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A mesoporous oxygen-vacancy-rich ZnO nanoplatform as a catalytic therapeutic hub for osteoarthritis therapy by TRIM15-targeted stem cell rejuvenation

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Abstract

Osteoarthritis (OA) lacks disease-modifying treatments, partly because aging creates an oxidative-senescent joint niche that accelerates cartilage degeneration and weakens endogenous MSC-mediated repair. We found that aged OA cartilage harboured TRIM15-high MSC-like cells, whereas aged joint-derived MSCs exhibited zinc depletion, defective mitophagy and impaired chondrogenesis. To address these abnormalities, we developed oxygen-vacancy-rich mesoporous ZnO (meso-ZnO_v) as an active therapeutic material capable of scavenging reactive oxygen species and restoring zinc homeostasis through Zn²⁺ release. Here, we incorporated a Zn²⁺-dependent TRIM15-targeting DNAzyme into meso-ZnO_v and coated this active core with an ITGA2-enriched hybrid membrane to generate ZDM. ZDM preferentially accumulated in damaged cartilage and enhanced tissue penetration. It alleviated oxidative stress and senescence and restored cartilage matrix homeostasis, while re-establishing zinc homeostasis and rejuvenating aged MSCs. Mechanistically, ZDM reduced TRIM15 abundance and YAP nuclear localisation and restored SOX9 expression and PINK1-dependent mitophagy, thereby reversing MSC senescence and promoting chondrogenesis. In aged mouse and beagle OA models, ZDM improved joint function, preserved cartilage and subchondral bone, and reduced synovial inflammation. These findings establish a meso-ZnO_v-based nanotherapeutic strategy that couples microenvironmental restoration with stem-cell rejuvenation, offering a promising disease-modifying approach for age-associated OA.

Introduction

Osteoarthritis (OA) is the most prevalent degenerative joint disease, affecting more than 500 million people worldwide¹. It is characterized by progressive articular cartilage destruction, synovial inflammation, aberrant subchondral bone remodelling, and chronic pain, ultimately leading to irreversible joint dysfunction^{2,3}. Despite its enormous clinical burden, no disease-modifying OA drugs (DMOADs) have been approved^{4,5}. Even with multiple candidates advancing into clinical evaluation, their transition into routine practice remains severely hampered by an inability to provide durable structural protection alongside symptomatic relief^{6,7}.

One major reason for the limited clinical benefit of current OA treatments is that OA progression is sustained by an aged and inflammatory joint microenvironment that disrupts cartilage homeostasis^{8–11}. Mesenchymal stem cells (MSCs) are an important endogenous repair population in the joint. Through self-renewal and differentiation towards chondrogenic lineages, MSCs support cartilage maintenance and repair^{12–14}. However, this reparative capacity is markedly weakened in OA. Chronic exposure to this highly oxidative and senescent milieu profoundly promotes MSC senescence, impairs chondrogenic differentiation, and disrupts mitochondrial homeostasis¹⁵. These changes are closely associated with sustained oxidative stress, mitochondrial damage, defective mitophagy, and blunted lineage-specific differentiation programmes^{16,17}. In this pathological niche, tripartite motif-containing 15 (TRIM15) has recently been identified as a regulator of chondrocyte senescence and OA progression. By promoting cellular senescence and SASP signalling, TRIM15 may amplify local inflammatory and oxidative stress, thereby further limiting MSC-mediated cartilage repair¹⁸.

To address this hostile microenvironment and reactivate endogenous repair, we developed a nanoplatform designed to restore the aged cartilage niche while improving the reparative fitness of aged MSCs. Because mitochondrial dysfunction and defective mitophagy are central features of aged MSC impairment, we focused on zinc homeostasis, which has been implicated in autophagy regulation and stem-cell function¹⁹. However, the direct intra-articular administration of free zinc salts is severely limited by rapid clearance and dose-dependent cytotoxicity. To safely harness its metabolic therapeutic potential, we engineered an oxygen-vacancy-rich mesoporous zinc oxide (meso-ZnO_v) nanocarrier. Rather than serving merely as an inert delivery vehicle, meso-ZnO_v acted as a primary microenvironmental modulator. Its abundant oxygen vacancies conferred nanozyme-like ROS-scavenging activity, directly reducing oxidative stress in the diseased niche. After cellular uptake, its degradation within acidic endo-lysosomes enabled Zn²⁺ release, supporting mitochondrial homeostasis in reparative MSCs^{19,20}. To further suppress TRIM15-driven senescence signalling, we loaded the meso-ZnO_v core with DNAzymes. Compared with conventional RNA interference, which is vulnerable to nuclease degradation in pathological joint fluids, DNAzymes offer superior extracellular and intracellular stability. As catalytically active single-stranded DNAs, they cleave target RNA without relying on host gene-silencing machinery^{21,22}. Notably, the

selected TRIM15-targeting DNAzyme requires Zn^{2+} for catalytic activity, allowing Zn^{2+} released from meso- ZnO_v degradation to serve not only as a bioactive ion for mitochondrial support but also as a local cofactor that activates TRIM15 RNA cleavage. Thus, the meso- ZnO_v -DNAzyme (ZD) core integrates ROS neutralization, metabolic support of MSCs and Zn^{2+} -assisted TRIM15 silencing.

To improve delivery through the permeable cartilage matrix, the meso- ZnO_v core was coated with an ITGA2-enriched chondrocyte–MSC hybrid membrane. Because COL1 is enriched in damaged OA cartilage²³, ITGA2–COL1 binding^{24,25}, promoted lesion-oriented localisation, while the MSC membrane component provided reparative-cell tropism^{26,27}. This coating enhanced cartilage penetration, enabling ZnO_v to remodel the oxidative-senescent microenvironment, restore PINK1–Parkin-associated mitochondrial homeostasis and improve aged MSC function. Consequently, ZDM preserved cartilage architecture and attenuated periarticular bone remodelling in aged OA mice and beagles (Fig. 1).

Results

Aged osteoarthritic joints harbour TRIM15-high MSC-like cells with impaired zinc homeostasis and mitophagy

To establish the pathological context for TRIM15 targeting, we first examined aged human cartilage and public transcriptomic data. X-ray imaging and histological staining showed typical OA-associated structural degeneration, including osteophyte formation, surface disruption and loss of proteoglycan-rich matrix in old human cartilage (Fig. 2a,b). Re-analysis of human cartilage RNA-seq data from OA and normal control samples identified broad transcriptional remodelling (Supplementary Fig. 1a,b), with significant enrichment of cellular senescence signatures (Supplementary Fig. 1c). Among the senescence-associated genes increased in OA cartilage, TRIM15 was prominently upregulated (Supplementary Fig. 1d). Because TRIM15 has been implicated in chondrocyte senescence¹⁸, we next validated its expression at the tissue level. Immunohistochemistry confirmed sparse TRIM15 staining in young human cartilage but pronounced TRIM15 accumulation in old cartilage (Fig. 2c and Supplementary Fig. 2a). Immunoblotting further showed reduced COL2 and SOX9 and increased MMP13 in old human cartilage

(Supplementary Fig. 2b), consistent with loss of anabolic cartilage identity.

We then asked whether the damaged cartilage niche retained reparative stromal cells that might be exposed to this TRIM15-high environment. In aged mouse joints, COL1A1 marked the damaged, fibrocartilage-like region, and PDGFR α -positive MSC-like cells accumulated around COL1A1-positive areas (Fig. 2d and Supplementary Fig. 2c). Co-staining further showed that PDGFR α -positive cells in aged cartilage displayed increased TRIM15 signal compared with young cartilage (Fig. 2e and Supplementary Fig. 2d). These observations indicate that aged damaged cartilage is not devoid of reparative cells; rather, it contains MSC-like cells positioned within a COL1-rich injury niche.

We next assessed whether aging also impaired the reparative phenotype of joint-resident MSCs. Cartilage from 21-month-old mice showed proteoglycan loss and higher histological degeneration scores than cartilage from 3-month-old mice (Supplementary Fig. 2e). It also showed higher TRIM15 and p21 staining (Fig. 2f,g), indicating that the aged mouse joint recapitulated the TRIM15-high and senescence-associated cartilage state observed in human samples. Because MSCs are a major endogenous reservoir for cartilage repair^{14,28–31}, we isolated MSCs from 3- and 21-month-old mouse knee joints and assessed whether aging compromised their reparative phenotype. Aged MSCs showed increased SA- β -gal staining and elevated γ -H2AX signal, indicating enhanced cellular senescence and DNA-damage-associated stress (Fig. 2h,i). Human MSCs from old donors also showed increased p53, p21 and p16 protein expression (Supplementary Fig. 2f). Aged mouse MSCs also showed higher TRIM15 expression and reduced mitochondrial membrane potential (Supplementary Fig. 3a,b). In parallel, SOX9 expression was markedly reduced in aged MSCs³², and toluidine blue staining showed weaker cartilage-like matrix deposition after chondrogenic induction (Fig. 2j,k and Supplementary Fig. 4a). Additional tri-lineage assays showed impaired chondrogenic and osteogenic differentiation together with increased adipogenic differentiation in aged MSCs (Supplementary Fig. 4a–c). These findings show that although MSC-like cells are present in damaged cartilage, MSCs in the aged joint display a senescent, metabolically impaired and poorly chondrogenic state.

We finally examined whether this impaired MSC state was associated with disrupted

zinc homeostasis and mitochondrial quality control. Consistent with the role of Zn^{2+} in cellular metabolism^{20,33}, FluoZin-3 imaging showed that labile Zn^{2+} levels were significantly lower in aged MSCs than in young MSCs (Fig. 2l). Additional zinc-responsive analyses in chondrocytes showed reciprocal regulation of COL2A1 protein expression (Supplementary Fig. 3c), further linking zinc availability to cartilage homeostatic markers. Mitophagy is a crucial cellular defence mechanism that helps maintain mitochondrial and cellular homeostasis^{34–37}. Mitophagy-related trafficking was also impaired, as shown by reduced colocalisation between PK-Mito-labelled mitochondria and LysoBrite-labelled lysosomes in aged MSCs (Fig. 2m). Additional serial-passage and marker analyses supported age-associated senescence in MSCs (Supplementary Figs. 5 and 6). Together, these data define a TRIM15-high, zinc-deficient and mitophagy-impaired MSC state within the aged osteoarthritic niche, providing the rationale for a therapeutic strategy that couples TRIM15 silencing with zinc-restorative mitochondrial support.

Synthesis and structural characterization of oxygen-vacancy-rich mesoporous ZnO nanoparticles

Oxygen-vacancy-rich mesoporous ZnO (meso- ZnO_v) was synthesized by a modified hard-template route reported in the literature. Powder X-ray diffraction (XRD) pattern showed that both meso- ZnO_v and meso-ZnO showed characteristic peaks consistent with the standard ZnO card, confirming successful synthesis of ZnO with a similar crystalline structure (Fig. 3a). Compared to meso-ZnO, the crystallinity of meso- ZnO_v was decreased, indicating its oxygen-vacancy-rich atomic structure. Transmission electron microscopy (TEM) images revealed that meso- ZnO_v exhibited a particulate morphology with a diameter of approximately 200 nm (Fig. 3b). The locally magnified TEM image along with its rainbow contrast analysis exhibited typical height differences, clearly indicating the presence of abundant mesopores (Fig. 3c). High-resolution TEM (HRTEM) further revealed atomic crystal structure of meso- ZnO_v (Fig. 3d). Uniform lattice fringes with a d-spacing distance of 0.235 nm corresponded to the plane of ZnO (Fig. 3d). Meanwhile, there were significant lattice distortions in meso- ZnO_v , further confirming the presence of abundant oxygen vacancies (Fig. 3d). Moreover, the energy-dispersive X-ray (EDX) elemental mapping

images demonstrated the uniform distribution of Zn and O throughout mesoporous nanoparticles (Fig. 3e). Similarly, electron paramagnetic resonance (EPR) measurements showed that meso-ZnO_v exhibited a strong characteristic signal at $g = 2.001$, which was attributed to unpaired electrons trapped by oxygen vacancies. The signal intensity was significantly higher than that of meso-ZnO, indicating the presence of abundant oxygen vacancies in meso-ZnO_v (Fig. 3g). Extended X-ray absorption fine structure (EXAFS) analysis and corresponding X-ray absorption near-edge structure (XANES) also revealed that the Zn–O coordination number decreased from 4.0 in ZnO to 3.5 in meso-ZnO_v (Supplementary Fig. 7). Compared to that of ZnO, the Zn K-edge white-line of meso-ZnO_v exhibited a distinct shift to the lower energy (Fig. 3h), indicating an increased electron density around the Zn sites. It further confirmed the local electronic redistribution between Zn and O atoms induced by oxygen vacancies. Moreover, the reduced intensity of the Zn–O bond signal in meso-ZnO_v further corroborated the decrease in oxygen coordination number (Supplementary Fig. 7), consistent with the weakened Zn–O coordination intensity observed in the wavelet transform (MWT) plots (Fig. 3i and Supplementary Fig. 8), thereby confirming the successful synthesis of meso-ZnO_v with abundant oxygen-vacancy-rich sites.

