axis.

Discussion

KOA is associated with various biological processes, including oxidative stress, inflammatory factors, apoptosis, and ferroptosis, which collectively contribute to cartilage degradation and joint dysfunction; research focusing on these biological mechanisms has been steadily increasing (Guan et al., 2022; Liu et al., 2022). For example, MnL4, a potent Superoxide dismutase (SOD) mimetic, can reduce pain, joint disorders, plasma TNF- α levels, and protein carbonylation in patients with osteoarthritis, which is highly beneficial for osteoarthritic disease (Di Cesare Mannelli et al., 2013). Baicalein could inhibit inflammation, chondrocyte apoptosis, and cartilage degradation in OA (Zhang et al., 2014). In addition, emerging evidence indicates that chondrocyte ferroptosis-induced cartilage degeneration is a critical factor in the development of OA (Ma et al., 2024). Therefore, targeting ECM degradation and chondrocyte ferroptosis may provide new ideas for treating KOA.

Posttranslational modification (PTM) is a crucial regulatory mechanism for proteins and plays a significant role in the onset and progression of degenerative diseases (Santiago-Mujika et al., 2022). Ubiquitination, an essential PTM, is a multistep enzymatic process that participates in numerous cellular biological activities (Sun et al., 2020). For instance, UCH37 was implicated in ADRM1-mediated ALK5 expression and OA progression by upregulating ALK5 deubiquitination (Zhu et al., 2023). Like other DUBs, UCHL1 performs its biological function by stabilizing and deubiquitinating downstream target proteins. For instance, Geng et al. elaborated that UCHL1 protects against ischemic heart injury by deubiquitinating HIF-1 α (Geng et al., 2022). Guo et al. revealed that UCHL1 promotes hippocampal dependent memory through deubiquitination of TrkB (Guo et al., 2017). However, whether UCHL1 influences KOA progression by regulating target proteins through deubiquitination is

unknown. Yan et al. demonstrated that UCHL1 has an alleviating effect on chondrocyte apoptosis (Yan et al., 2023). Moreover, Feng et al. confirmed that UCHL1 depletion facilitates bone loss under pathological conditions by accelerating osteoclast formation (Feng et al., 2023). Thus, we suspected that UCHL1 may play a vital role in the progression of KOA. Herein, we observed that UCHL1 was lowly expressed in MIA-induced KOA rats. Overexpression of UCHL1 restrained ferroptosis and ECM degradation both *in vivo* and *in vitro*. FOXO1 has been identified to prevent osteoarthritis by maintaining articular cartilage homeostasis (Wang et al., 2020). We found that *FOXO1* knockdown partially neutralized the suppression of ferroptosis and ECM degradation by UCHL1 overexpression in IL-1 β -triggered chondrocytes. These results demonstrated that FOXO1 was stabilized by UCHL1 through deubiquitination.

As a tumor suppressor gene, *TSC1* is implicated in the progression of many cancers (Yamada et al., 2014; Huang et al., 2022). In recent years, the functional role of *TSC1* in bone metabolic diseases has been gradually recognized. For example, Xu et al. uncovered that targeting *TSC1* restrains bone resorption and prevents bone lost-related diseases (Xu et al., 2018). Given that FOXO1 is a transcription factor regulating multifarious important biological functions, we used the JASPAR database to predict that the promoter of *TSC1* contains FOXO1 binding sites. We further demonstrated that FOXO1, as a transcription factor of *TSC1*, promotes *TSC1* transcription through ChIP, western blot, and dual-luciferase reporter assays. Moreover, *TSC1* knockdown partially reversed FOXO1-mediated inhibition of ferroptosis and ECM degradation in IL-1 β -stimulated chondrocytes. Furthermore, we demonstrated that UCHL1 relieves IL-1 β -induced chondrocyte injury by promoting *TSC1* transcription through increasing FOXO1 stability. These results demonstrated that UCHL1 inhibits ferroptosis and ECM degradation by regulating the FOXO1/TSC1 axis.

Conclusion

Our study demonstrated that UCHL1 suppressed ferroptosis and ECM degradation in KOA by deubiquitinating and stabilizing FOXO1, which subsequently upregulated *TSC1* expression, ultimately leading to chondroprotection. Our findings underscore the crucial role of the interactions between UCHL1, FOXO1, and *TSC1* in KOA pathogenesis, highlighting their potential as promising therapeutic targets.

