

Oxysophoridine Promotes Osteoarthritis Repair via GSH System Activation and ROS Suppression

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Abstract

Introduction: Osteoarthritis (OA) is a prevalent joint degenerative disease involving inflammation and oxidative stress, with reactive oxygen species (ROS) driving progression. Restoring joint redox balance mitigates cartilage damage. Osilyhizidine (OSR), from *Sophora alopecuroides*, has anti-inflammatory/antioxidant properties, but its OA-specific effects and mechanisms were unclear.

Material and Methods: In vitro experiments assessed OSR's impact on OA chondrocyte proliferation, repair, and inflammation, focusing on Glutathione peroxidase 4 (GPX4) and solute carrier family 7 member 11 (SLC7A11) regulation. A murine OA model validated findings in vivo.

Results: OSR showed anti-inflammatory, antioxidant effects and promoted cartilage repair, enhancing chondrocyte functions under inflammation and suppressing pro-inflammation. It upregulated GPX4 (improving ROS detoxification) and SLC7A11 (facilitating glutathione synthesis for redox balance) at transcriptional and protein levels. These were confirmed in mice.

Conclusions: OSR alleviates OA by activating GPX4/SLC7A11 to regulate ROS and oxidative stress, emerging as a promising OA therapeutic candidate, offering insights into redox-targeted interventions.

1. Introduction

Osteoarthritis (OA) develops as a degenerative musculoskeletal disorder in which articular cartilage gradually deteriorates while abnormal ossification occurs within the joint structures^{1–3}. Osteoarthritis represents the leading form of joint pathology across the globe, predominantly occurring in the ageing population⁴. World Health Organization (WHO) estimates indicate that among individuals older than 60 years, osteoarthritis prevalence reaches approximately 10% in men and 18% in women. Driven by demographic aging and the expanding prevalence of obesity, the burden of osteoarthritis has shown a continuous upward trend, emerging as an important issue within global public health⁵. Currently, osteoarthritis treatment primarily focuses on symptom relief and slowing disease progression, as no cure exists. Current therapeutic approaches to osteoarthritis integrate medication-based regimens (notably nonsteroidal anti-inflammatory agents), physiotherapy, lifestyle optimization, and various surgical techniques. However, these methods have limitations: pharmacological treatments mainly target pain relief with limited therapeutic efficacy; physical therapy and lifestyle changes have modest effects and are unlikely to reverse the disease; and surgical treatments carry higher risks, are expensive, and require lengthy recovery periods⁶. In summary, osteoarthritis is a complex and widespread chronic disease, highlighting the urgent need to develop novel, safe, and highly effective therapeutic drugs⁷.

Persistent inflammation is a key driver of articular cartilage degeneration and a hallmark of OA⁸. Among inflammatory cytokines, IL-1 β (Interleukin-1 β) is one of the most extensively studied in OA. Exposure of chondrocytes to IL-1 β results in pronounced up-regulation of matrix metalloproteinases—particularly MMP-1, MMP-3, and MMP-13—which catalyze the degradation of collagen and proteoglycans within the cartilage extracellular matrix, ultimately compromising its structural integrity. Concurrently, IL-1 β exposure diminishes the production of type II collagen and proteoglycans in chondrocytes, consequently aggravating the degenerative loss of cartilage matrix⁹. The interaction between OA and inflammatory cytokines is complex. These cytokines promote cartilage degradation, synovial inflammation, abnormal bone remodeling, and neuropathic sensitization, collectively driving the initiation, progression, and pain associated with OA¹⁰. Targeted anti-inflammatory therapies represent a critical direction in OA treatment. Ongoing and future studies should aim to delineate the detailed inflammatory pathways involved in OA, providing a foundation for the development of safer, more efficacious anti-inflammatory therapeutics and consequently improving patients' overall quality of life¹¹.

An imbalance between the generation of reactive oxygen species (ROS) and the neutralizing efficacy of endogenous antioxidants defines oxidative stress, resulting in oxidative modification of biomolecules and subsequent tissue impairment¹². In OA, oxidative stress is identified as a key pathological mechanism. Excessive ROS directly damages chondrocytes, leading to apoptosis or functional abnormalities. By activating pro-apoptotic signaling pathways (e.g., caspase, p53), ROS induces chondrocyte death, diminishing cell survival and repair capacity. ROS concurrently trigger intracellular signaling networks, including the NF- κ B pathway, thereby enhancing matrix metalloproteinase (MMP) expression and accelerating degradation of the cartilage matrix. ROS further compromise the biochemical and structural stability of the extracellular matrix by inducing oxidative alterations in collagen and proteoglycans¹³. Notably, a positive feedback loop exists between ROS and inflammatory cytokines. A reciprocal relationship exists between ROS and pro-inflammatory mediators, creating a continuous amplification cycle in which ROS provoke cytokine secretion—including IL-1 β and TNF- α —thereby stimulating additional ROS formation and driving progressive inflammation and joint matrix degeneration. Consequently, therapeutic strategies targeting oxidative stress—such as antioxidant therapies or the development of ROS scavengers—may provide novel approaches for treating osteoarthritis.

Oxysophoridine (OSR), an alkaloid obtained from *Sophora alopecuroides*, demonstrates significant pharmacological activities, notably anti-inflammatory actions, regulation of oxidative stress, and inhibition of apoptosis. Research indicates that OSR exhibits anti-inflammatory and antioxidant effects in various diseases¹⁴. According to Chen Jian-Yu et al., OSR counteracts oxidative injury and inflammatory activation during liver fibrosis progression by influencing the Nrf2 and NF- κ B signaling cascades. Mechanistically, OSR inhibits the induction of iNOS and COX-2, thereby dampening inflammatory signaling. Furthermore, by lowering malondialdehyde (MDA) levels and increasing glutathione (GSH) levels, OSR protects cell membranes from oxidative damage and decreases lactate dehydrogenase (LDH) release, thus exerting antioxidant effects. Evidence from Wang Hongbo et al. indicates that OSR confers neuroprotection in murine cerebral ischemia/reperfusion models, primarily by attenuating oxidative stress and repressing the expression of the NMDA receptor subunit NR1¹⁵. Consistent with these findings, Wang Yong-Sheng and co-workers revealed that OSR confers neuroprotection against ischemic damage in murine models through modulation of inflammatory mediators and suppression of the NF- κ B signaling cascade. These findings highlight OSR's potential for treating diseases caused by inflammation and oxidative stress¹⁶. Although OSR exhibits promising pharmacological effects, its specific molecular mechanisms in osteoarthritis pathogenesis have yet to be comprehensively clarified, underscoring the need for further in-depth research.

Our research focused on evaluating how OSR modulates IL-1 β -induced responses in osteoarthritic chondrocytes to elucidate its protective potential for cartilage cells. In addition, we clarified the mechanisms through which OSR counteracts inflammatory signaling and oxidative stress triggered by IL-1 β stimulation. Furthermore, we evaluated OSR's chondroprotective effects in vivo using a DMM-induced OA mouse model, confirming that OSR reduces inflammation and oxidative stress, resulting in a therapeutic benefit for osteoarthritis.

2. Materials and methods

2.1. Reagents

OSR was acquired from TopScience (Shanghai, China), and recombinant human IL-1 β was obtained from PeproTech (Rocky Mount, NJ, USA). Dimethyl sulfoxide (DMSO) was provided by Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies recognizing GPX4 (ab125066), SLC7A11 (ab307601), and goat anti-rabbit IgG-HRP (ab150077) were obtained from Abcam (Cambridge, UK). The GAPDH antibody (R24404) was supplied by ZenBio (Chengdu, China), while antibodies against IL-6 (21865-1-AP), Ki67 (27309-1-AP), TNF- α (26405-1-AP), COX2 (27308-1-AP), and MMP13 (18165-1-AP) were purchased from Proteintech (Wuhan, China).