Engineering a hybrid membrane nanoplatform with enhanced cartilage targeting and penetration

Integrin $\alpha 2$ (ITGA2) is a well-established collagen receptor that directly binds type I collagen (COL1), whereas COL1 is preferentially enriched in damaged, fibrocartilage-like osteoarthritic regions rather than in healthy hyaline cartilage^{24,38,39}. We therefore exploited the ITGA2–COL1 interaction for lesion-oriented delivery. Molecular docking predicted a favourable binding interface between ITGA2 and COL1 (Fig. 4a), and molecular dynamics simulations supported the stability of the complex (Supplementary Fig. 9a). ITGA2 overexpression and its predominant plasma membrane localisation in chondrocytes were confirmed by immunofluorescence and DiO colocalisation, respectively (Supplementary Fig. 9b,c). The identities of the chondrocytes and MSCs used for membrane preparation were verified using representative markers, and co-immunoprecipitation confirmed the association between ITGA2 and COL1 (Supplementary Fig.

9d,e). Membranes from ITGA2-overexpressing chondrocytes were subsequently fused with MSC membranes to generate a chondrocyte–MSC hybrid membrane (MC-HM), with ITGA2 enrichment confirmed by immunoblotting (Supplementary Fig. 9f).

MC-HM was then used to coat DNAzyme-loaded meso-ZnO_v (ZD), generating ZDM. Coomassie staining showed that the overall membrane protein profile was retained after membrane fusion and nanoparticle coating (Fig. 4b). Immunoblotting further detected representative membrane proteins on ZDM, including ITGA2, integrin β 1, integrin α 3, CD44, N-cadherin, E-cadherin and Na⁺/K⁺-ATPase (Fig. 4c). Consistent with successful coating, ZDM exhibited a modestly increased hydrodynamic diameter and a more negative surface potential than ZD (Supplementary Fig. 10). We also verified the Zn²⁺-dependent activity of the DNAzyme cargo. Increasing Zn²⁺ concentrations enhanced cleavage of the TRIM15 RNA substrate (Fig. 4d), and DNAzyme-containing formulations reduced cellular TRIM15 expression (Supplementary Fig. 11).

Molecular dynamics simulations were used to assess how hybrid membrane coating affected nanoparticle interactions with a phospholipid bilayer. Both ZD and ZDM traversed the model bilayer, with ZDM completing translocation earlier (Fig. 4e,f). Although ZDM exhibited a lower diffusion coefficient, it maintained more sustained interactions with surrounding phospholipids, suggesting reduced random motion during bilayer engagement (Fig. 4g). Interaction-energy and force analyses revealed distinct membrane-interaction profiles, while phospholipid density maps showed local lipid redistribution around both formulations (Supplementary Fig. 12a–d). These results indicate that MC-HM alters nanoparticle mobility and promotes sustained bilayer interactions while preserving translocation. Electron microscopy and elemental mapping confirmed the morphology and elemental composition of ZD and ZDM (Fig. 4h). TEM further revealed a peripheral coating layer surrounding ZDM, consistent with a core–shell architecture (Supplementary Fig. 12e).

We next assessed lesion-associated cartilage targeting. Picrosirius red staining revealed pronounced collagen remodelling at the OA cartilage surface. At 6 h after intra-articular injection, Cy5-labelled ZDM accumulated along the damaged surface and penetrated significantly deeper into DMM cartilage than into sham cartilage (Fig. 4i). By contrast, ZD showed limited penetration without a significant difference between sham and DMM cartilage (Supplementary

Fig. 14b). These findings are consistent with COL1-associated lesion targeting conferred by the ITGA2-enriched hybrid membrane.

The tissue-penetrating capacity of ZDM was further evaluated in three-dimensional chondrocyte spheroids. After 12 h, ZD remained largely confined to the spheroid periphery, whereas ZDM was detected throughout optical sections at depths of 25-100µm and reached the spheroid interior (Fig. 4j). Fluorescence intensity profiles confirmed the broader distribution of ZDM. Following cellular uptake, ZDM initially colocalised with lysosomes at 1 h, followed by reduced overlap at 6 h, suggesting reduced lysosomal retention and subsequent intracellular redistribution (Fig. 4k). Progressive nanoparticle degradation was observed at later time points (Supplementary Fig. 13). Similarly, ZDM penetrated deeper into ex vivo cartilage explants, whereas ZD remained largely restricted to the superficial layer (Fig. 4l and Supplementary Fig. 14a). In multicellular co-culture, ZDM uptake was greater in chondrocytes and MSCs than in NIH3T3 cells (Fig. 4m,n). No overt histological toxicity was observed after treatment (Supplementary Fig. 15a,b). Collectively, these findings show that the hybrid membrane functions primarily as a delivery interface that enhances lesion-associated localisation, cartilage penetration and cellular uptake of the meso-ZnO_v-based therapeutic core.

ZDM activates zinc-responsive reparative programmes and promotes MSC migration

To gain insight into the reparative activity of ZDM, we performed integrated transcriptomic and proteomic profiling in MSCs treated with ZDM (Supplementary Fig. 16a). Differential analysis revealed broad molecular remodelling following treatment (Fig. 5a,b). Cross-comparison of enriched terms showed that ZDM activated programmes associated with chondrocyte differentiation, positive regulation of cell migration and response to zinc ion, while suppressing extracellular matrix disassembly, inflammatory responses and collagen catabolic processes (Fig. 5c). Consistent with this, transcriptomic and proteomic analyses further supported activation of anabolic, redox-homeostatic and stem cell-related programmes, together with repression of inflammatory and catabolic pathways (Supplementary Figs. 16–19). These data suggest that ZDM reprogrammes cells towards a more pro-reparative state that may favour cell migration and cartilage regeneration. We therefore directly examined whether ZDM could enhance MSC migration and chondrogenic differentiation. Because the hybrid membrane

incorporates an MSC-derived membrane component, which is expected to endow biomimetic nanocarriers with stem-cell-like homing features^{40–42}, we examined whether ZDM could enhance MSC migration to the injured joint. Both Transwell and wound-healing assays showed that ZDM promoted cell migration, with greater efficacy than ZD or MC-HM alone (Fig. 5d). To determine whether this translated into stem cell migration in vivo, fluorescence-labelled MSCs were administered intravenously via the tail vein. Longitudinal in vivo imaging showed that ZDM increased MSC accumulation in the joint, indicating enhanced homing of circulating MSCs (Fig. 5e). Consistent with this, CD105 staining at 72 h revealed a pronounced increase in the ZDM-treated joints (Fig. 5f and Supplementary Fig. 20a). Together, these findings demonstrate that ZDM enhances MSC migratory competence in vitro and increases the joint accumulation of administered MSCs in vivo.

ZDM restores MSC chondrogenic capacity through Zn²⁺-enabled TRIM15-YAP-SOX9 regulation

Because aged OA cartilage retains MSC-like cells, we next asked whether ZDM could restore the chondrogenic capacity of these locally available cells. In microsphere culture, ZDM enhanced cartilage-like matrix formation, as evidenced by stronger Safranin O and toluidine blue staining together with increased ACAN and COL2A1 signals (Fig. 5g and Supplementary Fig. 20b). ZDM also increased expression of the chondrogenic transcription factor SOX9 in MSCs (Fig. 5h) and elevated SOX9 in chondrocytes (Supplementary Fig. 20c). In line with these findings, ZDM enhanced extracellular matrix deposition in both monolayer and microsphere cultures (Supplementary Fig. 21a,b). Moreover, ZDM restored an anabolic phenotype while suppressing catabolic markers, with increased SOX9 and COL2A1 and reduced MMP13 and MMP3 expression (Supplementary Fig. 22). We then investigated the mechanism by which ZDM promoted chondrogenesis. A previous study showed that TRIM15 interacts with YAP and facilitates its nuclear localisation by regulating YAP ubiquitination, thereby contributing to chondrocyte senescence and osteoarthritis¹⁸. In parallel, YAP has been identified as a negative regulator of MSC chondrogenesis, and YAP can repress SOX9 expression and chondrogenic differentiation³². We first confirmed the interaction between TRIM15 and YAP by co-immunoprecipitation (Fig. 5i). To identify ZDM-associated proteins of the nanoplatform, ZDM

was incubated with MSC lysates, and immunoblotting of bound proteins showed affinity of ZDM for both TRIM15 and YAP (Fig. 5i). We next assessed whether ZDM influenced YAP subcellular localisation. Under IL-1 β stimulation, YAP exhibited prominent nuclear accumulation, whereas treatment with ZDM markedly reduced nuclear YAP and promoted its redistribution to the cytoplasm (Fig. 5j and Supplementary Fig. 23a). At the protein level, ZDM decreased TRIM15 abundance while increasing SOX9 and COL2A1 expression (Fig. 5k and Supplementary Fig. 22). Consistent with the reported mechanism linking TRIM15 to YAP ubiquitination¹⁸, additional validation showed that YAP ubiquitination was regulated under inflammatory stimulation (Supplementary Fig. 23b). Collectively, these data support a model in which ZDM promotes chondrogenesis by suppressing TRIM15, relieving YAP-dependent repression of SOX9, and thereby driving matrix anabolism and cartilage-lineage differentiation (Fig. 5l).

ZDM remodels the oxidative-senescent cartilage and rejuvenates aged MSCs through restoration of mitochondrial homeostasis

Because a degenerative cartilage niche characterized by oxidative stress and senescence is unfavourable for reparative stem-cell function, we first asked whether ZDM could alleviate the hostile microenvironment of aged chondrocytes. In aged chondrocytes, total ROS, superoxide and hydroxyl radical-associated signals were all markedly elevated, whereas treatment with ZDM substantially reduced these oxidative stress readouts (Fig. 6a and Supplementary Fig. 24a). Consistent with this, aged chondrocytes displayed increased expression of the senescence-associated markers p16 and p21, together with elevated γ -H2AX; these changes were all attenuated by ZDM (Fig. 6b and Supplementary Fig. 24b). We next sought to understand how ZDM influenced aged MSCs at the molecular level. Enrichment analysis of upregulated ZDM-responsive targets identified regulation of mitophagy as a prominent process in MSCs (Fig. 6c). Mitophagy is a central mitochondrial quality-control mechanism that preserves stem-cell fitness by eliminating damaged mitochondria and thereby supports self-renewal, differentiation capacity, and resistance to aging-associated stress^{37,43,44}. We therefore examined whether ZDM could restore mitochondrial fitness in aged MSCs. Compared with young controls, aged MSCs exhibited a marked loss of mitochondrial membrane potential, as shown by a reduced JC-1

329 aggregate/monomer ratio, together with increased SA- β -gal positivity; both abnormalities
330 were substantially reversed by ZDM (Fig. 6d,e). In parallel, ZDM also lowered
331 mitochondrial ROS, as indicated by reduced MitoSOX fluorescence, and partially restored
332 proliferative capacity, as evidenced by increased EdU incorporation (Supplementary Fig.
333 25a,b). We then examined whether this recovery was associated with improved
334 mitochondrial turnover. Aged MSCs showed impaired mitochondria-lysosome
335 colocalisation, whereas ZDM restored this interaction, consistent with enhanced
336 mitophagy-related trafficking (Fig. 6f and Supplementary Fig. 26).

337 This was accompanied by recovery of intracellular ATP levels (Fig. 6g), supporting
338 improved mitochondrial function. At the protein level, ZDM increased the expression of
339 PINK1, Parkin, LC3B, SIRT1, PGC1 α and TFAM, while reducing p62, p53, p21 and p16
340 (Fig. 6h), indicating coordinated restoration of mitophagy, mitochondrial biogenesis and
341 senescence control. Seahorse analysis further showed that aged MSCs had markedly
342 reduced oxygen consumption, whereas ZDM restored their respiratory activity, including
343 maximal respiration, towards a young-like state (Fig. 6i,j). Transcriptomic comparison
344 showed that ZDM partially reversed the aged MSC expression programme, downregulating
345 a subset of genes elevated during aging while reactivating genes suppressed in the aged
346 state (Fig. 6k,l). Enrichment analysis of these restored genes highlighted processes related
347 to autophagy of mitochondrion, cellular respiration, response to oxidative stress, regulation
348 of cellular senescence, cartilage development and mitochondrial gene expression (Fig.
349 6m). qPCR validation further confirmed that ZDM increased the expression of Pink1, Prkn,
350 Tfam, Ppargc1a and Sirt1, while suppressing the senescence-associated transcript
351 Cdkn1a in aged MSCs (Fig. 6n). Consistently, ZDM dose-dependently enhanced PINK1-
352 Parkin-associated mitophagy signalling, as shown by increased PINK1, Parkin and LC3B
353 together with reduced p62 (Supplementary Fig. 27a). To determine whether PINK1-
354 dependent mitophagy was required for the rejuvenation-like effects of ZDM, we silenced
355 PINK1 in aged MSCs. Notably, siPINK1 markedly blunted the ability of ZDM to reduce SA-
356 β -gal positivity (Supplementary Fig. 27b), restore mitophagy-related proteins
357 (Supplementary Fig. 27c), and enhance mitochondria-lysosome colocalisation
358 (Supplementary Fig. 27d). These findings indicate that the beneficial effects of ZDM on

aged MSCs depended, at least in part, on PINK1-mediated mitophagy rather than merely accompanying mitochondrial improvement.

