

ARTICLE OPEN



Comparative phenotypic and molecular profiling of replicative and chemically-induced senescence in articular chondrocytes

Maria Belen Arteaga^{1,3}, Karyna Tarasova^{1,3}, Angkana Kidtiwong¹, Sinan Gültekin¹, Iris Gerner^{1,2,4} and Florian Jenner^{1,2,4}

© The Author(s) 2026

Osteoarthritis (OA) is a degenerative joint disease characterized by the accumulation of senescent chondrocytes, which drive inflammation and cartilage degradation. However, in vitro models often fail to recapitulate the complexity of OA-associated senescence. This study compares three senescence induction strategies in chondrocytes—replicative senescence (HP), and stress-induced premature senescence (SIPS) via doxorubicin (DOX) and dexamethasone (DEX)—to establish a physiologically relevant in vitro model for OA research. To this end ovine chondrocytes ($n = 3$) were subjected to serial passaging (to P40) or exposed to optimized concentrations of DOX (50 nM) or DEX (1 μ M). Low passage (P3) cells served as controls. Cellular senescence was assessed via proliferation assays, cell cycle analysis, SA- β -gal activity, telomere length, ROS levels, mitochondrial function, transcriptomic profiling (NGS), and high-resolution mass spectrometry proteomic analysis. All models induced key senescence hallmarks including cell cycle and proliferation arrest, increased SA- β -gal activity, and mitochondrial dysfunction. HP cells showed telomere shortening, ROS accumulation, ATP depletion, and SASP secretion. DOX induced strong DNA damage responses and elevated apoptosis markers, while DEX induced senescence without significant ROS or apoptosis, suggesting distinct SIPS mechanisms. Transcriptomics revealed convergent downregulation of oxidative phosphorylation and selenoamino acid metabolism pathways, implicating mitochondrial dysfunction and redox imbalance as shared features. However, HP induced broad transcriptional suppression, also of inflammatory pathways, while DOX and DEX activated immune and SASP-related pathways. Proteomics confirmed divergent secretory profiles, with DOX/DEX increasing SASP-factors and HP enriching matrix proteins. In summary, although all models recapitulate fundamental aspects of senescence, they diverge in stress responses, immune signaling, and apoptosis profiles. HP most closely mimics aging-associated senescence, whereas DOX and DEX model distinct SIPS relevant to oxidative or pharmacological stress. These findings underscore the importance of model selection in senescence-focused OA research and highlight mitochondrial dysfunction as a central mechanistic hub across senescence pathways.

Cell Death Discovery (2026)12:106; <https://doi.org/10.1038/s41420-026-02961-y>

INTRODUCTION

Osteoarthritis (OA), the most prevalent musculoskeletal disease, affects over 654 million individuals globally [1–3]. Characterized by progressive cartilage degeneration and articular inflammation, OA manifests clinically with pain, stiffness and limited mobility [1–3]. Despite its high prevalence and impact, effective curative treatments remain elusive, in part due to its clinical and molecular heterogeneity. Notably, osteoarthritic joints, regardless of the underlying molecular pathomechanisms (endotypes), consistently show increased levels of senescent cells, highlighting cellular senescence as a unifying pathological mechanism and a potential therapeutic target.

While chronological aging is the predominant risk factor for OA, articular cartilage also exhibits signs of premature aging, including cellular senescence, oxidative stress, and mitochondrial dysfunction, that both contribute to and reflect OA pathogenesis [4–17]. Indeed, OA joints exhibit an increased burden of senescent cells, and their presence correlates with disease severity [4–17]. Beyond their intrinsic dysfunction, senescent chondrocytes develop a