2.2. Ethical Statement

Human articular cartilage samples were collected from individuals diagnosed with osteoarthritis who underwent total knee arthroplasty (TKA) at Xuzhou Mining Group General Hospital. Written informed consent was obtained from all participants prior to sample collection, and all procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Approval for the use of human tissues was granted by the Biomedical Research Ethics Committee of Xuzhou Mining Group General Hospital (Approval No. [2024]100901).

All animal experimental protocols were reviewed and approved by the Laboratory Animal Ethics Committee of Anhui Medical University (Approval No. LLSC20252136). All animal studies were performed in accordance with the institutional guidelines for the care and use of laboratory animals, national regulations, and the ARRIVE guidelines (<https://arriveguidelines.org>).

Osteoarthritis was induced in six-week-old male C57BL/6 mice through destabilization of the medial meniscus (DMM) surgery. Animals were randomly divided into five groups ($n = 6$ per group): Sham, DMM, DMM + DMSO, DMM + DMSO + OSR-down (25 mg/kg), and DMM + DMSO + OSR-up (50 mg/kg). The Sham and DMM groups received intraperitoneal injections of normal saline, the DMM + DMSO group received equivalent volumes of DMSO solvent, and the OSR-treated groups received intraperitoneal injections of OSR solution once per week for eight weeks.

At the end of the experimental period, mice were deeply anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal) prior to euthanasia. After confirming complete loss of reflexes, cervical dislocation was performed to ensure death. This method of euthanasia complies with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020) and was chosen to minimize animal pain and distress. All possible efforts were made to reduce the number of animals used and to alleviate suffering.

2.3. Cell Isolation, Identification, and Culture

Cartilage specimens obtained from osteoarthritis patients during total knee arthroplasty were subjected to enzymatic digestion for chondrocyte isolation. Following recovery, cells were maintained in DMEM/F-12 supplemented with fetal bovine serum, L-glutamine, and antibiotics under standard incubator conditions (37 °C, 5% CO₂, humidified). Their morphology and identity were characterized through methylene blue cytochemical staining and ultrastructural evaluation using electron microscopy. The C28/I2 chondrocyte line was maintained in parallel under identical culture parameters.

2.4. Cell Viability Assay (CCK-8)

Stock preparations of OSR (10, 20, and 40 mM) were generated in DMSO, and recombinant human IL-1 β was adjusted to a final concentration of 10 ng/mL. Chondrocytes (3×10^3 per well) were cultured in 96-well plates for 24, 48, or 72 hours under standard conditions. OSR was administered at 10–40 μ M concentrations in DMEM/F-12 medium. To induce OA-like phenotypes, cells were preincubated with IL-1 β for 2 hours prior to OSR exposure. After the designated treatments, 10 μ L of CCK-8 reagent was added, and absorbance was quantified spectrophotometrically at 450 nm.

2.5. EdU Proliferation Assay

To simulate inflammatory conditions, chondrocytes were stimulated with IL-1 β (10 ng/mL) for 2 h prior to exposure to OSR at graded concentrations of 10, 20, and 40 μ M for a 24-h incubation period. EdU assays (Beyotime) followed the manufacturer's protocol. 2 $\times$ EdU was mixed 1:1 with medium and applied to cells. After the 2-h pretreatment period, cells underwent fixation (15 min), washing, and permeabilization steps, followed by incubation with EdU solution for 30 min protected from light. After final washes, DAPI staining was done for 10 minutes before microscopy.

2.6. Western Blotting

To assess protein expression following exposure to OSR and IL-1 β , chondrocytes were lysed using RIPA lysis buffer enriched with protease inhibitors, and total protein concentration was measured.

2.7. RT-qPCR

Total RNA from chondrocyte lysates was extracted using the TRIzol Reagent (Thermo Fisher Scientific, USA) according to the manufacturer's protocol. Complen

GAPDH (human): Forward 5'-GTCTCCTCTGACTTCAACAGCG-3'; Reverse 5'-ACCACCCTGTTGCTGTAGCCAA-3'.

MMP-13 (human): Forward 5'-CCTTGATGCCATTACCACTCTCC-3'; Reverse 5'-AAACAGCTCCGCATCAACCTGC-3'.

TNF- α (human): Forward 5'-CCTCTCTCTAATCAGCCCTCTG-3'; Reverse 5'-GAGGACCTGGGAGTAGATGAG-3'.

COX-2 (human): Forward 5'-TGAAACCCACTCCAAACACAG-3'; Reverse 5'-TCTCCTATGAGTATGAGTCTGCTGG-3'.

IL-6 (human): Forward 5'-ACTCACCTCTTCAGAACGAATTG-3'; Reverse 5'-CCATCTTTGGAAGGTTCAAGTTG-3'.

2.8. Lipid peroxidation assay

Lipid peroxidation levels in IL-1 β -exposed chondrocytes subjected to graded OSR treatments were determined using the oxidation-sensitive fluorescent probe C11-BODIPY581/591 (Thermo Fisher Scientific). After 24 hours of incubation, cells were labeled with 10 μ M C11-BODIPY at 37 °C for 30 minutes in darkness, subsequently rinsed with pre-chilled PBS, harvested, and analyzed for fluorescence intensity via CytoFLEX flow cytometry (Beckman Coulter).

2.9. Determination of GSH, MDA, and 4-HNE levels in chondrocytes.

GSH, MDA, and 4-hydroxynonenal (4-HNE) levels in chondrocytes and DMM mouse cartilage were measured using commercial kits. GSH and MDA kits were from Nanjing Jiancheng, and 4-HNE ELISA kits from Sinobest Bio. Procedures followed manufacturers' instructions.

2.10. Molecular markers and Flow cytometry

Human articular chondrocytes and C28/I2 cells were plated in 6-well plates and cultured for 24 h prior to treatment. The cells were then stimulated with IL-1 β (10 ng/mL) for 2 h and subsequently incubated with graded concentrations of OSR (10, 20, or 40 μ M).

2.11. Molecular docking

The ligand structure of OSR (PubChem CID 114850) was acquired from the PubChem compound repository in SDF file format for subsequent computational analysis. Hydrogens were added and energy minimized (Amber10-EHT, rigid water solvent, RMS gradient 0.1 kcal/mol/Å, no constraints). The optimized structure underwent conformation searching (MOE, LowModeMD). Docking to Protein.pdb was performed in MOE using TriangleMatcher and London δ G scoring. PLIF clustering was applied, and optimal docking conformations were selected based on score and binding mode. The S value indicates protein–compound affinity (lower S = stronger binding).

2.12. Cellular Thermal Transfer Assay (CETSA)

Human primary chondrocytes and C28/I2 cell lines were cultured in complete medium under typical conditions (37 °C, 5% CO₂, humidified atmosphere) until reaching a cell density of approximately 90%. Following lysis, the cell lysates were combined and portioned (1 mL per sample) into DMSO and OSR experimental groups. Each aliquot was incubated for 2 hours at room temperature with either 1 μ L DMSO or 1 μ L of 500 mM DDZ. Subsequently, 150 μ L supernatant from each treatment was collected, exposed to incremental heating at 50, 55, 60, 65, 70, and 75 °C for 5 minutes, and immediately cooled on ice to halt denaturation. Protein samples were then prepared for Western blot analysis to assess GPX4 expression and thermal stability.

2.13. Animal Experiments

Six-week-old male C57BL/6 mice were randomly assigned to five treatment groups (n = 6 per group): sham-operated, DMM model, DMM + DMSO vehicle, DMM + OSR, and DMM + DDZ. Body weights were tracked every three days during the study period. After 90 days of treatment, animals were humanely euthanized, and knee joint tissues were harvested, fixed, decalcified, paraffin-embedded, sectioned, and examined using hematoxylin-eosin staining, immunohistochemical analysis, and immunofluorescence microscopy.