Collectively, these data show that ZDM alleviates oxidative-senescent stress in cartilage and induces a rejuvenation-like recovery of aged MSCs, at least in part through restoration of PINK1–Parkin-dependent mitochondrial homeostasis.

ZDM restrains OA progression and restores cartilage homeostasis in aged DMM mice

To determine whether the protective effects observed in vitro and ex vivo translated into therapeutic benefit in vivo, we performed DMM surgery in 21-month-old mice, while 3-month-old mice were included as a young reference group. Intra-articular treatment was initiated 1 week after DMM surgery, and joints were harvested at week 8 for micro-CT and histological analyses (Fig. 7a). Gait analysis showed that aged DMM mice displayed a marked reduction in stride length compared with both young mice and aged sham controls, indicating impaired joint function in the aged OA setting. Intra-articular administration of ZDM significantly improved stride length, whereas ZD produced a weaker effect (Fig. 7b).

Because periarticular bone remodelling is a key component of OA progression, we next assessed osteophyte formation and subchondral bone architecture by micro-computed tomography. Representative 2D sections and 3D reconstructions showed pronounced osteophyte formation, irregular bony contours and severe subchondral structural deterioration in aged DMM joints, whereas these changes were visibly alleviated after ZDM treatment (Fig. 7c,d). Additional pseudocolour and greyscale coronal sections further supported the structural rescue of subchondral bone by ZDM (Supplementary Fig. 28). Quantitative analysis showed that aged DMM mice exhibited reduced trabecular thickness (Tb.Th) and bone volume fraction (BV/TV), together with increased osteophyte number and bone surface-to-volume ratio (BS/BV), relative to young controls and aged sham mice. These pathological changes were partially improved by ZD and more effectively corrected by ZDM (Fig. 7c), indicating that ZDM attenuated OA-associated periarticular bone remodelling in aged mice. We next assessed cartilage structural integrity by histological staining. Safranin O staining showed marked surface fibrillation and proteoglycan loss in aged DMM cartilage, whereas ZDM treatment preserved cartilage architecture and matrix staining intensity to a substantially greater extent than ZD (Fig.

7e). Consistently, OARSI scores were significantly elevated in aged DMM joints and were reduced after treatment, with the strongest improvement observed in the ZDM group (Fig. 7f). Under polarized light microscopy, picrosirius red staining revealed prominent accumulation of abnormal collagen fibrils in aged sham and, more markedly, aged DMM cartilage, whereas ZDM markedly diminished this signal (Fig. 7e,f). In parallel, immunohistochemical analysis showed that ZDM restored anabolic matrix markers, as evidenced by increased COL2A1-positive area, while suppressing the catabolic marker MMP13 (Fig. 7e,f). Independent whole-joint Safranin O staining further confirmed the chondroprotective effect of ZDM in vivo (Supplementary Fig. 29a).

Given the age-dependent context of this model, we further examined senescence- and mitochondrial quality control-related markers in cartilage. Aged sham cartilage already displayed increased p16- and p21-positive chondrocytes relative to young mice, and these senescence-associated signals remained elevated after DMM surgery. Both markers were significantly reduced by ZDM treatment, with a greater effect than ZD (Fig. 7e,f). In addition, the COL1 signal, which was elevated in aged cartilage and remained high after DMM, was attenuated after treatment. By contrast, the mitochondrial quality-control marker PINK1, which was suppressed in aged cartilage, was restored by ZDM (Fig. 7e,f). Consistent with the intended genetic intervention, TRIM15 immunostaining was also reduced in ZDM-treated joints (Supplementary Fig. 29b,c). Notably, CD90 staining was increased in ZDM-treated joints (Supplementary Fig. 29b,c), consistent with enhanced accumulation of reparative stromal or MSC-associated cells in the joint after treatment. Together, these findings show that ZDM effectively suppresses OA progression in aged mice by improving joint function, mitigating periarticular bone remodelling, preserving cartilage matrix homeostasis and reducing senescence-associated cartilage deterioration. Importantly, repeated intra-articular administration of ZDM did not produce overt systemic toxicity. Serum biochemical indices remained comparable among groups, and histological examination of major organs, including the heart, lung, liver, kidney and spleen, revealed no obvious pathological abnormalities after treatment (Supplementary Fig. 30). These data support the in vivo biosafety of the platform.

ZDM confers disease-modifying efficacy in beagle dogs and human cartilage explants

To further evaluate the translational potential of ZDM beyond murine models, we tested its

therapeutic efficacy in a clinically relevant beagle OA model. Aged beagle dogs underwent surgery-induced OA, followed by monthly intra-articular administration of ZD or ZDM at months 1, 2 and 3, and joints were collected 4 months after surgery for imaging and histological analyses (Fig. 8a). Radiographic examination showed that OA joints exhibited marked narrowing of the medial joint space compared with sham controls, whereas ZDM treatment significantly restored medial joint space width; ZD produced only partial improvement (Fig. 8b).

We next performed multimodal imaging to assess structural protection at the joint level. Representative micro-computed tomography and magnetic resonance images revealed cartilage thinning, effusion-synovitis and subchondral bone deterioration in OA joints, whereas these pathological changes were visibly alleviated after ZDM treatment (Fig. 8c). Quantitative analysis confirmed that ZDM significantly reduced the effusion-synovitis score and increased cartilage thickness relative to untreated OA joints (Fig. 8d,e). Micro-computed tomography further showed that OA induced severe subchondral bone loss and trabecular architectural disruption, whereas ZDM improved bone microarchitecture, as reflected by increased bone volume fraction and trabecular number, together with reduced trabecular separation (Fig. 8f). In parallel, synovial fluid analysis showed that OA markedly elevated MMP13, IL-1 β and TNF- α , whereas these inflammatory and catabolic mediators were significantly reduced after ZDM treatment and were only modestly affected by ZD (Fig. 8g). Histological assessment supported these imaging and biochemical findings. ZDM preserved Safranin O-positive cartilage matrix and surface integrity (Fig. 8h) and reduced synovial hyperplasia and inflammatory infiltration in the synovium (Fig. 8i). Gross cartilage scoring, OARSI grading and synovitis scoring further confirmed the superior disease-modifying efficacy of ZDM over ZD in beagle dogs (Supplementary Fig. 31). Repeated intra-articular administration did not induce obvious histopathological abnormalities in major organs, supporting the systemic biosafety of the treatment (Supplementary Fig. 32). Together, these data indicate that ZDM exerts disease-modifying activity in a large-animal OA model by simultaneously protecting cartilage, mitigating synovitis and preserving subchondral bone homeostasis.

To determine whether these therapeutic effects extended to human tissue, we

established an ex vivo explant culture system using paired intact and damaged cartilage specimens obtained from patients undergoing joint replacement surgery (Fig. 8j). Consistent with the in vivo findings, damaged human cartilage explants displayed marked loss of proteoglycan-rich matrix, as evidenced by reduced Safranin O and Alcian blue staining, whereas ZDM treatment restored matrix staining intensity (Fig. 8k). Immunohistochemical analysis further showed that ZDM increased the hyaline cartilage-associated marker COL2A1 and reduced the fibrocartilage-associated marker COL1 in damaged explants (Fig. 8l). ZDM also suppressed inflammatory and senescence-associated signals, as indicated by decreased IL-1 β and p21 staining (Fig. 8m). Additional analyses showed restoration of ACAN and COL2A1, together with reductions in MMP13, COL1A1, TNF- α , p16 and TRIM15 signals (Supplementary Figs. 33 and 34). Because durable cartilage repair also depends on the functional competence of reparative progenitors, we next examined whether ZDM could reverse age-associated dysfunction in human MSCs. Human MSCs isolated from young and aged donors were cultured from passage 1 to passage 6 in the presence or absence of ZDM (Supplementary Fig. 35a). Aged human MSCs displayed marked senescence, as shown by increased SA- β -gal staining, whereas ZDM substantially reduced this phenotype (Supplementary Fig. 35b). Consistent with impaired chondrogenic potential in aged human MSCs, toluidine blue staining showed reduced cartilage-like matrix deposition, which was restored by ZDM treatment (Supplementary Fig. 35c). Multilineage differentiation assays further supported an age-associated drift in lineage propensity that was partially normalized by ZDM (Supplementary Fig. 36a,b). At the molecular level, ZDM restored SOX9 expression and intracellular labile Zn²⁺ levels, reduced p21 expression and increased EdU incorporation in aged human MSCs (Supplementary Fig. 37a,b).

Collectively, these findings extend the translational relevance of ZDM beyond murine systems and support its capacity not only to protect diseased cartilage in vivo and ex vivo but also to rejuvenate aged human MSCs towards a more reparative state.

Conclusion

In this study, we developed ZDM, a hybrid membrane-coated meso-ZnO_v that integrates

damaged-cartilage targeting, microenvironmental restoration and rejuvenation of aged MSCs for OA therapy. ITGA2-COL1 recognition promoted lesion-associated localisation, while the hybrid membrane enhanced cartilage penetration and cellular uptake. At the damaged joint, meso-ZnO_v acted as an active therapeutic core by scavenging reactive oxygen species and releasing Zn²⁺, which restored zinc homeostasis and served as a catalytic cofactor for the TRIM15-targeting DNAzyme. In aged MSCs, ZDM reduced TRIM15 abundance and YAP nuclear localisation, restored SOX9 expression and PINK1-dependent mitophagy, thereby reversing senescence and recovering chondrogenic capacity. These coordinated effects restored cartilage matrix homeostasis and improved OA outcomes in aged mouse and beagle models. This work establishes a ZnO-centred nanotherapeutic strategy that combines lesion-oriented delivery with MSC rejuvenation, providing a potential disease-modifying approach for age-associated OA.

Materials and Methods

Synthesis of meso-ZnO_v and meso-ZnO

Synthesis of KIT-6: Mesoporous KIT-6 was synthesized according to previously reported procedures⁴⁵.

Synthesis of meso-ZnO_v: 1.0 mmol of Zn(NO₃)₂·6H₂O and 0.30 g of KIT-6 were added to a beaker containing 15 mL of ethanol, stirred for 30 min and then heated at 60 °C until the ethanol was removed. The mixture was then transferred to a muffle furnace and heated at 550 °C for 6 h to obtain the oxide/KIT-6 hybrid. The oxide/KIT-6 hybrid was transferred to a tube furnace and reacted under an H₂/N₂ atmosphere (5:95) at 300 °C for 3 h, followed by etching in a 5 wt% HF/20 wt% NH₄F buffer solution for 20 min. After washing with water and ethanol, the meso-ZnO_v was finally obtained.

Synthesis of meso-ZnO: meso-ZnO was synthesized using a similar route but without tube-furnace reduction under an H₂/N₂ atmosphere (5:95) at 300 °C for 3 h.

Isolation of MSCs from mouse knee joints

MSCs were isolated from the knee joints of young (3-month-old) and aged (21-month-old) mice. Briefly, after removal of the skin and surrounding muscle, the knee joints were

exposed under sterile conditions, and intra-articular soft tissues were carefully collected. Tissues were minced at 37 °C with gentle agitation. The cell suspension was filtered through a 70-µm cell strainer, centrifuged and resuspended in complete culture medium. Adherent fibroblast-like cells were expanded and used for subsequent analyses. These cells are referred to throughout the manuscript as MSCs. MSC identity was further confirmed by tri-lineage differentiation assays and marker analysis as described below.

Isolation of chondrocyte and MSC membranes

Plasma membranes were isolated separately from ITGA2-overexpressing primary chondrocytes and MSCs by differential centrifugation. Cells were washed with PBS, collected by gentle scraping and centrifuged at 800 × g for 5 min. Cell microspheres were resuspended in ice-cold isolation buffer (75 mM sucrose, 225 mM mannitol, 30 mM Tris–HCl, pH 7.4, 0.5% (w/v) bovine serum albumin, 0.5 mM EGTA, and protease/phosphatase inhibitors) and disrupted by ultrasonication (30 W) for 5 min in an ice-water bath. Homogenates were centrifuged at 3,000 × g for 5 min at 4 °C to remove nuclei and cell debris, followed by centrifugation at 10,000 × g for 10 min at 4 °C to remove mitochondria and other heavy organelles. The supernatants were then ultracentrifuged at 100,000 × g for 2 h at 4 °C to collect plasma membrane fractions. Membrane microspheres were resuspended in 0.2 mM EDTA and quantified by BCA assay. Chondrocyte membranes and MSC membranes were subsequently mixed at the indicated protein ratio and extruded to generate hybrid membrane vesicles (MC-HM), which were used immediately for nanoparticle coating or stored at –80 °C until use.