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Patient consent for publication. Not applicable.

Availability of data and materials. The datasets used and/or analyzed

during the current study are available from the corresponding author upon reasonable request.

Author's contributions. YW and JL designed this study. CG and ZX performed all the experiments, analyzed the data, and prepared the figures. ZX and BG drafted the initial manuscript. TJ reviewed and revised the manuscript. All authors read and approved the final manuscript.

References

- Bijlsma J.W., Berenbaum F. and Lafeber F.P.J.G. (2011). Osteoarthritis: An update with relevance for clinical practice. *Lancet* 377, 2115-2126.
- Dixon S.J., Lemberg K.M., Lamprecht M.R., Skouta R., Zaitsev E.M., Gleason C.E., Patel D.N., Bauer A.J., Cantley A.M., Yang W.S., Morrison B. 3rd. and Stockwell B.R. (2012). Ferroptosis: An iron-dependent form of nonapoptotic cell death. *Cell* 149, 1060-1072.
- Di Cesare Mannelli L., Bani D., Bencini A., Brandi M.L., Calosi L., Cantore M., Carossino A.M., Ghelardini C., Valtancoli B. and Failli P. (2013). Therapeutic effects of the superoxide dismutase mimetic compound MnIIIme2DO2A on experimental articular pain in rats. *Mediators Inflamm.* 2013, 905360.
- Feng Z., Tao S., Huang Z., Zheng B., Kong X., Xiang Y., Zhang Q., Song H., Xu Z., Wei X., Zhao F. and Chen J. (2023). The deubiquitinase UCHL1 negatively controls osteoclastogenesis by regulating TAZ/NFATC1 signalling. *Int. J. Biol. Sci.* 19, 2319.
- Gao H., Freeling J., Wu P., Liang A.P., Wang X. and Li Y. (2019). UCHL1 regulates muscle fibers and mTORC1 activity in skeletal muscle. *Life Sci.* 233, 116699.
- Gao H., Antony R., Srinivasan R., Wu P., Wang X. and Li Y. (2020). UCHL1 regulates oxidative activity in skeletal muscle. *PLoS One* 15, e0241716.
- Geng B., Wang X., Park K.H., Lee K.E., Kim J., Chen P., Zhou X., Tan T., Yang C., Zou X., Janssen P.M., Cao L., Ye L., Wang X., Cai C. and Zhu H. (2022). UCHL1 protects against ischemic heart injury via activating HIF-1 α signal pathway. *Redox Biol.* 52, 102295.
- Gu Y., Huang J., Guo H., Song X., Li J., Shi Y. and Xie X. (2020). A randomized controlled study for Yuanhu Zhitong dropping pills in the treatment of knee osteoarthritis. *Medicine* 99, e20666.
- Guan T., Ding L.-G., Lu B.-Y., Guo J.-Y., Wu M.-Y., Tan Z.-Q. and Hou S.-Z. (2022). Combined administration of curcumin and chondroitin sulfate alleviates cartilage injury and inflammation via NF- κ B pathway in knee osteoarthritis rats. *Front. Pharmacol.* 13, 882304.
- Guo Y.-Y., Lu Y., Zheng Y., Chen X.-R., Dong J.-L., Yuan R.-R., Huang S.-H., Yu H., Wang Y., Chen Z.-Y. and Su B. (2017). Ubiquitin C-terminal hydrolase L1 (UCHL1) promotes hippocampus-dependent memory via its deubiquitinating effect on TrkB. *J. Neurosci.* 37, 5978-5995.
- Haq S., Suresh B. and Ramakrishna S. (2018). Deubiquitylating enzymes as cancer stem cell therapeutics. *Biochim. Biophys. Acta Rev. Cancer* 1869, 1-10.
- Hu Z., Chen L., Zhao J., Zhang W., Jin Z., Sun Y., Li Z., Chang B., Shen P. and Yang Y. (2024). Lipoxin A4 ameliorates knee osteoarthritis progression in rats by antagonizing ferroptosis through activation of the ESR2/LPAR3/Nrf2 axis in synovial fibroblast-like synoviocytes. *Redox Biol.* 73, 103143.