2.14. Statistical Analysis

Quantitative data are expressed as mean values $\pm$ standard deviation (SD). Group differences were evaluated by one-way analysis of variance (ANOVA) using Graph

3. Results

3.1. OSR promotes chondrocyte activity under inflammatory conditions.

The structural formula of OSR is shown in Fig. 1A. Human chondrocytes were isolated from total knee replacement samples and identified by morphology (Fig. 1B1) and toluidine blue staining, which revealed elliptical or spindle-shaped cells with purple-stained cytoplasmic proteoglycans (Fig. 1B2). To mimic inflammation-induced injury, IL-1 β -treated chondrocytes (including C28/I2 cells) were exposed to graded concentrations of OSR. CCK-8 and EdU assays demonstrated that OSR dose-dependently restored chondrocyte viability impaired by IL-1 β (Fig. 1C–E). Collectively, OSR protects chondrocytes from inflammation-induced impairment.

3.2. OSR inhibits inflammation and reduces oxidative stress in chondrocytes

Inflammation is central to osteoarthritis, making anti-inflammatory actions critical for potential therapeutics. IL-1 β stimulation markedly increased IL-6, TNF- α , COX-2, and MMP13 expression in chondrocytes, whereas OSR treatment suppressed these markers in a dose-dependent manner (Fig. 2A), indicating notable anti-inflammatory activity. Because oxidative stress contributes to osteoarthritis progression, the lipid peroxidation probe C11-BODIPY581/591 was used to assess lipid ROS, and OSR significantly decreased lipid ROS levels in IL-1 β -treated chondrocytes (Fig. 2B–C). OSR also elevated GSH concentrations while reducing MDA and 4-HNE levels (Fig. 2D–F). These data demonstrate that OSR mitigates both inflammatory and oxidative-stress responses in chondrocytes.

3.3. OSR inhibits oxidative stress in chondrocytes by upregulating GPX4 expression

Lipid peroxidation reflects oxidative stress, and GPX4 serves as a key enzyme that reduces lipid hydroperoxides via GSH to limit ROS damage. OSR treatment increased GPX4 mRNA and protein levels in both human chondrocytes and C28/I2 cells (Fig. 3A–C), as further confirmed by flow cytometry (Fig. 3D–E). Molecular docking (PLIF clustering) revealed that OSR directly binds GPX4 with a binding energy of -5.21 kcal/mol, suggesting enhanced protein stability (Fig. 3F). The CETSA assay demonstrated that OSR protected GPX4 from heat-induced degradation (Fig. 3G–H). Together, these results indicate that OSR alleviates oxidative stress through dual regulation of GPX4—enhancing its expression and stabilizing the protein.

3.4. OSR suppresses oxidative stress in osteoarthritis by activating the GSH synthesis-related pathways.

GSH, a major intracellular antioxidant, maintains redox balance through regulation of genes such as SLC7A11, GCLC/GCLM, GSS, and SLC3A2. IL-1 β -induced chondrocytes (primary and C28/I2) displayed decreased SLC7A11 and GCLC/GCLM expression, whereas GSS and SLC3A2 levels remained unchanged (Fig. 4A). Under oxidative stress, OSR treatment significantly upregulated SLC7A11 expression at both mRNA and protein levels, thus enhancing antioxidant capacity (Fig. 4A–C). Flow cytometry confirmed that OSR elevated SLC7A11 expression, attenuated oxidative stress, and alleviated inflammation (Fig. 4D–E). Therefore, OSR promotes GSH biosynthesis via SLC7A11 activation, sustaining redox equilibrium under osteoarthritic conditions.

3.5. OSR efficiently and safely restores chondrocyte activity in DMM model mice

To evaluate OSR's chondroprotective and anti-osteoarthritic effects in vivo, a DMM model was established in C57BL/6 mice with five groups: Sham, DMM, DMSO, OSR 25 mg/kg, and OSR 50 mg/kg (Fig. 5A). Immunohistochemistry revealed increased Ki67 expression in OSR-treated cartilage, particularly at 50 mg/kg, indicating enhanced chondrocyte proliferation (Fig. 5B–C). TUNEL staining confirmed higher cell viability in the OSR groups compared with the DMM group (Fig. 5D–E). OSR treatment did not significantly affect body weight (Fig. 5F), and H&E staining showed no evident organ toxicity (Fig. 5G). These findings demonstrate that OSR effectively restores chondrocyte activity and exhibits good in vivo safety.

3.6. OSR alleviates inflammation and oxidative stress in DMM model mice

Immunohistochemical analysis showed that DMM and DMSO groups displayed elevated IL-6, TNF- α , COX-2, and MMP13 expression in mouse knee cartilage. OSR administration—especially at 50 mg/kg—significantly reduced these inflammatory markers, approaching levels observed in the Sham group (Fig. 6A–B), thereby confirming strong in vivo anti-inflammatory activity. Consistent with the in vitro findings, OSR increased GPX4 and SLC7A11 expression in cartilage (Fig. 7A–B) and improved oxidative-stress parameters, including elevated GSH and reduced MDA and 4-HNE levels (Fig. 7C–G). Collectively, OSR exerts potent anti-inflammatory and antioxidant effects by upregulating GPX4 and SLC7A11, thereby decelerating osteoarthritis progression (Fig. 8).

4. Discussion

The present study demonstrates that OSR exerts protective effects on osteoarthritic chondrocytes by restoring cellular activity under inflammatory stress conditions. Exposure to OSR significantly attenuated the IL-1 β -induced reduction in cell viability in both human primary chondrocytes and C28/I2 cells, reflecting a reduced inflammatory response. Persistent inflammatory signaling is a hallmark of OA progression, mediated by elevated production of IL-6, TNF- α , COX-2, and MMP13, which together accelerate cartilage degradation and joint deterioration^{17–19}. OSR substantially downregulated pro-inflammatory factor expression in IL-1 β -stimulated chondrocytes, underscoring its potential as an emerging candidate for osteoarthritis therapy.

Excessive oxidative stress is another central driver of OA pathogenesis, amplifying inflammation and accelerating chondrocyte apoptosis through ROS-induced cellular injury and activation of degenerative signaling cascades^{20–22}. Mutual reinforcement between oxidative stress and inflammation establishes a pathological feedback loop that aggravates cartilage damage and joint deterioration^{23,24}. In this study, OSR efficiently reduced intracellular ROS accumulation and enhanced intrinsic antioxidant defenses, thereby breaking this pathological feedback loop and improving cellular redox homeostasis.

Mechanistically, the antioxidant capacity of

OSR is largely attributed to its ability to induce and stabilize GPX4, a selenium-dependent enzyme that neutralizes lipid peroxides and serves as a central safeguard a

Simultaneously, OSR upregulated SLC7A11, a critical subunit of system Xc⁻, which mediates cystine–glutamate exchange and regulates cystine uptake, the rate-limiting step in GSH synthesis^{28,29}. GSH serves as a major cellular antioxidant and an essential cofactor for GPX4 activity^{30,31}. Through restoration of SLC7A11 activity, OSR facilitated greater cystine uptake and GSH accumulation, while simultaneously lowering the levels of lipid products such as MDA and 4-HNE. These findings highlight OSR's dual ability to augment antioxidant capacity and mitigate oxidative stress-induced chondrocyte damage.

In vivo experiments using the DMM mouse model corroborated the in vitro results, demonstrating that OSR mitigates joint degeneration in an experimental setting. OSR administration significantly increased cartilage cell proliferation (Ki67 expression) and reduced chondrocyte apoptosis (TUNEL-positive cells), collectively indicating functional repair of OA-damaged cartilage. Immunohistochemical analysis confirmed that OSR downregulated inflammatory mediators

and oxidative stress markers while upregulating GPX4 and SLC7A11 expression in joint tissues. Furthermore, OSR demonstrated excellent systemic tolerability, with consistent body weight and preserved histoarchitecture across major organs, indicating minimal off-target toxicity^{32,33}.