Human cartilage samples

Human articular cartilage specimens and tissues for MSC isolation were obtained from young and old donors at the Department of Orthopaedics, West China Hospital, Sichuan University (Supplementary Table 1). Donors were stratified into young and old groups according to age at collection, and these group labels were used consistently for human cartilage and MSC analyses. The old OA cartilage cohort used for paired intact/damaged explant experiments comprised 14 patients undergoing total knee arthroplasty, including 8 male and 6 female patients. All procedures involving human samples were conducted in

539 accordance with the Declaration of Helsinki and were approved by the Institutional Ethics
540 Committee of West China Hospital, Sichuan University (No. 20241464). Written informed
541 consent was obtained from all participants before sample collection. Osteoarthritis was
542 diagnosed on the basis of clinical presentation and standing lower-limb radiographs
543 according to standard diagnostic criteria. For explant experiments, articular cartilage
544 specimens were macroscopically evaluated and divided into relatively intact (I) and
545 damaged (D) regions according to the local degree of cartilage degeneration. For age-
546 comparison analyses, cartilage and MSC samples were processed according to donor age
547 group. For molecular analyses, freshly isolated cartilage samples were snap-frozen in
548 liquid nitrogen immediately after collection and stored until protein extraction. Parallel
549 specimens for histological and morphological analyses were fixed in 10% neutral-buffered
550 formalin.

551 Human cartilage explant treatment

552 For ex vivo therapeutic testing, paired relatively intact and damaged cartilage regions from
553 old OA donors were trimmed into explants and equilibrated in culture medium. Damaged
554 explants were treated with vehicle or ZDM, whereas relatively intact explants served as
555 paired tissue controls. After culture, explants were fixed, embedded and analysed by
556 Safranin O, Alcian blue, immunohistochemistry or immunofluorescence to assess matrix,
557 inflammatory, senescence-associated and TRIM15-related markers.

559 Human MSC expansion and functional assays

560 Human MSCs isolated from young and old donors were expanded from passage 1 to
561 passage 6 with vehicle or ZDM treatment, as indicated in the corresponding experiments.
562 Cellular senescence, proliferation, labile Zn^{2+} , SOX9 and p21 were assessed by SA- β -gal
563 staining, EdU incorporation and fluorescence imaging. Chondrogenic, adipogenic and
564 osteogenic differentiation were evaluated using toluidine blue, Oil Red O and Alizarin Red
565 S staining, respectively.

567 Animal experiments

All animal procedures were conducted in accordance with institutional guidelines for animal care and use and were approved before study initiation. Mouse experiments were approved under protocol No. 20240527004, and beagle dog experiments were approved under protocol No. 20260206009. Animals were monitored throughout the study, and all surgical and intra-articular procedures were performed under appropriate anaesthesia and aseptic conditions to minimise pain and distress.

Animals

For the murine efficacy study, 21-month-old C57BL/6 mice underwent destabilization of the medial meniscus (DMM) surgery to induce osteoarthritis. Intra-articular injections of ZD or ZDM were performed at weeks 1, 3, 5 and 7 after surgery, and mice were euthanized at week 8. Three-month-old mice served as young controls, and 21-month-old sham-operated mice served as age-matched surgical controls. Gait behaviour was assessed at baseline and week 7, and knee joints were collected for micro-CT and histological analyses. For the large-animal efficacy study, male beagle dogs underwent surgery-induced knee osteoarthritis, followed by monthly intra-articular administration of ZD or ZDM at months 1, 2 and 3 after surgery. Sham-operated and untreated OA animals were included as controls. Knee joints and synovial fluid were collected 4 months after surgery for radiography, magnetic resonance imaging, micro-CT, biochemical analysis and histological assessment.

Histological analysis

Human cartilage and murine knee joints were fixed in 10% neutral-buffered formalin, decalcified in 0.5 M EDTA (pH 7.2) for 2 weeks, embedded in paraffin and sectioned coronally at 5 µm. For murine joints, serial sections spanning the entire medial compartment were collected from the first appearance to the disappearance of the medial meniscus. Safranin O/Fast Green staining (Solarbio, G1371) was performed according to the manufacturer's protocol. Cartilage degeneration at the medial tibial plateau was quantified using the OARSI scoring system (0–6). All cartilage scoring was performed independently by two blinded observers.

In vivo micro-CT analysis

For micro-computed tomography (micro-CT) analysis, knee joint samples were fixed in 4% paraformaldehyde for 48 h and scanned using a high-resolution micro-CT system (SkyScan 1275). The region of interest was defined to encompass the entire medial compartment of the tibial subchondral bone. Three-dimensional microarchitectural parameters, including BV/TV, Tb.Th, Tb.N, Tb.Sp, BS/BV and osteophyte-related measurements, were calculated using the manufacturer's software.

SA- β -gal staining

Cellular senescence was assessed using a senescence-associated β -galactosidase (SA- β -gal) staining kit (Beyotime). For age-comparison experiments, young and aged MSCs were stained after culture under the indicated conditions. For treatment experiments, MSCs were exposed to the indicated formulations for the indicated duration before staining. Cells were fixed with 0.5% glutaraldehyde for 10 min and washed three times with PBS, followed by incubation with SA- β -gal staining solution at 37 °C overnight at pH 6.0. SA- β -gal-positive cells were identified under an inverted phase-contrast microscope (Nikon). Quantification was performed by counting total cells and SA- β -gal-positive cells in each dish using ImageJ (v2.9.0).

Western blotting

Total protein was extracted from primary mouse chondrocytes and human articular cartilage using RIPA buffer (Thermo Fisher Scientific) supplemented with protease and phosphatase inhibitors. Protein concentrations were determined using a BCA Protein Assay Kit (Thermo Fisher Scientific). Equal amounts of protein (20–40 μ g per lane) were separated by SDS–PAGE and transferred to 0.22- μ m polyvinylidene difluoride membranes (Millipore). Membranes were blocked with 5% non-fat milk in TBST for 2 h at room temperature and incubated overnight at 4 °C with the indicated primary antibodies. After washing in TBST, membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Signals were visualized using enhanced chemiluminescence substrate (Biosharp, BL520A) and imaged with a ChemiDoc system (Bio-Rad). Antibody information is provided in Supplementary Table 2.

Immunoprecipitation (IP) assay

For co-immunoprecipitation (co-IP), cells were lysed on ice for 30 min in IP lysis buffer (Thermo Scientific, 87787) supplemented with protease and phosphatase inhibitors. Lysates were clarified by centrifugation at $12,000 \times g$ for 10 min at 4 °C, and 5% of the supernatant was retained as input. The remaining lysate was incubated with the indicated antibodies or antibody-conjugated magnetic beads for 3 h at 4 °C with rotation. Isotype-matched IgG was used as a negative control where appropriate. Beads were washed three times with cold IP buffer, and bound proteins were eluted by boiling in 2× SDS loading buffer at 95 °C for 10 min. Eluates and input samples were analysed by western blotting.

Immunohistochemistry (IHC)

Paraffin-embedded sections (5 µm) were deparaffinized, rehydrated and subjected to heat-induced antigen retrieval for 15 min. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide for 10 min. After blocking with 5% bovine serum albumin for 1 h, sections were incubated with primary antibodies overnight at 4 °C. Signals were detected using an HRP-conjugated secondary antibody polymer system and visualized with 3,3'-diaminobenzidine (DAB). Sections were counterstained with haematoxylin, dehydrated and mounted. Whole-slide images were acquired using an Olympus VS200 slide scanner. Positive cells and integrated optical density were quantified in at least five randomly selected fields per section using ImageJ (v1.53c, NIH) by two observers blinded to group allocation. Antibody information is provided in Supplementary Table 2.

Immunofluorescence (IF) staining

Cells grown on glass-bottom dishes or coverslips were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.3% Triton X-100 in PBS for 3 min. After blocking with 10% normal goat serum for 1 h, samples were incubated with primary antibodies overnight at 4 °C, followed by fluorescent secondary antibodies for 1 h at room temperature in the dark. Nuclei were counterstained with DAPI for 5 min. Images were acquired by confocal microscopy, and fluorescence intensity and colocalisation were analysed using ImageJ.

Molecular dynamics simulation

Molecular dynamics (MD) simulations of the ITGA2–COL1 complex were performed using the Desmond module in Schrödinger Suite (release 2021-4). The top-ranked docking pose was used as the initial structure. The complex was placed in an orthorhombic box with a 10 Å buffer, solvated with the TIP3P explicit water model, neutralized with counterions, and supplemented with 0.15 M NaCl. Simulations were conducted using the OPLS4 force field. After the default Desmond relaxation protocol, a 100-ns production run was carried out in the NPT ensemble at 300 K and 1.01325 bar using the Nosé–Hoover chain thermostat and Martyna–Tobias–Klein barostat. Trajectory frames were saved every 250 ps. Structural stability and flexibility were assessed by calculating the root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of protein C α atoms. Protein–protein interactions were analysed using the Desmond Simulation Interaction Diagram.

RT-qPCR analysis

Total RNA was isolated from cells using TRIzol reagent (Thermo Fisher Scientific) following the manufacturer's instructions. RNA (2 μ g) was reverse transcribed into first-strand complementary DNA (cDNA) using a cDNA synthesis kit (Takara, catalogue number RR036A). Real-time PCR was then performed using the FastStart Universal SYBR Green master kit (Roche, catalogue number 4913914001) on an Applied Biosystems 7500 real-time PCR system (Life Technologies) to determine relative mRNA expression levels. Fold changes in target-gene expression were calculated with the $2^{-\Delta\Delta C_t}$ method using β -actin as a reference control. Primer sequences are provided in Supplementary Table 3.

In vitro stem cell migration assay

Briefly, the lower chamber was filled with 200 μ l of serum-free medium containing the indicated formulation. MSCs were then seeded into the upper insert (8- μ m pore size, 24-well plate) at a density of 1×10^4 cells per well. The chamber was incubated at 37 °C in a 5% CO $_2$ incubator for 4 h to allow for MSC migration. After incubation, the cells that had migrated to the lower chamber were fixed with 4% paraformaldehyde for 10 min. Following fixation, the cells were stained with 0.1% crystal violet solution for 10 min. The stained cells were then washed with

PBS to remove excess dye and imaged under a microscope. Images were analysed using ImageJ software.

Intracellular ROS-scavenging assay in vitro

The antioxidative capacity of ZD and ZDM was assessed by measuring intracellular total ROS, hydroxyl radicals ($\cdot\text{OH}$) and superoxide ($\text{O}_2\cdot^-$) in primary chondrocytes. Cells were seeded in confocal dishes (1×10^5 cells per dish) overnight and then exposed to inflammatory or oxidative stimulation together with the indicated treatments. Control cells received fresh medium alone. After incubation, cells were washed and stained with DCFH-DA (10 μM , 20 min) to detect total ROS. In parallel assays, $\cdot\text{OH}$ and $\text{O}_2\cdot^-$ were detected using APF (10 μM , 30 min) and DHE (10 μM , 30 min), respectively. Nuclei were counterstained with DAPI, and fluorescence signals were acquired by confocal laser scanning microscopy and quantified using ImageJ.

Mitochondrial function and proliferation assays

Mitochondrial membrane potential was assessed using JC-1 staining, mitochondrial ROS was detected with MitoSOX, and intracellular ATP was quantified using a luminescence-based ATP assay. Labile intracellular Zn^{2+} was measured with FluoZin-3, and mitochondria-lysosome colocalisation was evaluated after PK-Mito and LysoBrite staining. Cellular proliferation was assessed using EdU incorporation. Oxygen consumption rate was measured using a Seahorse extracellular flux analyser after sequential addition of oligomycin, FCCP and rotenone/antimycin A.

Synthesis of TRIM15 DNAzyme

Three species-matched TRIM15-targeting DNAzymes were designed for human, mouse and dog TRIM15 transcripts, respectively, to support the corresponding in vitro, murine and beagle experiments. Candidate cleavage sites were selected within accessible regions of the TRIM15 mRNA sequence, and each DNAzyme was designed to contain a central catalytic core flanked by two antisense binding arms complementary to the species-specific TRIM15 target sequence (Supplementary Table 4). Oligonucleotides were chemically synthesized and purified by high-performance liquid chromatography. Lyophilized DNAzymes were dissolved in nuclease-free

water to prepare stock solutions and stored at -20°C . Before use, DNazymes were diluted in the indicated buffer or culture medium and mixed with meso-ZnO_v to generate ZD, allowing the released Zn²⁺ from meso-ZnO_v to serve as the catalytic cofactor for TRIM15 mRNA cleavage.