- Huang Q., Li F., Hu H., Fang Z., Gao Z., Xia G., Ng W.-L., Khodadadi-Jamayran A., Chen T., Deng J., Zhang H., Almonte C., Labbe K., Han H., Geng K., Tang S., Freeman G.J., Li Y., Chen H. and Wong K.-K. (2022). Loss of *TSC1/TSC2* sensitizes immune checkpoint blockade in non-small cell lung cancer. *Sci. Adv.* 8, eabi9533.
- Kurakazu I., Akasaki Y., Hayashida M., Tsushima H., Goto N., Sueishi T., Toya M., Kuwahara M., Okazaki K., Duffy T., Lotz M.K. and Nakashima Y. (2019). FOXO1 transcription factor regulates chondrogenic differentiation through transforming growth factor β 1 signaling. *J. Biol. Chem.* 294, 17555-17569.
- Lee K.I., Choi S., Matsuzaki T., Alvarez-Garcia O., Olmer M., Grogan S.P., D'Lima D.D. and Lotz M.K. (2020). FOXO1 and FOXO3 transcription factors have unique functions in meniscus development and homeostasis during aging and osteoarthritis. *Proc. Natl. Acad. Sci. USA* 117, 3135-3143.
- Li C., Zhu C., Song K., Xiang X., Wang X., Hu J., Li Z., Deng Y., Jiang D., Guo L., Ren T., Zhu L., Ai H., Zhang S., Liu Z., Yang Y., Zhu S. and He C. (2025). An evidence-based tailored eHealth patient education tool for patients with knee osteoarthritis: A randomized controlled trial. *Digit. Health* 11, 20552076251317230.
- Lino V.T., Marinho D.S., Rodrigues N.C.P. and Andrade C.A.F. (2024). Efficacy and safety of ozone therapy for knee osteoarthritis: An umbrella review of systematic reviews. *Front. Physiol.* 15, 1348028.
- Liu F., Yang H., Li D.-Z., Wu X. and Han Q.-C. (2021). Punicalagin attenuates osteoarthritis progression via regulating Foxo1/Prg4/HIF3 α axis. *Bone* 152, 116070.
- Liu L., Luo P., Yang M., Wang J., Hou W. and Xu P. (2022). The role of oxidative stress in the development of knee osteoarthritis: A comprehensive research review. *Front. Mol. Biosci.* 9, 1001212.
- Ma J., Yu P., Ma S., Li J., Wang Z., Hu K., Su X., Zhang B., Cheng S. and Wang S. (2024). Bioinformatics and integrative experimental method to identifying and validating co-expressed ferroptosis-related genes in OA articular cartilage and synovium. *J. Inflamm. Res.* 12, 957-980.
- Matsuzaki T., Alvarez-Garcia O., Mokuda S., Nagira K., Olmer M., Gamini R., Miyata K., Akasaki Y., Su A.I., Asahara H. and Lotz M.K. (2018). FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. *Sci. Transl. Med.* 10, eaan0746.
- Mochizuki M., Okumo T., Takemura H., Izukashi K., Tatsuo T., Ikemoto H., Adachi N., Kawate N. and Sunagawa M. (2024). Suppressive activity of boiogito, a japanese traditional kampo medicine, on periostin secretion in human fibroblast-like synoviocytes *in vitro*. *Cureus* 16, e57690.
- Ohzono H., Hu Y., Nagira K., Kanaya H., Okubo N., Olmer M., Gotoh M., Kurakazu I., Akasaki Y., Kawata M., Chen E., Chu A.C., Johnson K.A. and Lotz M.K. (2023). Targeting FoxO transcription factors with HDAC inhibitors for the treatment of osteoarthritis. *Ann. Rheum. Dis.* 82, 262-271.
- Santiago-Mujika E., Luthi-Carter R., Giorgini F. and Mukaetova-Ladinska E.B. (2022). Tubulin isotypes and posttranslational modifications in vascular dementia and alzheimer's disease. *J. Alzheimers Dis. Rep.* 6, 739-748.
- Shi S., Chu G., Zhang L., Yuan H., Madaniyat M., Zhou X., Wang L., Cai C., Pang W., Gao L. and Yang G. (2023). Deubiquitinase UCHL1 regulates estradiol synthesis by stabilizing voltage-dependent anion channel 2. *J. Biol. Chem.* 299, 105316.
- Shim S., Kwon Y.-B., Yoshikawa Y. and Kwon J. (2008). Ubiquitin C-terminal hydrolase L1 deficiency decreases bone mineralization. *J. Vet. Med. Sci.* 70, 649-651.
- Stockwell B.R., Angeli J.P.F., Bayir H., Bush A.I., Conrad M., Dixon S.J.,