Current pharmacological treatments for OA primarily provide symptomatic relief rather than disease modification. While NSAIDs and analgesics provide transient pain relief, they are ineffective in rebuilding cartilage or interrupting the chronic inflammatory and oxidative stress cycle. Moreover, long-term NSAID use carries notable safety concerns, including an increased risk of gastrointestinal damage and cardiovascular complications³⁵. These limitations underline the critical need for agents capable of targeting molecular mechanisms that drive OA development instead of providing transient relief.

Despite promising results from preclinical studies, translation of antioxidant or anti-inflammatory therapies into clinical success has been hindered by ambiguous molecular mechanisms and inconsistent safety profiles^{36,37}.

OSR, a quinolizidine alkaloid purified from *Sophora alopecuroides*, has been reported to exert potent anti-inflammatory and antioxidative effects in experimental models⁴⁰; however, its specific role in OA had not been characterized before this study. Our findings reveal that OSR addresses fundamental pathogenic mechanisms of OA by restoring redox homeostasis through the GPX4/SLC7A11-GSH axis, thereby simultaneously attenuating inflammation and oxidative stress while promoting cartilage regeneration.

Taken together, these results position OSR as a promising dual-action candidate for OA therapy, providing complementary anti-inflammatory and antioxidant properties superior to single-target interventions. Future research should focus on pharmacokinetic profiling, dose optimization, and genetic model validation (e.g., GPX4 or SLC7A11 knockout mice) to fully define OSR's regulatory network. Ultimately, well-designed clinical trials will be necessary to confirm its therapeutic efficacy and safety in human OA patients.

5. Conclusion

Taken together, our findings reveal that OSR restores cartilage homeostasis in osteoarthritis by dampening ROS-driven oxidative stress and enhancing the glutathione antioxidant network through GPX4 stabilization and SLC7A11 activation. By coupling antioxidant reinforcement with anti-inflammatory effects, OSR preserves chondrocyte survival and tissue architecture. These results underscore OSR as an innovative and safe therapeutic candidate with strong potential for clinical translation in osteoarthritis management.

Abbreviations

CETSA, Cellular Thermal Shift Assay; COX-2, Cyclooxygenase-2; DMSO, Dimethyl sulfoxide; GPX4, Glutathione peroxidase 4; GSH, Glutathione; H&E, Hematoxylin and eosin; IL-6, Interleukin-6; MDA, Malondialdehyde; MMP-13, Matrix metalloproteinase 13; OA, Osteoarthritis; OSR, Oxsophoridine; ROS, Reactive oxygen species; SLC7A11, Solute carrier family 7 member 11; TNF- α , Tumor necrosis factor- α ; 4-HNE, 4-Hydroxynonenal.

Declarations

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Consent for Publication

Written informed consent for publication was obtained from all participants who provided human cartilage samples. All identifying information has been removed to ensure anonymity, and signed consent forms are available for review by the journal's editorial office upon request.

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Competing Interests

The authors declare that they have no competing interests.

Authors' contributions

Jun Tu (First Author): Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft, Writing - Review & Editing;

Zhiwei Peng: Data Curation, Writing - Original Draft, Writing - Review & Editing;

Haobo Liu: Visualization, Investigation, Writing - Review & Editing;

Xiyang Sun: Visualization, Writing - Review & Editing;

Jianqi Zhao: Resources, Supervision, Writing - Review & Editing;

Bin Xu: Software, Validation, Writing - Review & Editing;

XuZhu: Conceptualization, Methodology, Writing – review & Editing.

Baofang Liu: (Corresponding Author): Conceptualization, Funding Acquisition, Resources, Supervision, Writing - Review & Editing.

All authors agreed on the journal for submission, reviewed and approved all versions of the manuscript, and accept responsibility for the published content.

Data Availability Statement

All data generated or analyzed during this study are available from the corresponding author upon reasonable request. The datasets include raw Western blot images, RT-qPCR Ct values, flow cytometry files, histological and immunohistochemical images, and molecular docking results. All data have been carefully validated, and the authors affirm their accuracy and reliability. Requests for access should be directed to the corresponding author.

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Figures

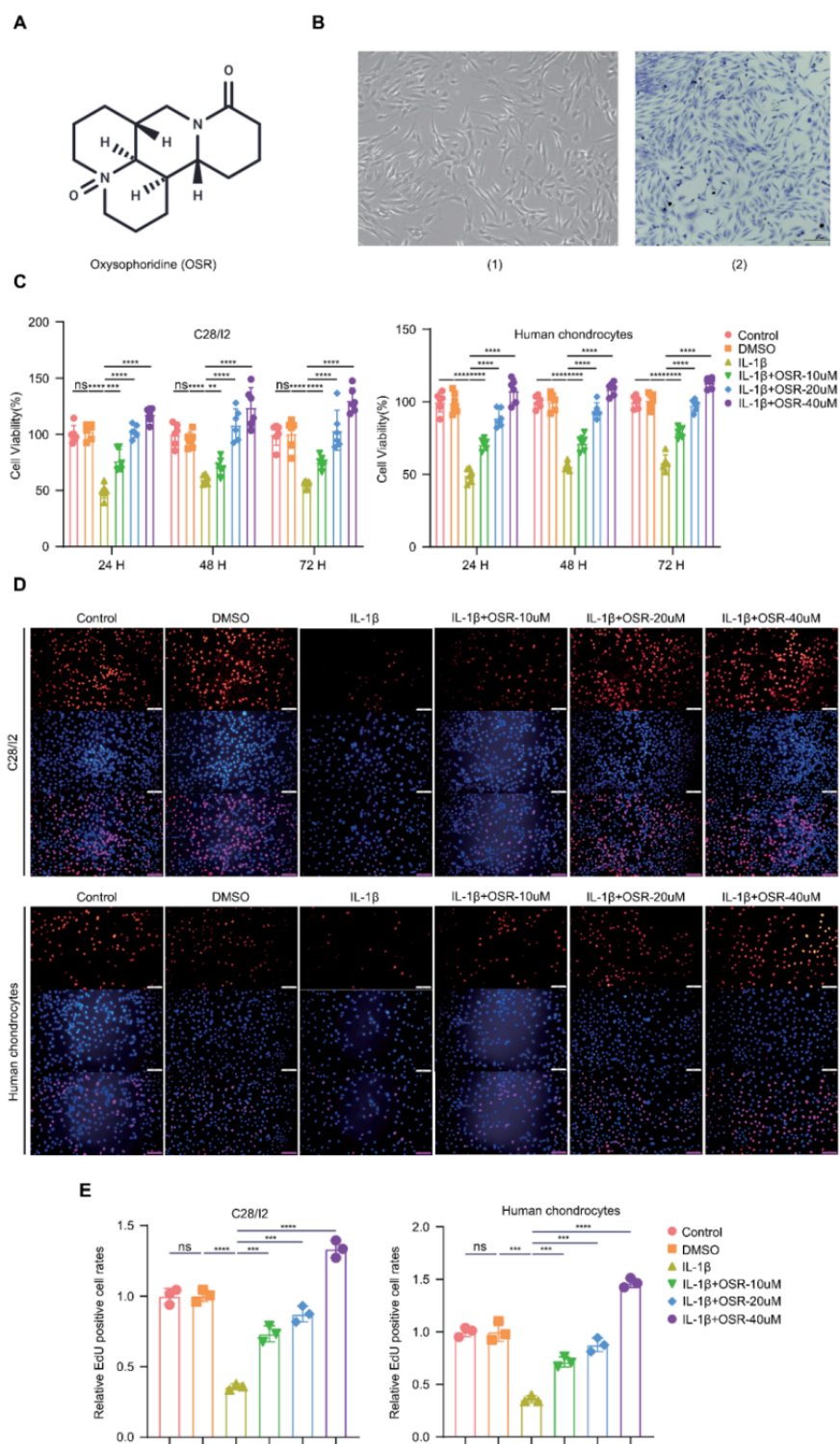


Figure 1

Oxsophoridine (OSR) mitigates IL-1 β -induced inflammatory responses in chondrocytes. (A) Structural representation of OSR. (B) Morphological features of human primary chondrocytes observed under (1) light microscopy and (2) toluidine blue staining (scale bar = 50 μ m). (C) CCK-8 assay results showing the effects of different OSR concentrations on the viability of human primary chondrocytes and C28/I2 cells after 24, 48, and 72 h (n = 6). (D) EdU live/dead staining images of both cell types following 24 h of OSR exposure (scale bar = 200 μ m). (E) Quantitative evaluation of EdU-positive cells using ImageJ (n = 3). Values are presented as the mean $\pm$ SD from three or six independent experiments. ****p < 0.0001, ***p < 0.001, **p < 0.01, ns > 0.05.