Multicellular cartilage spheroids

Multicellular cartilage spheroids were generated using the hanging-drop method. Briefly, primary chondrocytes were suspended in DMEM containing 1.2% methylcellulose at a density of 1×10^6 cells ml⁻¹. Aliquots (20 μl) were placed on the lid of a culture dish, which was then inverted over the dish and incubated at 37°C in 5% CO₂. After 3 days, the resulting spheroids were collected and incubated with Cy5-labelled nanoparticles (200 $\mu\text{g ml}^{-1}$) for 12 h. After three washes with PBS, spheroids were transferred to confocal dishes and imaged by confocal microscopy using Z-stack acquisition with 25- μm intervals from apex to equator. Penetration depth and fluorescence intensity were quantified using ImageJ.

Cartilage explant permeation

Human cartilage explants were prepared as described above for ex vivo cartilage analyses. After equilibration in culture medium, explants were incubated with Cy5-labelled ZD or ZDM at a final concentration of 200 $\mu\text{g ml}^{-1}$ for 3 days. After incubation, samples were washed thoroughly with PBS to remove unbound nanoparticles, embedded in Tissue-Tek optimum cutting temperature compound, and cryosectioned into 10- μm slices. Sections were mounted with DAPI Fluoromount-G mounting medium and imaged immediately using a confocal fluorescence microscope (Zeiss LSM 710, Zeiss). ZD was used as the control formulation for comparison of cartilage penetration.

In vivo fluorescence tracking and cartilage penetration assay

For in vivo fluorescence tracking, 50 μl of sterile isotonic saline containing Cy5-labelled ZD or ZDM (5 mg ml⁻¹) was injected into the right knee joint of mice. Fluorescence signals in knee joint sections were examined at the indicated time points by confocal fluorescence imaging to assess cartilage localisation and penetration. Fluorescence intensity and penetration depth within cartilage were quantified using ImageJ.

749

750 Cellular uptake of ZDM

751 To assess lysosomal escape, cells were seeded on glass-bottom dishes and cultured overnight.
752 Cy5-labelled ZDM was added and incubated with cells for 1 h or 6 h. At the indicated time points,
753 lysosomes were labelled with LysoBrite according to the manufacturer's protocol. Cells were
754 then washed with PBS, fixed with 4% paraformaldehyde for 15 min and counterstained with
755 DAPI. Confocal images were acquired using a Zeiss LSM 710 microscope. Colocalisation
756 between Cy5-labelled ZDM and lysosomes was quantified using ImageJ. Early colocalisation
757 with lysosomes followed by reduced overlap at later time points was used as evidence of
758 lysosomal escape.

759

760 RNA sequencing

761 Cells were assigned to two groups, control and ZDM, with three independent biological
762 replicates per group. Total RNA was extracted using TRIzol reagent (Invitrogen) according to
763 the manufacturer's instructions. RNA concentration and purity were assessed using a
764 NanoDrop spectrophotometer, and RNA integrity was evaluated using an Agilent 2100
765 Bioanalyser. Samples with an RNA integrity number greater than 7.0 were used for subsequent
766 library construction. Sequencing libraries were prepared using the NEBNext Ultra RNA Library
767 Prep Kit (New England Biolabs) following the manufacturer's protocol and sequenced on an
768 Illumina NovaSeq 6000 platform to generate paired-end reads. Raw reads were processed to
769 remove adaptor sequences, low-quality reads and reads containing excessive ambiguous
770 bases, yielding clean reads for downstream analysis.

771

772 Proteomic analysis

773 Primary mouse chondrocytes were assigned to two groups, control and ZDM, with three
774 independent biological replicates per group. Cells were lysed in protein extraction buffer
775 containing protease inhibitors, and protein concentrations were determined using a BCA
776 Protein Assay Kit. Equal amounts of protein from each sample were reduced, alkylated and
777 digested with trypsin using standard in-solution digestion procedures. The resulting peptides
778 were desalted and subjected to liquid chromatography–tandem mass spectrometry (LC–

MS/MS) analysis. Peptide separation was performed on an EASY-nLC 1200 system coupled to a Q Exactive HF-X mass spectrometer (Thermo Fisher Scientific). Raw mass spectrometry data were processed using MaxQuant software against the UniProt mouse protein database.

Statistical analysis

Statistical analyses were performed using GraphPad Prism (v.10.1.2). To ensure objectivity, sample selection and histological quantification were performed using a randomised and blinded approach. The normality of continuous data distributions was assessed using the Shapiro–Wilk test. Comparisons between two groups were performed using a two-sided unpaired Student's t-test. Comparisons among multiple groups of continuous data were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. Ordinal scoring data, including OARSI grade, synovitis score and macroscopic score, were analysed using the Kruskal–Wallis test followed by Dunn's multiple-comparison test. Continuous data are presented as mean $\pm$ s.d., whereas ordinal scoring data are presented as median with interquartile range. Each data point represents one biologically independent sample unless otherwise indicated. Technical replicates or multiple imaging fields were averaged to generate one value for each biological replicate. Differences were considered statistically significant at $P < 0.05$.

Conflict of Interest

The authors declare no competing interests.

Author Contributions Statement

Z.Z., C.Z., P.L., Z.X., B.L., and C.H. conceived and designed the study. H.C., H.Z., B.L., and C.H. secured funding and supervised the study. Z.Z., C.Z., P.L., Z.X., Q.Z., and Y.L. developed the methodology. Z.Z., C.Z., P.L., and Z.X. performed the experiments, analysed the data, and interpreted the results. Z.Z., C.Z., P.L., Z.X., Q.Z., and Y.L. generated the visualisations. Z.Z., C.Z., P.L., and Z.X. drafted the initial manuscript. H.C., H.Z., B.L., and C.H. critically revised the manuscript. All authors reviewed and approved the final version.

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Data availability

The transcriptomic data generated in this study have been deposited in the NCBI Gene Expression Omnibus under accession number GSE334288. The proteomics data have been deposited in the ProteomeXchange Consortium via the iProX repository under accession number PXD078178. All other data supporting the findings of this study are available within the article or from the corresponding authors upon reasonable request.

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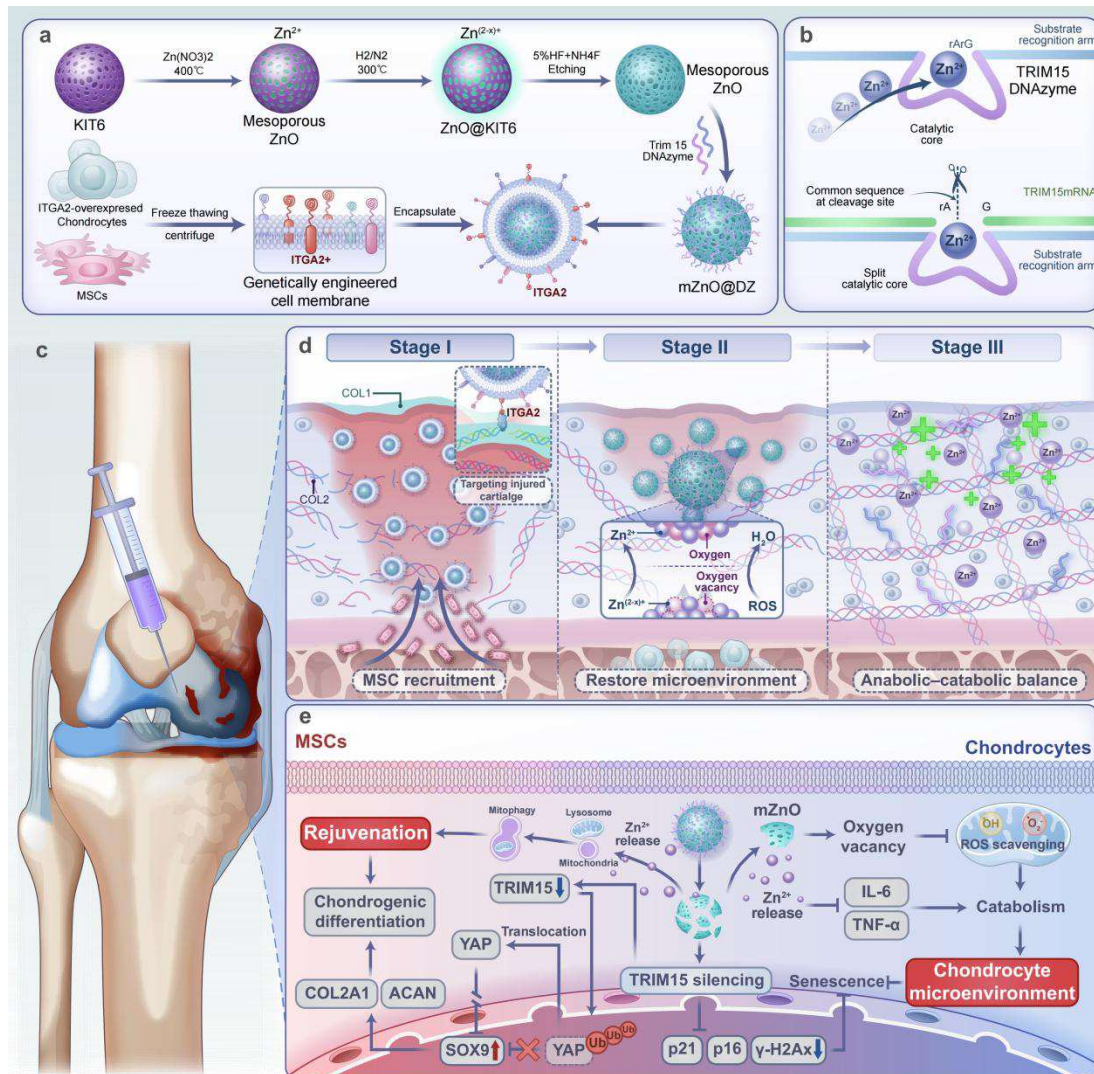


Fig. 1 Schematic illustration of the biomimetic ZDM nanoplatform for osteoarthritis therapy.

a, Fabrication of the membrane-camouflaged nanotherapeutic, where mesoporous ZnO (meso-ZnO_v) loaded with TRIM15 DNAzyme is encapsulated by a genetically engineered (ITGA2-overexpressed) hybrid cell membrane derived from chondrocytes and MSCs.

b, Zn^{2+} -dependent cleavage of TRIM15 mRNA by the DNAzyme.

c,d, **Intra-articular** administration and the in vivo three-stage tissue repair process: targeted MSC migration, ROS scavenging, and microenvironment restoration.

e, Intracellular mechanisms of ZDM. ZDM alleviates oxidative stress, inflammation and senescence in chondrocytes. In aged MSCs, it restores PINK1–Parkin-dependent mitophagy and mitochondrial homeostasis, while TRIM15 silencing reduces YAP nuclear localisation and relieves YAP-mediated repression of SOX9, thereby promoting chondrogenic differentiation and MSC rejuvenation.

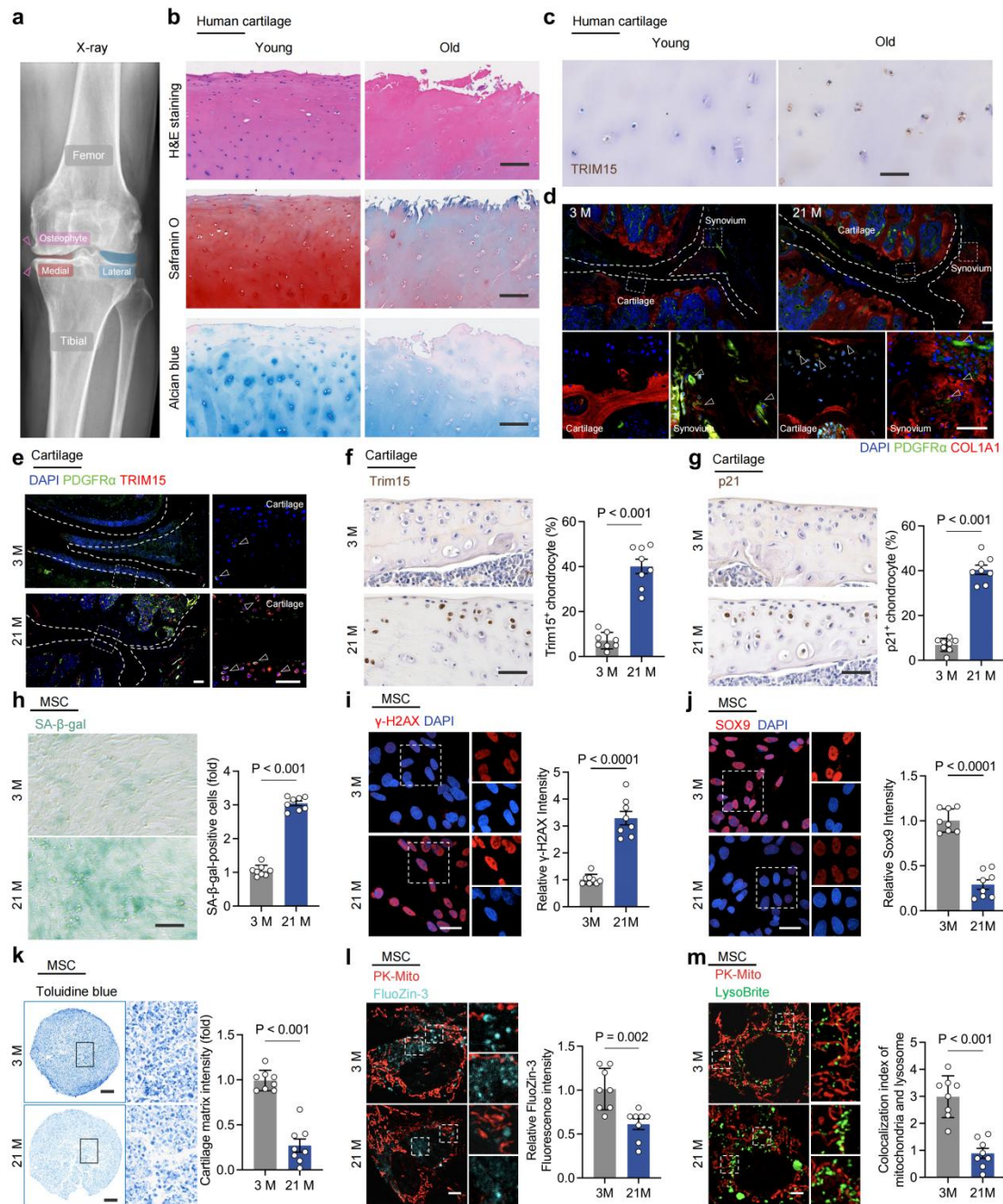


Fig. 2 TRIM15-high aged cartilage harbours MSC-like cells with senescence-associated dysfunction.