- Fulda S., Gascón S., Hatzios S.K., Kagan V.E., Noel K., Jiang X., Linkermann A., Murphy M.E., Overholtzer M., Oyagi A., Pagnussat G.C., Park J., Ran Q., Rosenfeld C.S., Salnikow K., Tang D., Torti F.M., Torti S.V., Toyokuni S., Woerpel K.A. and Zhang D.D. (2017). Ferroptosis: A regulated cell death nexus linking metabolism, redox biology, and disease. *Cell* 171, 273-285.
- Sun T., Liu Z. and Yang Q. (2020). The role of ubiquitination and deubiquitination in cancer metabolism. *Mol. Cancer* 19, 146.
- Tang D., Chen X., Kang R. and Kroemer G. (2021). Ferroptosis: Molecular mechanisms and health implications. *Cell Res.* 31, 107-125.
- Wang C., Shen J., Ying J., Xiao D. and O'Keefe R. (2020). FoxO1 is a crucial mediator of TGF- β /TAK1 signaling and protects against osteoarthritis by maintaining articular cartilage homeostasis. *Proc. Natl. Acad. Sci. USA* 117, 30488-30497.
- Xu S., Zhang Y., Wang J., Li K., Tan K., Liang K., Shen J., Cai D., Jin D., Li M., Xiao G., Xu J., Jiang Y. and Bai X. (2018). TSC1 regulates osteoclast podosome organization and bone resorption through mTORC1 and Rac1/Cdc42. *Cell Death Differ.* 25, 1549-1566.
- Yamada D., Hoshii T., Tanaka S., Hegazy A.M., Kobayashi M., Tadokoro Y., Ohta K., Ueno M., Ali M.A.E. and Hirao A. (2014). Loss of Tsc1 accelerates malignant gliomagenesis when combined with oncogenic signals. *J. Biochem.* 155, 227-233.
- Yan Q., Shi S., Ge Y., Wan S., Li M. and Li M. (2023). UCHL1 alleviates apoptosis in chondrocytes via upregulation of HIF-1 α -mediated mitophagy. *Int. J. Mol. Med.* 52, 1-13.
- Ye Y. and Liu A. (2023). The effectiveness of tai chi for knee osteoarthritis: An overview of systematic reviews. *Int. J. Gen. Med.* 16, 4499-4514.
- Yuan H., Yi N., Li D., Xu C., Yin G.-R., Zhuang C., Wang Y.-J. and Ni S. (2024). PPAR γ regulates osteoarthritis chondrocytes apoptosis through caspase-3 dependent mitochondrial pathway. *Sci. Rep.* 14, 11237.
- Zhang X., Zhu Y., Chen X., Zhang Y., Zhang Y., Jia Y., Wang H., Liu Y. and Xiao L. (2014). Baicalein ameliorates inflammatory-related apoptotic and catabolic phenotypes in human chondrocytes. *Int. Immunopharmacol.* 21, 301-308.
- Zhang S., Xu J., Si H., Wu Y., Zhou S. and Shen B. (2022). The role played by ferroptosis in osteoarthritis: Evidence based on iron dyshomeostasis and lipid peroxidation. *Antioxidants* 11, 1668.
- Zhu M., Cao Z., Mo F., Shi S., Hu J., Xu Q., Quan K., Liang J., Li W., Hong X., Zhang B., Liu X. and Dai M. (2023). ADRM1/RPN13 attenuates cartilage extracellular matrix degradation via enhancing UCH37-mediated ALK5 deubiquitination. *Int. J. Biol. Macromol.* 247, 125670.

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