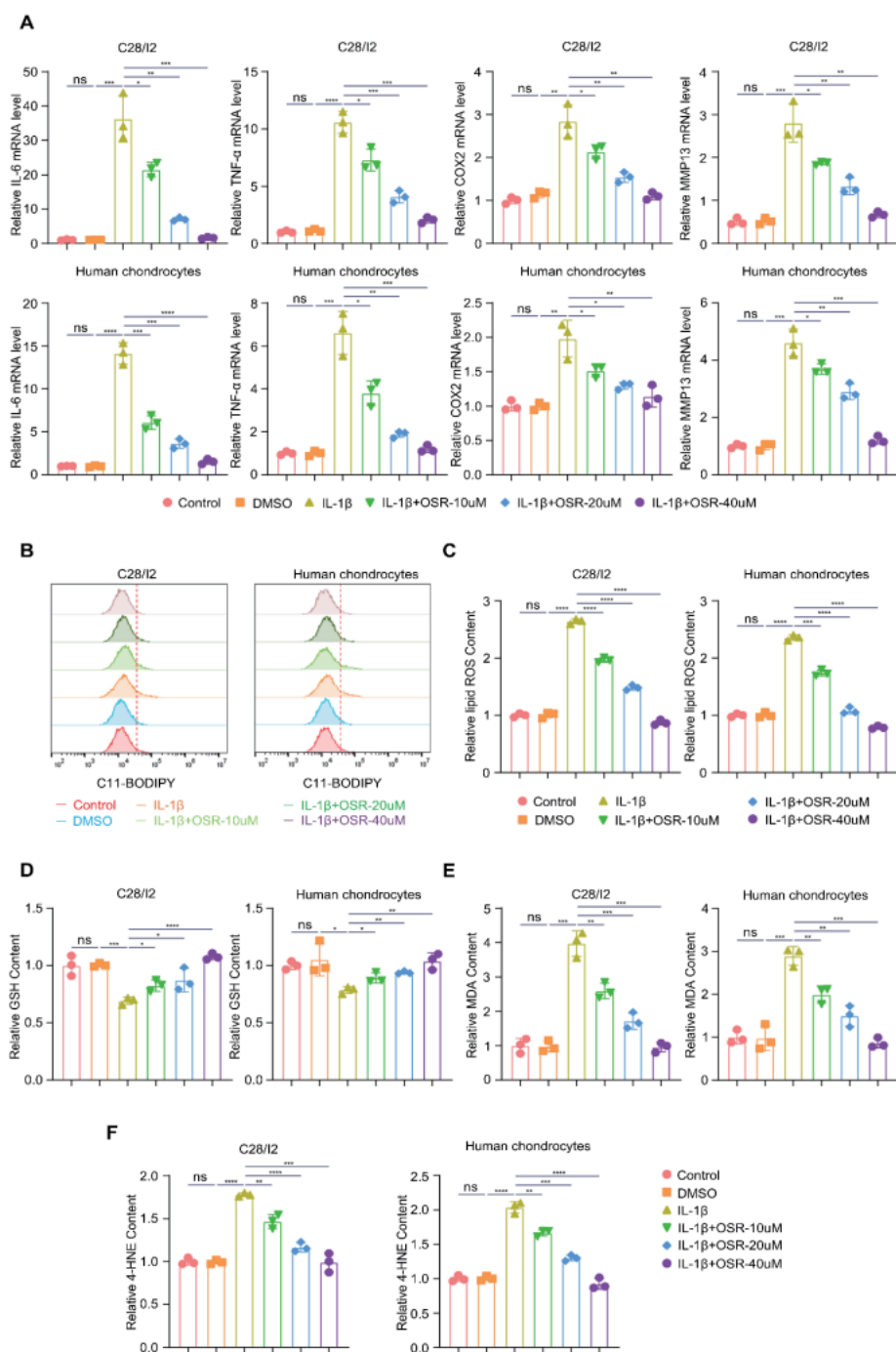


Figure 2

OSR suppresses inflammation by inhibiting ROS production in chondrocytes. IL-1 β -stimulated human primary chondrocytes and C28/I2 cells were cultured with OSR for 24 h. (A) RT-qPCR analysis of IL-6, TNF- α , and IL-1 β mRNA expression. (B) Intracellular lipid ROS levels measured by flow cytometry after C11-BODIPY581/591 staining. (C) Quantification of lipid ROS. (D) GSH levels assessed with a glutathione assay kit. (E) MDA content measured by MDA assay kit. (F) 4-HNE expression determined by ELISA. Data are mean $\pm$ SD from three independent experiments. **** P <0.0001, *** P <0.001, ** P <0.01, * P <0.05, ns>0.05.