a, Representative X-ray image of the knee joint from an aged patient with osteoarthritis.

b, Representative haematoxylin and eosin (H&E), Safranin O and Alcian blue staining images of human articular cartilage from young and old donors.

c, Representative immunohistochemical staining images of TRIM15 in human articular cartilage from young and old donors.

d, Representative immunofluorescence images showing COL1A1-positive damaged cartilage regions and PDGFR α -positive MSC-like cells in knee joint sections from 3-month-old (3M) and 21-month-old (21M) mice. Arrowheads indicate PDGFR α -positive cells.

e, Representative immunofluorescence images showing TRIM15 expression in PDGFR α -positive MSC-like cells in cartilage from 3M and 21M mice. Arrowheads indicate representative PDGFR α -positive/TRIM15-positive cells.

f, Representative immunohistochemical staining images and quantification of TRIM15-positive chondrocytes in cartilage from 3M and 21M mice.

g, Representative immunohistochemical staining images and quantification of p21-positive chondrocytes in cartilage from 3M and 21M mice.

h, Representative senescence-associated β -galactosidase (SA- β -gal) staining images and quantification in MSCs isolated from 3M and 21M mice.

i, Representative immunofluorescence images and quantification of γ -H2AX in MSCs isolated from 3M and 21M mice.

j, Representative immunofluorescence images and quantification of SOX9 in MSCs isolated from 3M and 21M mice.

k, Representative toluidine blue staining images and quantification of cartilage matrix formation in chondrogenic microspheres derived from MSCs isolated from 3M and 21M mice.

l, Representative fluorescence images and quantification of labile Zn²⁺ levels in MSCs from 3M and 21M mice using FluoZin-3, with mitochondria labelled by PK-Mito.

m, Representative fluorescence images and quantification of mitochondria–lysosome colocalisation in MSCs from 3M and 21M mice using PK-Mito and LysoBrite.

Continuous data are presented as mean $\pm$ s.d. (**f–m**). Each dot represents one biologically independent mouse (**f,g**) or one independent cell preparation (**h–m**). *P* values were determined using a two-sided unpaired Student's *t*-test (**f–m**). Scale bars, as indicated.

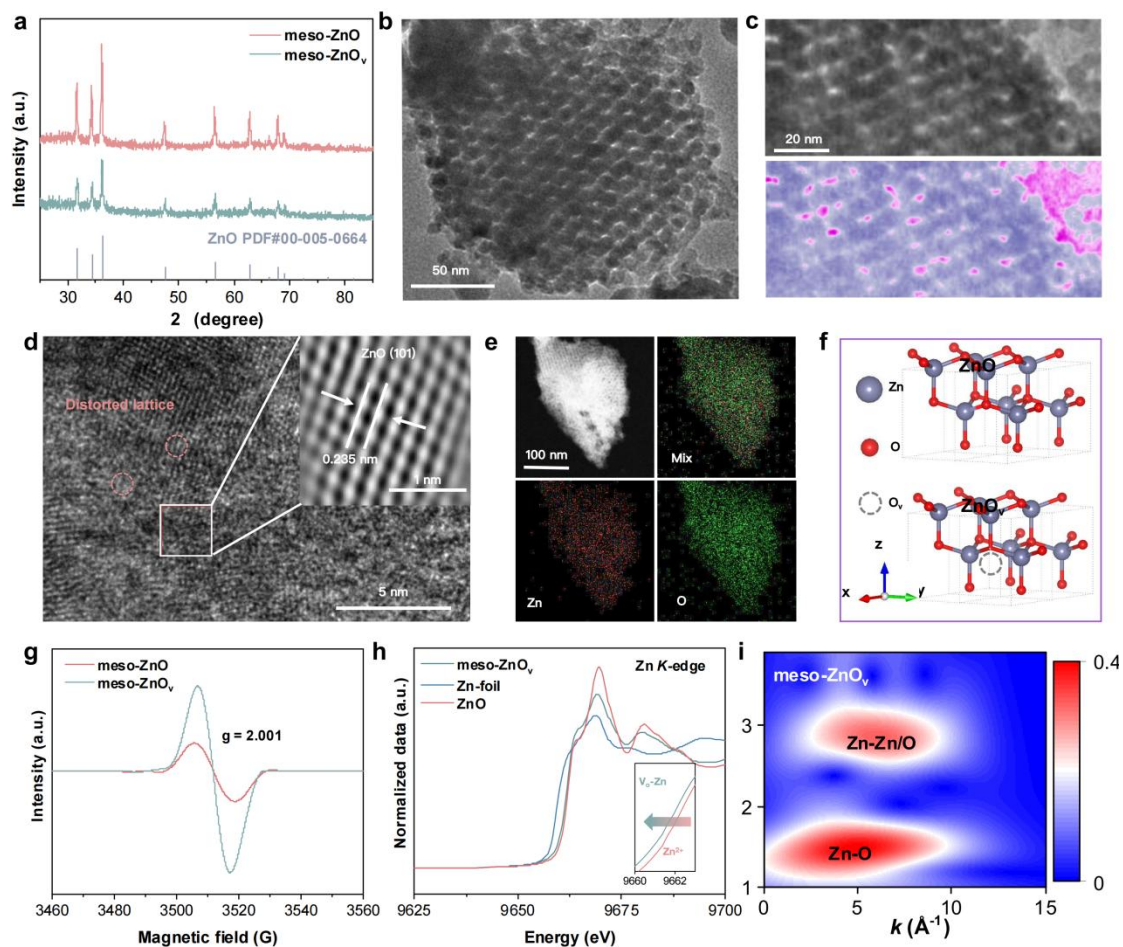


Fig. 3 Physicochemical characterization of meso-ZnO_v.

a, XRD patterns of meso-ZnO_v and meso-ZnO.

b, Low-magnification TEM image.

c, High-magnification TEM image and corresponding equalised false-colour image.

d, High-resolution TEM images.

e, HAADF-STEM EDX mapping images.

f, Structural illustrations of meso-ZnO_v and meso-ZnO.

g, EPR spectrum.

h, XANES spectra of the Zn K-edge.

i, Wavelet transform plots for meso-ZnO_v. Scale bars, 50 nm (**b**), 20 nm (**c**), 5 nm and 1 nm (**d**), and 100 nm (**e**).

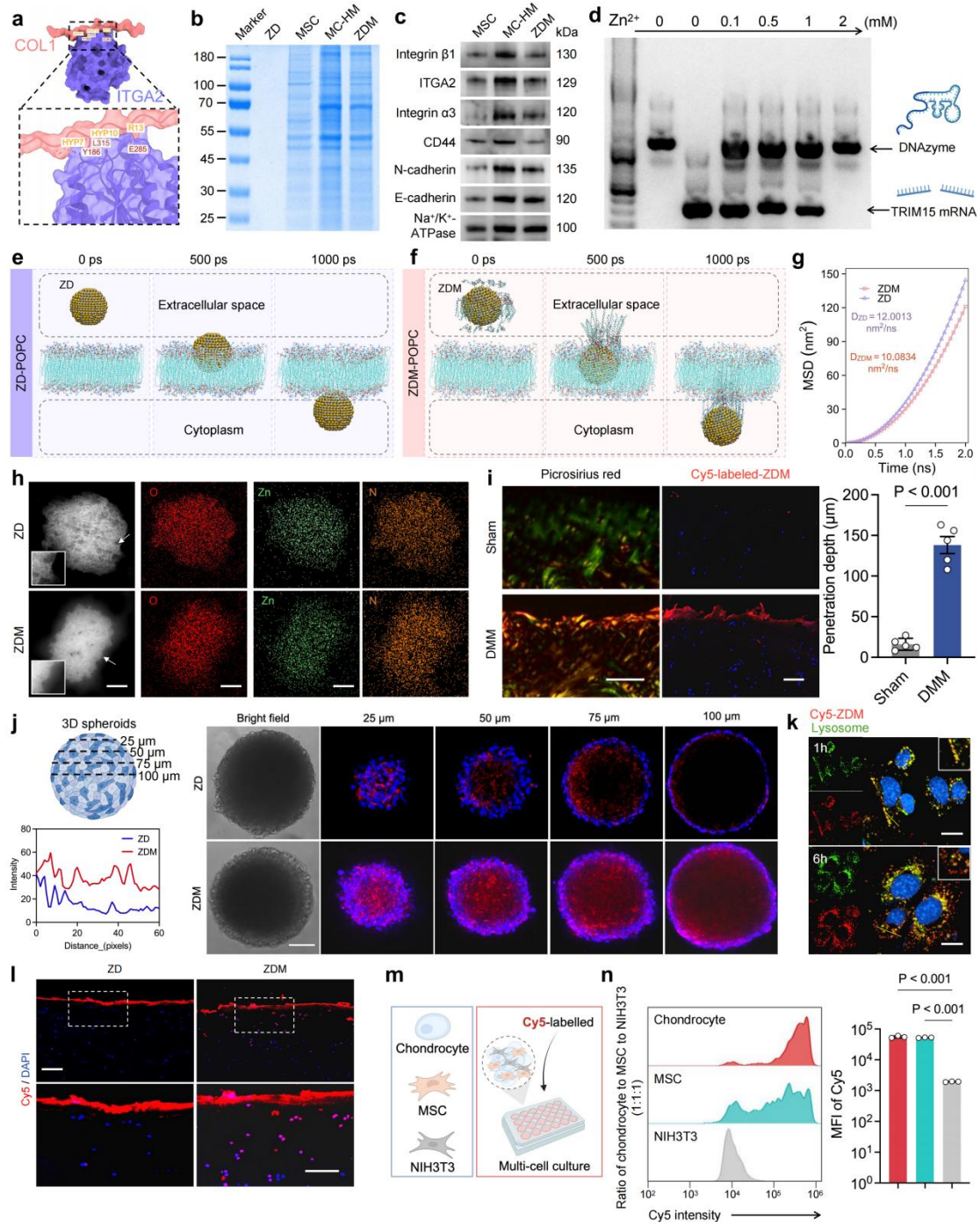


Fig. 4 Engineering a hybrid membrane nanoplateform with enhanced cartilage targeting and penetration.

a, Molecular docking model showing the predicted interaction interface between ITGA2 and COL1.

b, Coomassie brilliant blue staining showing the protein profiles of ZD, MSC membranes, MC-HM and ZDM.

c, Immunoblot analysis of representative membrane-associated proteins in MSC membranes, MC-HM and ZDM.

d, Polyacrylamide gel electrophoresis showing Zn²⁺-dependent cleavage of TRIM15 mRNA by the TRIM15-targeting DNAzyme.

e,f, Representative molecular dynamics simulation snapshots showing the interactions and translocation of ZD (e) and ZDM (f) across a POPC phospholipid bilayer at the indicated time points.

g, Mean square displacement curves and calculated diffusion coefficients of ZD and ZDM during the molecular dynamics simulations.

h, Representative electron microscopy images and elemental maps of O, Zn and N in ZD and ZDM. Scale bars, 100 nm.

i, Representative picosirius red staining and fluorescence images showing the distribution of Cy5-labelled ZDM in sham and DMM cartilage after intra-articular injection, together with quantification of penetration depth. Scale bars, 100 μ m.

j, Schematic of the three-dimensional chondrocyte spheroid model, line-scan fluorescence intensity profiles, bright-field images and representative confocal images showing the penetration of Cy5-labelled ZD and ZDM at the indicated optical depths. Scale bars, 100 μ m.

k, Representative confocal fluorescence images showing the intracellular colocalisation of Cy5-labelled ZDM with lysosomes at 1 and 6 h. Boxed regions are shown at higher magnification. Scale bars, 10 μ m.

l, Representative confocal fluorescence images showing the penetration of Cy5-labelled ZD and ZDM into cartilage explants. Scale bars, 100 μ m.

m, Schematic of the multicellular co-culture system comprising chondrocytes, MSCs and NIH3T3 cells for evaluating the cellular preference of Cy5-labelled ZDM.

n, Representative flow cytometry histograms and quantification of the mean Cy5 fluorescence intensity in chondrocytes, MSCs and NIH3T3 cells after incubation with Cy5-labelled ZDM. Data are presented as mean $\pm$ s.d. (**i,n**). Each dot represents one biologically independent mouse (**i**) or one independent cell preparation (**n**). Statistical significance was determined using a two-sided unpaired Student's t-test (**i**) or one-way ANOVA followed by Tukey's multiple-comparison test (**n**).

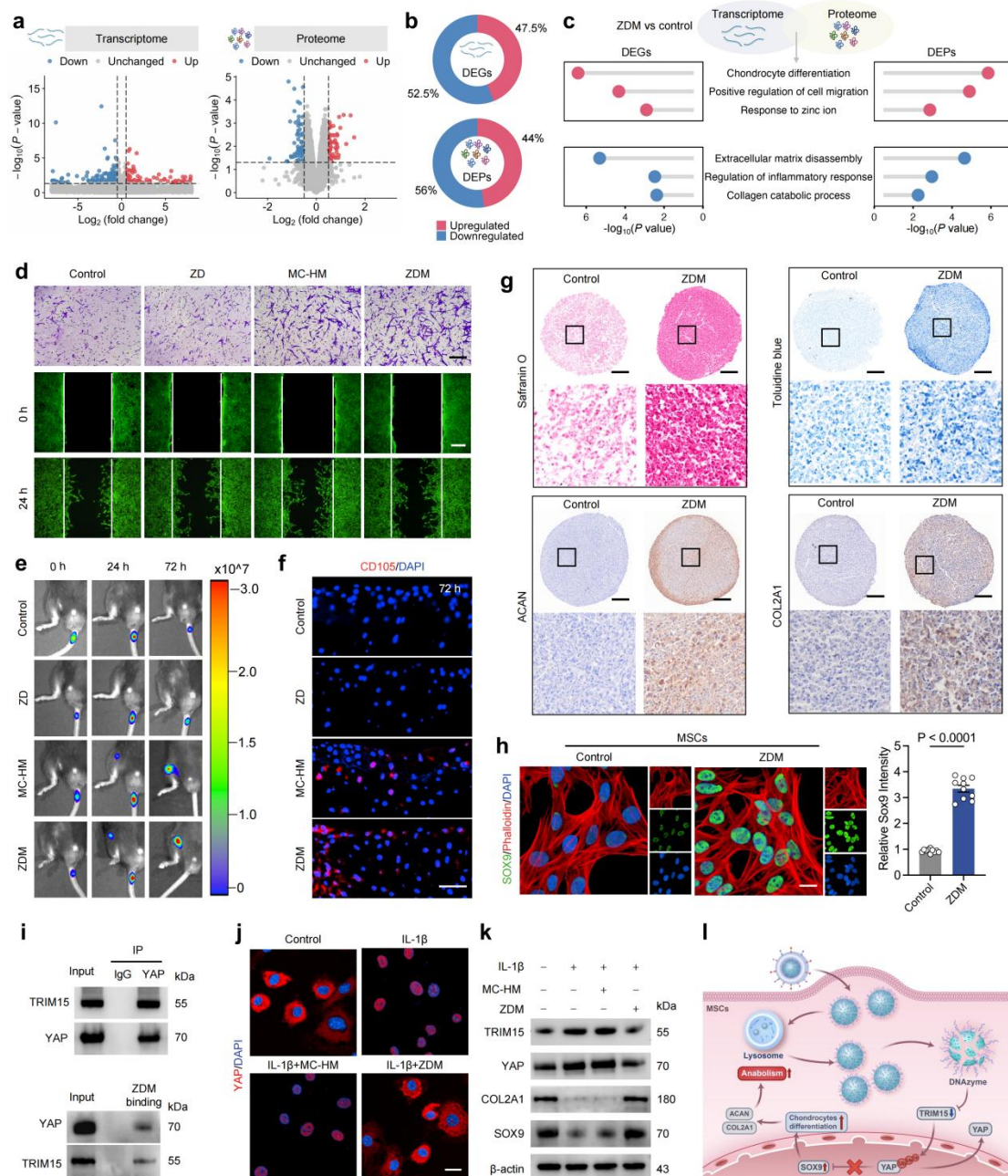


Fig. 5 ZDM activates zinc-responsive MSC repair and promotes TRIM15–YAP–SOX9 regulated chondrogenesis.

a, Volcano plots showing differentially expressed genes (DEGs) in the transcriptome and differentially expressed proteins (DEPs) in the proteome of cells after ZDM treatment.

b, Pie charts showing the proportions of upregulated and downregulated DEGs and DEPs.

c, Cross-comparison of enriched biological processes from transcriptomic and proteomic analyses, showing activation of chondrocyte differentiation, positive regulation of cell migration and response to zinc ion, together with suppression of extracellular matrix disassembly, inflammatory responses and collagen catabolism.

d, Representative Transwell migration assay images (top) and wound-healing assay images at 0 h and 24 h (bottom) for control, ZD, MC-HM and ZDM treatment groups. Scale bars, 100 μm .

e, Representative in vivo fluorescence images showing accumulation of intravenously injected

fluorescence-labelled MSCs in the knee joint at 0 h, 24 h and 72 h after treatment with control, ZD, MC-HM or ZDM.

f, Representative immunofluorescence images of CD105 in knee joint sections at 72 h after treatment with control, ZD, MC-HM or ZDM. Scale bars, 100 μ m.

g, Representative Safranin O, toluidine blue, ACAN and COL2A1 staining images of chondrogenic microspheres from control and ZDM-treated groups. Scale bars, 100 μ m.

h, Representative immunofluorescence images and quantification of SOX9 in MSCs from control and ZDM-treated groups. Scale bars, 10 μ m.

i, Co-immunoprecipitation analysis showing the interaction between TRIM15 and YAP, and immunoblot detection of TRIM15 and YAP in ZDM-associated protein fractions.

j, Representative immunofluorescence images showing YAP subcellular localisation in control, IL-1 β , IL-1 β + MC-HM and IL-1 β + ZDM groups. Scale bars, 10 μ m.

k, Immunoblot analysis of TRIM15, YAP, COL2A1 and SOX9 in IL-1 β -stimulated cells treated with MC-HM or ZDM.

l, Schematic illustration of the proposed mechanism by which ZDM promotes MSC migration and chondrogenic differentiation through suppression of the TRIM15–YAP axis.

Continuous data are presented as mean $\pm$ s.d. (h). Each dot represents one independent cell preparation. *P* values were determined using a two-sided unpaired Student's t-test (h).

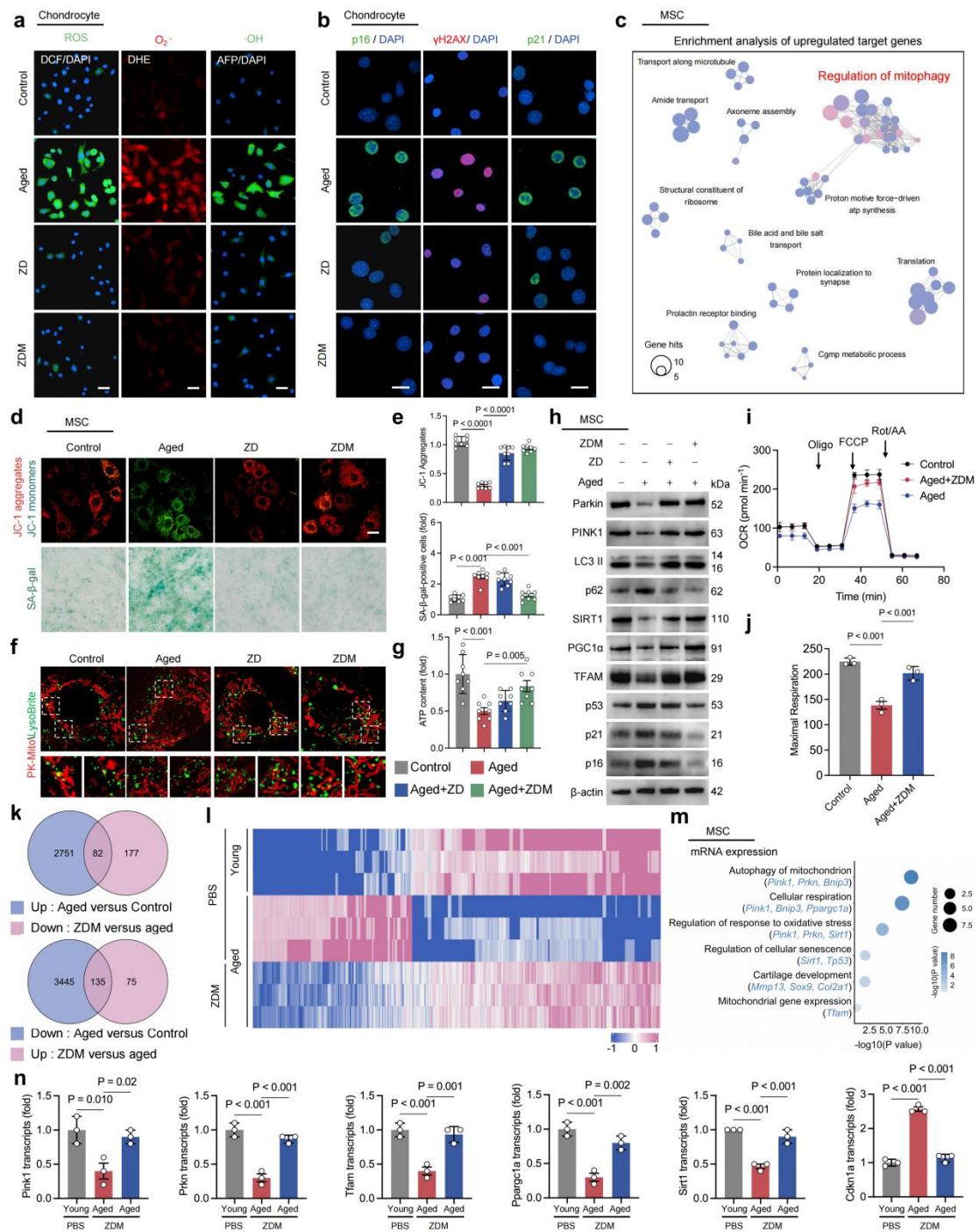


Fig. 6 ZDM remodels the oxidative-senescent cartilage milieu and induces rejuvenation-like recovery of aged MSCs through restoration of mitochondrial homeostasis.

a, Representative fluorescence images showing total ROS, superoxide and hydroxyl radical-associated signals in chondrocytes from control, aged, aged + ZD and aged + ZDM groups. Scale bars, 10 μ m.

b, Representative immunofluorescence images showing p16, γ -H2AX and p21 in chondrocytes from control, aged, aged + ZD and aged + ZDM groups. Scale bars, 10 μ m.

c, Enrichment network analysis of upregulated ZDM-responsive targets in MSCs, highlighting regulation of mitophagy-related processes.

d, Representative JC-1 staining images showing mitochondrial membrane potential (top) and

senescence-associated β -galactosidase (SA- β -gal) staining images (bottom) in MSCs from control, aged, aged + ZD and aged + ZDM groups. Scale bars, 10 μ m (top); 100 μ m (bottom).

e, Quantification of the JC-1 aggregate/monomer ratio and SA- β -gal-positive cells corresponding to **d**.

f, Representative fluorescence images showing mitochondria-lysosome colocalisation in MSCs from control, aged, aged + ZD and aged + ZDM groups using PK-Mito and LysoBrite. Scale bars, 10 μ m.

g, Quantification of intracellular ATP levels in MSCs from control, aged, aged + ZD and aged + ZDM groups.

h, Immunoblot analysis of mitophagy-, mitochondrial biogenesis- and senescence-associated proteins in MSCs from control, aged, aged + ZD and aged + ZDM groups.

i, Representative Seahorse oxygen consumption rate (OCR) traces in MSCs from control, aged and aged + ZDM groups.

j, Quantification of maximal respiration corresponding to **i**.

k, Venn diagrams showing overlap between genes upregulated in aged versus control MSCs and downregulated in ZDM-treated versus aged MSCs (top), and between genes downregulated in aged versus control MSCs and upregulated in ZDM-treated versus aged MSCs (bottom).

l, Heatmap showing partial reversal of the aged MSC transcriptional programme by ZDM treatment.

m, Gene ontology enrichment analysis of restored target genes in MSCs, highlighting autophagy of mitochondrion, cellular respiration, response to oxidative stress, regulation of cellular senescence, cartilage development and mitochondrial gene expression.

n, Quantitative PCR analysis of Pink1, Prkn, Tfam, Ppargc1a, Sirt1 and Cdkn1a transcripts in MSCs from young, aged and aged + ZDM groups.