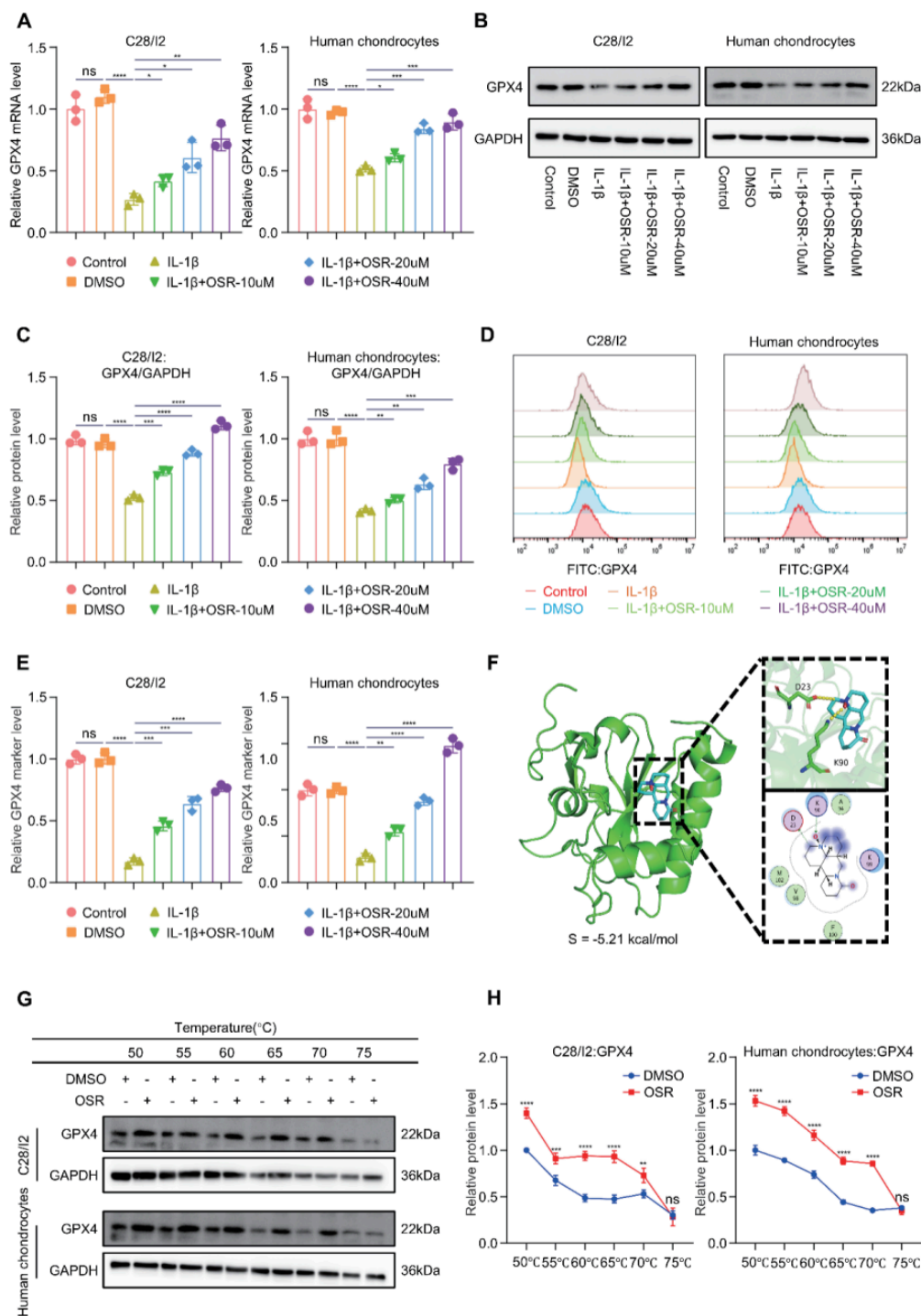


Figure 3

OSR reduces oxidative stress in chondrocytes by upregulating GPX4. (A–E) IL-1 β -stimulated chondrocytes (primary and C28/I2) were treated with OSR (24 h), and GPX4 levels were analyzed by qPCR (A), Western blot (B–C), and flow cytometry (D–E), quantified with ImageJ. (F) Binding conformation of OSR and GPX4 shown in 3D/2D views, highlighting hydrogen bonds. (G–H) CETSA/Western blot analysis showed OSR stabilizes GPX4 at increasing temperatures, quantified by ImageJ. Data: mean $\pm$ SD (n=3); significance: ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns>0.05.

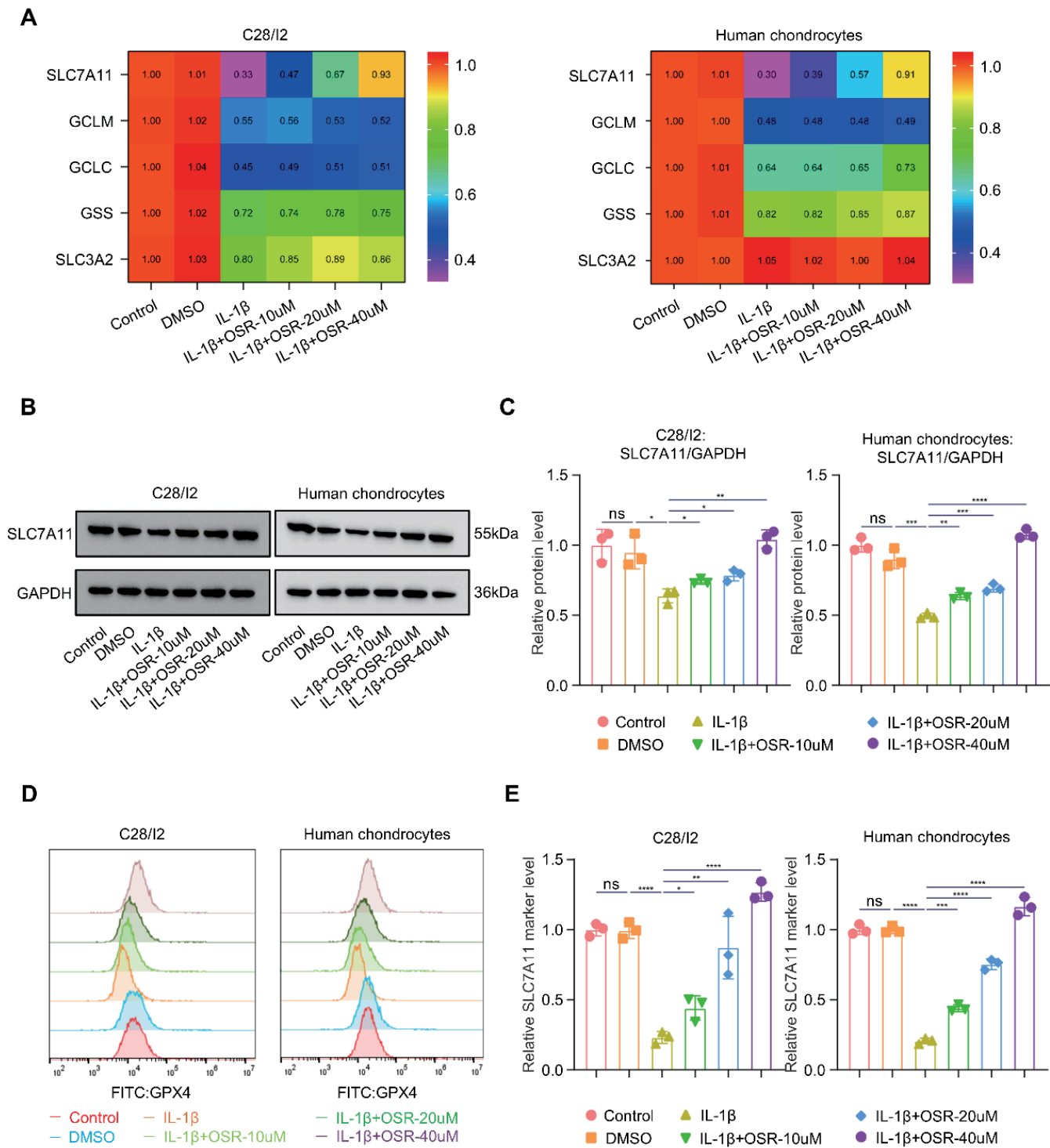


Figure 4

OSR suppresses osteoarthritis oxidative stress and inflammation by activating GSH synthesis genes. (A) RT-qPCR heatmap shows mRNA levels of SLC7A11, GCLC/GCLM, GSS, and SLC3A2. (B–C) Western blot and ImageJ quantification of SLC7A11 protein. (D–E) Flow cytometry of SLC7A11 in IL-1 β -induced chondrocytes treated with OSR; data shown as histograms. Results: mean $\pm$ SD (n=3); ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns>P 0.05.