Continuous data are presented as mean $\pm$ s.d. (e,g,j,n). Each dot represents one independent cell preparation. *P* values were determined using one-way ANOVA followed by Tukey's multiple-comparison test (e,g,j,n).

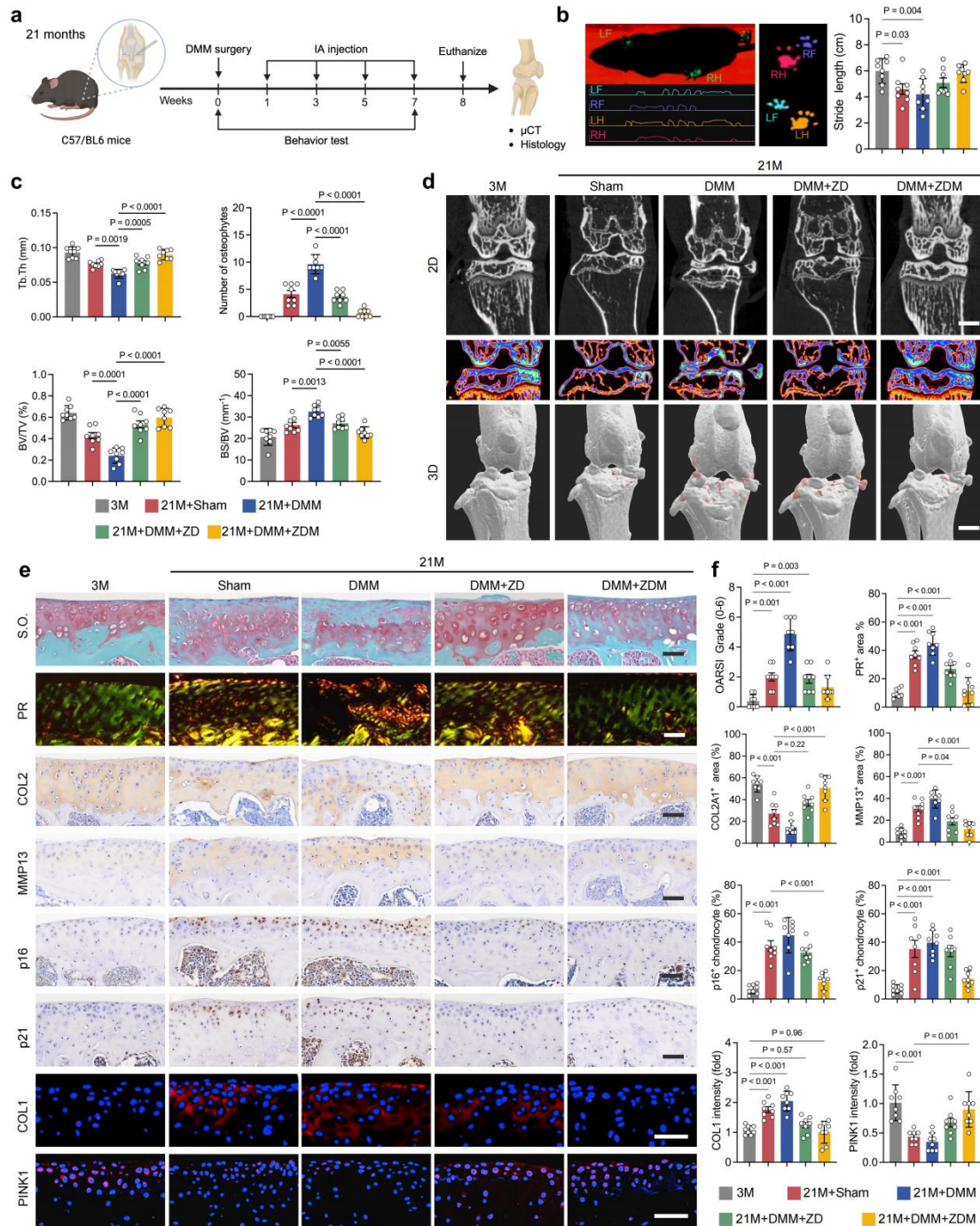


Fig. 7 ZDM attenuates structural degeneration and preserves joint homeostasis in aged osteoarthritic mice.

a, Schematic illustration of the experimental design. Twenty-one-month-old C57BL/6 mice underwent destabilization of the medial meniscus (DMM) surgery, followed by intra-articular (IA) injection at the indicated time points and endpoint analyses including behavioural testing, micro-computed tomography (micro-CT) and histology. Three-month-old (3M) mice were used as young controls.

b, Representative gait analysis images and quantification of stride length in 3M, 21M + Sham, 21M + DMM, 21M + DMM + ZD and 21M + DMM + ZDM groups.

c, Quantitative analysis of trabecular thickness (Tb.Th), osteophyte number, bone volume

fraction (BV/TV) and bone surface-to-volume ratio (BS/BV) in the indicated groups.

d, Representative 2D micro-CT images, corresponding pseudocolour reconstructions and 3D reconstructed images of knee joints from 3M, 21M + Sham, 21M + DMM, 21M + DMM + ZD and 21M + DMM + ZDM groups. Red markings indicate osteophyte formation. Scale bars, 1 mm.

e, Representative Safranin O (S.O.) staining, picrosirius red (PR) staining under polarized light, immunohistochemical staining for COL2 and MMP13, immunohistochemical staining for p16 and p21, and immunofluorescence staining for COL1 and PINK1 in knee cartilage sections from the indicated groups. Scale bars, 100 μ m.

f, Quantification of OARSI grade, PR-positive area, COL2A1-positive area, MMP13-positive area, percentages of p16-positive and p21-positive chondrocytes, COL1 fluorescence intensity and PINK1 fluorescence intensity in the indicated groups.

Continuous data are presented as mean $\pm$ s.d. for stride length, micro-CT parameters and imaging-based quantification (b,c,f), whereas OARSI grades (f) are presented as median with interquartile range. Each dot represents one biologically independent mouse. *P* values for continuous variables were determined using one-way ANOVA followed by Tukey's multiple-comparison test; OARSI grades were analysed using the Kruskal–Wallis test followed by Dunn's multiple-comparison test.

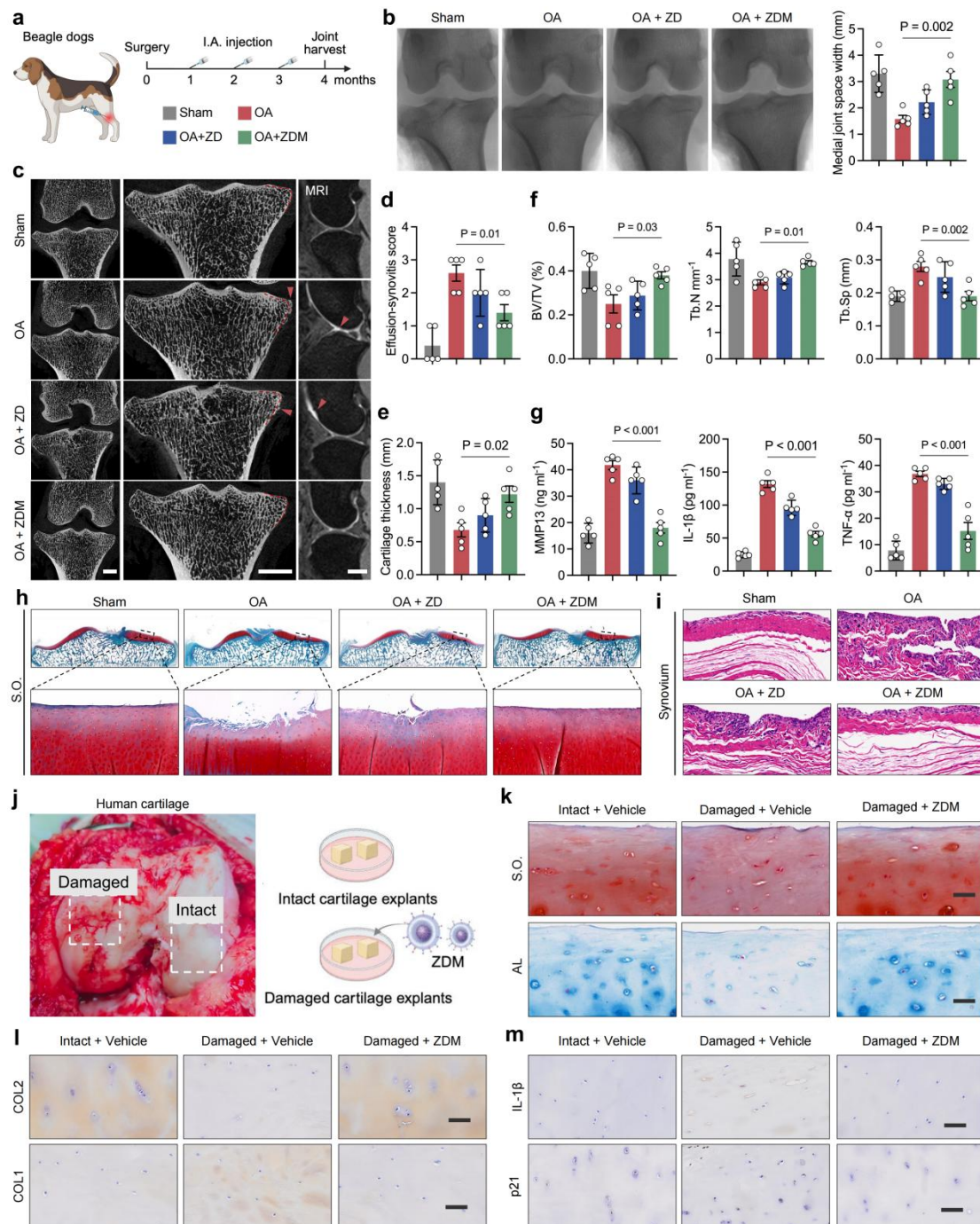


Fig. 8 ZDM confers disease-modifying efficacy in beagle dogs and human cartilage explants.

a, Schematic illustration of the experimental design in aged male beagle dogs. Osteoarthritis was induced surgically, followed by monthly intra-articular injection of ZD or ZDM at months 1, 2 and 3. Knee joints were harvested 4 months after surgery for endpoint analyses.

b, Representative X-ray images of knee joints from Sham, OA, OA + ZD and OA + ZDM beagle dogs, and quantification of medial joint space width.

c, Representative micro-CT and magnetic resonance images of knee joints from the indicated groups. Red dashed lines and arrowheads indicate representative structural

lesions or regions of interest. Scale bars, 5 mm.

d,e, Quantification of effusion-synovitis score (d) and cartilage thickness (e) based on magnetic resonance imaging.

f, Quantification of subchondral bone parameters, including bone volume fraction (BV/TV), trabecular number (Tb.N) and trabecular separation (Tb.Sp), based on micro-computed tomography.

g, Synovial fluid levels of MMP13, IL-1 β and TNF- α in the indicated groups.

h, Representative Safranin O staining images of beagle knee cartilage from Sham, OA, OA + ZD and OA + ZDM groups. Scale bars, 100 μ m.

i, Representative haematoxylin and eosin staining images of synovium from Sham, OA, OA + ZD and OA + ZDM beagle dogs. Scale bars, 100 μ m.

j, Representative gross image of human articular cartilage showing paired intact and damaged regions and a schematic of the human cartilage explant culture system.

k, Representative Safranin O (S.O.) and Alcian blue (AL) staining images of intact and damaged human cartilage explants treated with vehicle or ZDM. Scale bars, 100 μ m.

l, Representative immunohistochemical staining images of COL2 and COL1 in intact and damaged human cartilage explants treated with vehicle or ZDM. Scale bars, 100 μ m.

m, Representative immunohistochemical staining images of IL-1 β and p21 in intact and damaged human cartilage explants treated with vehicle or ZDM. Scale bars, 100 μ m.

Continuous data are presented as mean $\pm$ s.d. for medial joint-space width, cartilage thickness, subchondral bone parameters and synovial-fluid biomarkers (b,e–g), whereas effusion-synovitis scores (d) are presented as median with interquartile range. Each dot represents one biologically independent beagle dog. *P* values for continuous variables were determined using one-way ANOVA followed by Tukey's multiple-comparison test; effusion-synovitis scores were analysed using the Kruskal–Wallis test followed by Dunn's multiple-comparison test.

Supplementary Files

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