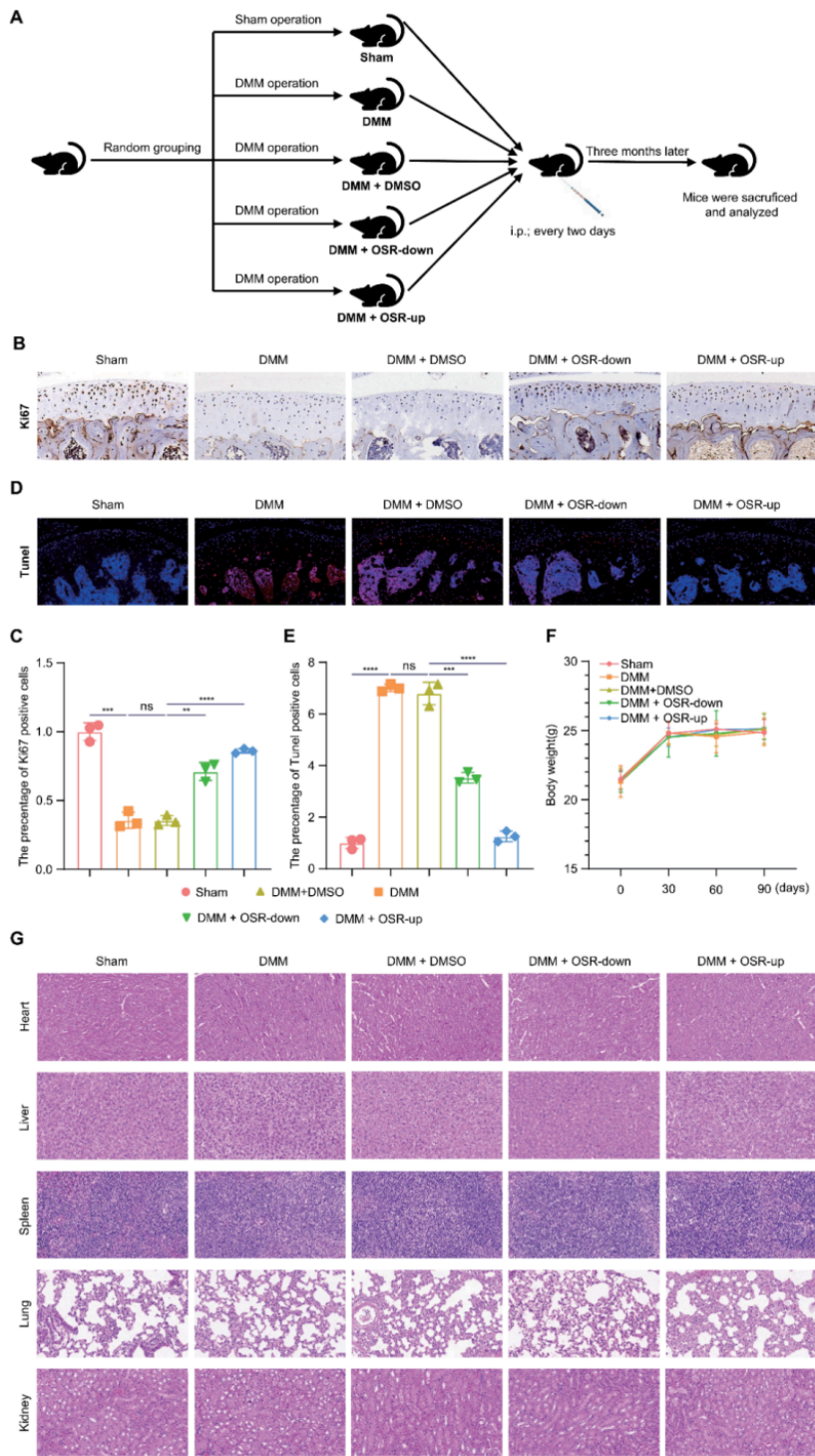


Figure 5

OSR enables effective and safe cartilage repair in DMM mice. Five groups: Sham, DMM, DMSO, OSR 25 mg/kg, and OSR 50 mg/kg. (A) Experimental schematic. (B–C) Ki67 immunohistochemistry of cartilage, quantified by ImageJ. (D–E) TUNEL immunofluorescence of cartilage, quantified by ImageJ. (F) Mouse body weights recorded every 30 days (n=6). (G) H&E staining of heart, liver, spleen, lung, and kidney. Scale bar: 100 μ m. B–E: mean $\pm$ SD (n=3); F: mean $\pm$ SD (n=6). Significance: ****P<0.0001, ***P<0.001, **P<0.01, ns>P 0.05.

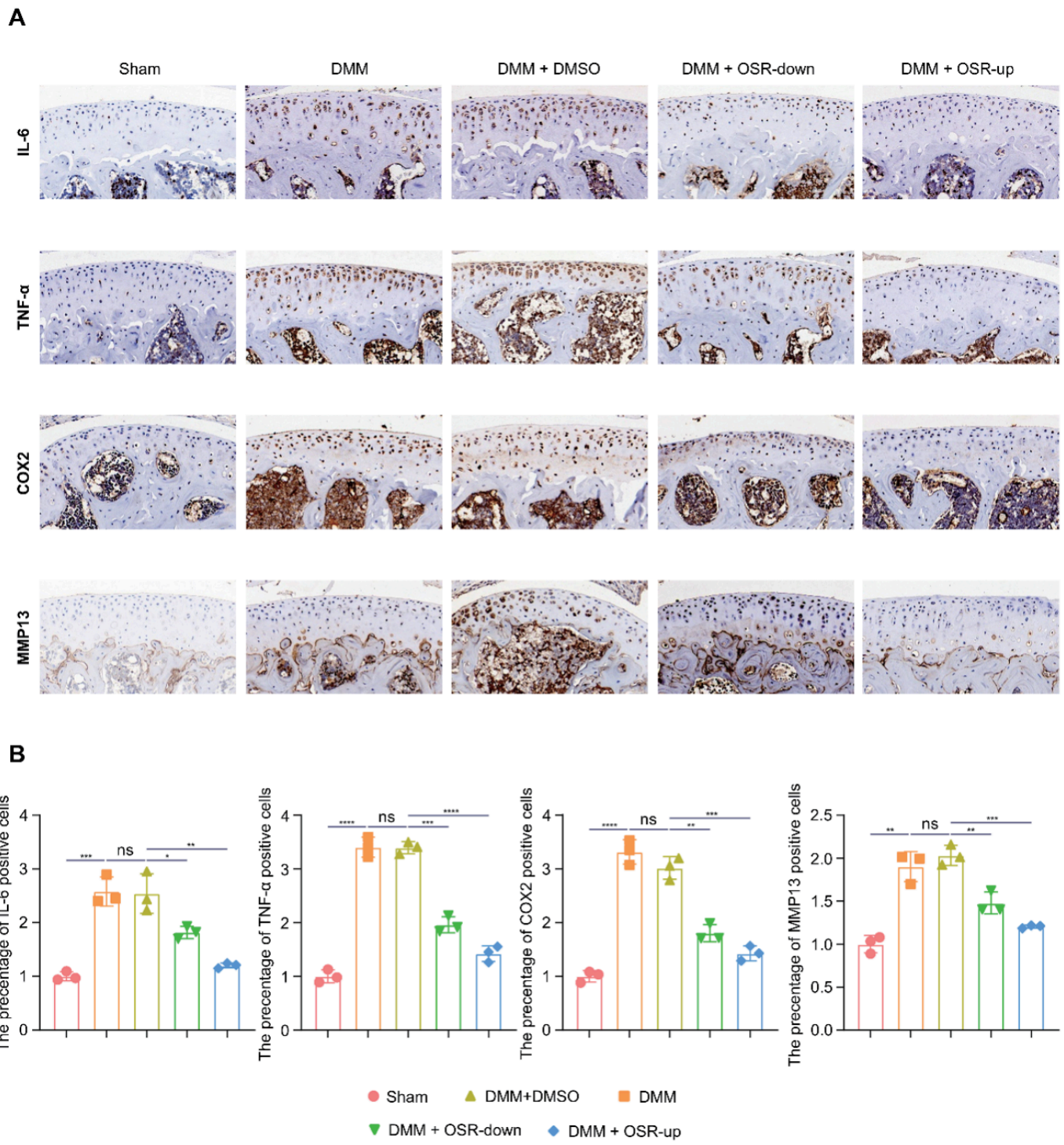


Figure 6

OSR reduces inflammation in DMM model mice. Five groups: Sham, DMM, DMSO, OSR 25 mg/kg, and OSR 50 mg/kg. (A–B) Immunohistochemical analysis of IL-6, TNF-α, COX-2, and MMP13 in cartilage, quantified by ImageJ. Scale bar: 100 μm. Data are mean ± SD from three independent experiments. Significance: ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, ns>P 0.05.

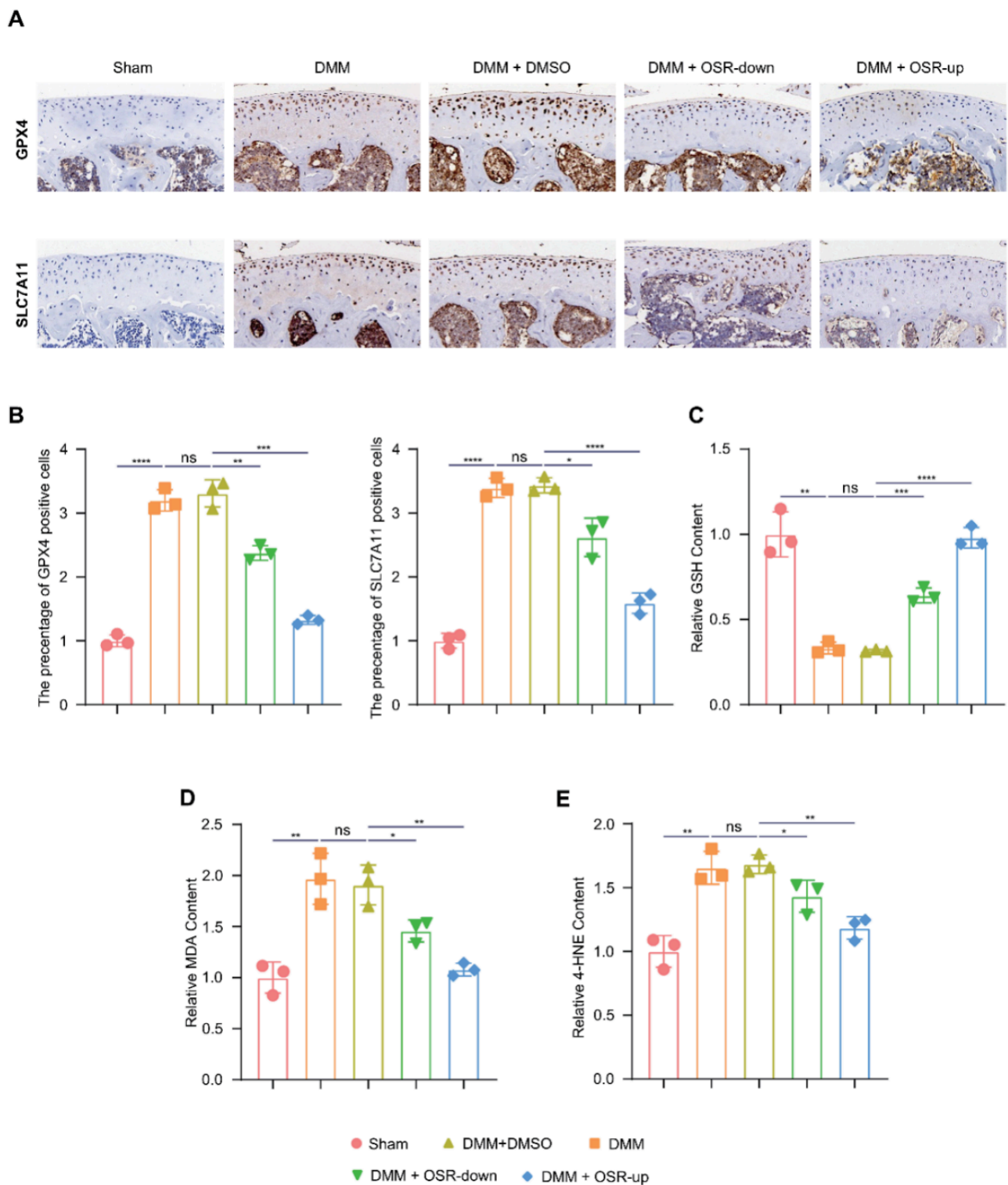


Figure 7

OSR reduces oxidative stress in DMM model mice. Five groups: Sham, DMM, DMSO, OSR 25 mg/kg, and OSR 50 mg/kg. (A–B) Immunohistochemistry of GPX4 and SLC7A11 in cartilage chondrocytes, quantified by ImageJ. Scale bar: 100 μ m. (C–E) Levels of GSH, MDA, and 4-HNE in chondrocytes. Data are mean $\pm$ SD from three independent experiments. Significance: **** P <0.0001, *** P <0.001, ** P <0.01, * P <0.05, ns> P 0.05.

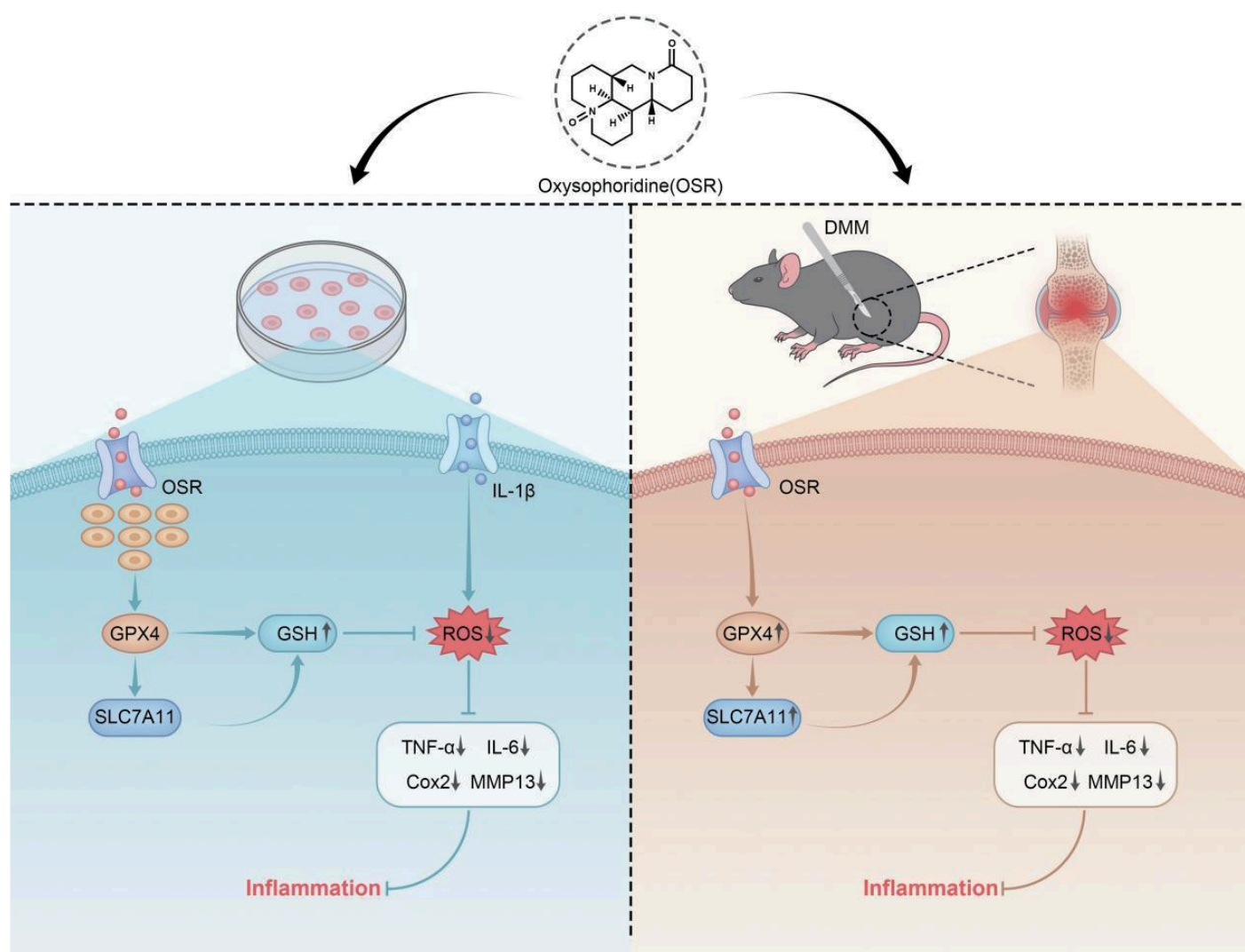


Figure 8

Schematic illustration of OSR's protective mechanism in osteoarthritis. OSR mitigates cartilage damage from inflammatory factors in vivo and in vitro by suppressing ROS-induced oxidative stress and regulating GPX4 activity, thereby preserving cartilage integrity.