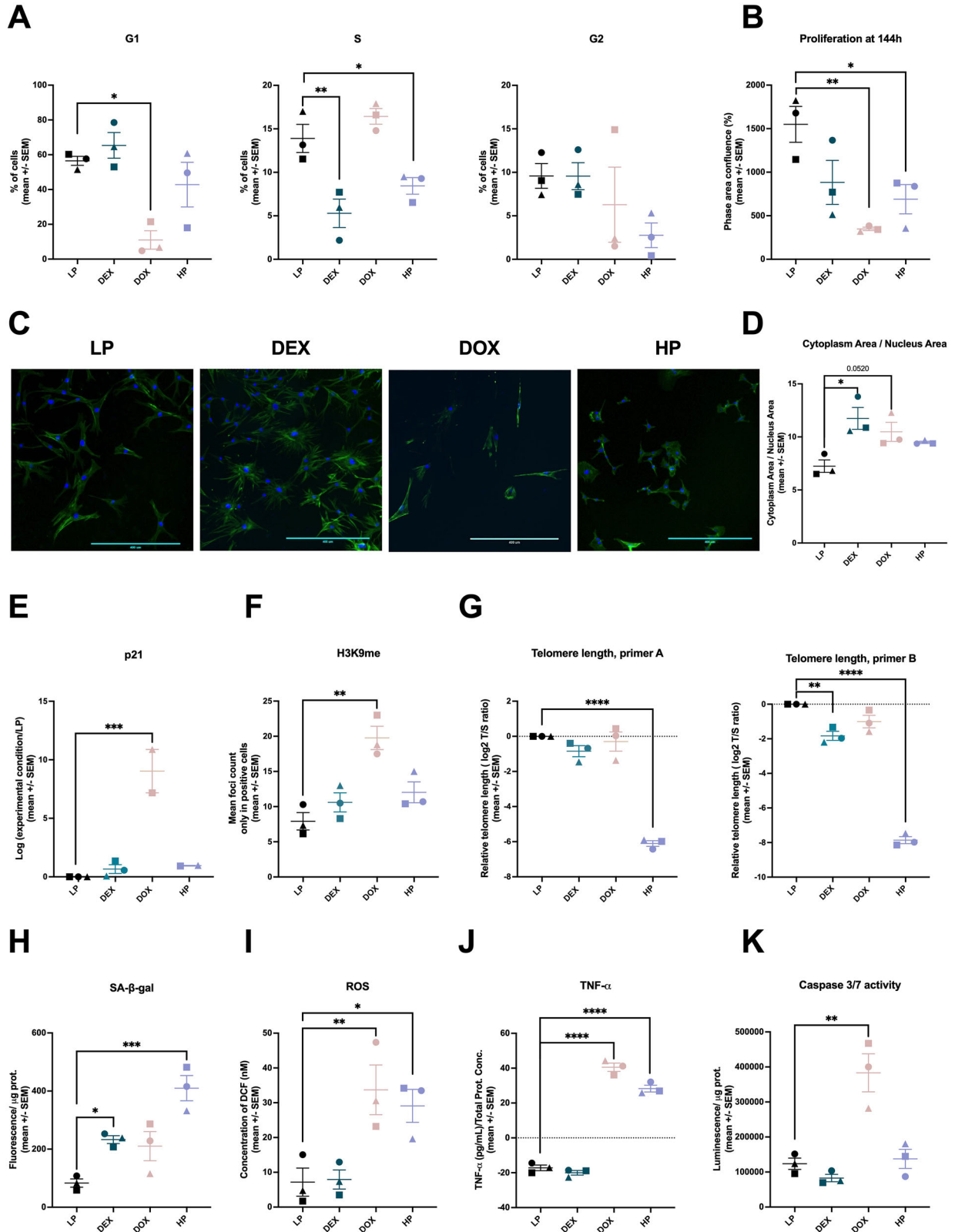
senescence-associated secretory phenotype (SASP), characterized by hypersecretion of pro-inflammatory cytokines, matrix-degrading enzymes, and reactive oxygen species (ROS) [4–19]. The SASP autocrinally and paracrinally reinforces senescence, promoting extracellular matrix breakdown and chronic inflammation, establishing a self-perpetuating cycle of senescence and inflammation that drives OA progression [4–19]. The causal role of senescent cells in OA is further supported by studies showing that intra-articular transplantation of senescent chondrocytes induces OA-like changes, whereas their targeted clearance reduces disease burden [9, 15].

Chondrocyte senescence can arise from replicative mechanisms or stress-induced premature senescence (SIPS) due to extrinsic factors like oxidative stress, mechanical overload, or chronic inflammation [4–19]. The precise pathophysiological relevance of replicative senescence in OA remains debated, given the generally postmitotic and quiescent nature of articular chondrocytes and the uncertainty over whether in vivo cell turnover is sufficient to cause significant telomere erosion [12, 17, 20–23]. Nevertheless,

¹Department for Small Animals and Horses, Centre for Equine Health and Research, Equine Surgery Unit, Veterinary Tissue Engineering and Regenerative Medicine Laboratory, University of Veterinary Medicine Vienna, Vienna, Austria. ²Austrian Cluster for Tissue Regeneration, Vienna, Austria. ³These authors contributed equally: Maria Belen Arteaga, Karyna Tarasova. ⁴These authors jointly supervised this work: Iris Gerner, Florian Jenner. ✉email: florian.jenner@vetmeduni.ac.at

Received: 1 August 2025 Revised: 27 November 2025 Accepted: 9 February 2026

Published online: 23 February 2026



during the early stages of OA, chondrocytes can re-enter the cell cycle and exhibit transient increases in proliferation and metabolic activity as part of a short-lived reparative response [24, 25]. Consequently, cumulative replication over decades, particularly in the context of cartilage injury or articular inflammation, where

mitotic activity may increase severalfold, could contribute to telomere attrition [12, 17, 20–23]. Although chondrocytes' telomeres shorten with age, suggesting a role for replicative aging in cartilage degeneration, the rate of erosion appears insufficient to fully account for the extent of chondrocyte

Fig. 1 Effect of chemically induced senescence by Doxorubicin (DOX) and Dexamethasone (DEX) and serial passaging (HP) on chondrocytes (Senescence Hallmarks). **A** DOX -treated cells showed a significant decrease in G1 phase ($p = 0.0227$), a non-significant accumulation in the S ($p = 0.3837$) and a non-significant decrease in the G2 ($p = 0.7515$) phase compared to LP cells. DEX led to a non-significant accumulation of cells in the G1 ($p = 0.8071$) phase, a significant decrease in the S phase ($p = 0.0057$) and no change in the G2 ($p > 0.999$) phase. HP cells showed non-significant decrease in the G1 phase ($p = 0.5716$), a significant decrease in the S ($p = 0.0437$) and non-significant decrease in the G2 phase ($p = 0.2911$). **B** Cell proliferation after 144 h in culture was significantly impaired in DOX stimulated ($p = 0.0076$) and in HP cells ($p = 0.0339$), while non-significant decreased in DEX ($p = 0.0880$) compared to LP chondrocytes. **C** Phalloidin staining of F-actin (green) and Dapi of nucleus (blue). Typical fluorescence images show LP, HP, DEX and DOX stimulated cells (magnification 10X). **D** The ratio cytoplasm area/ nucleus area was significantly increased in DEX stimulated cells (DEX: $p = 0.0129$) and non-significantly increased in DOX ($p = 0.0520$) and in HP ($p = 0.1736$) compared to LP cells. **E** p21 gene expression was significantly upregulated in in DOX-stimulated chondrocytes ($p = 0.0004$), with non-significant increases observed in DEX- ($p = 0.8291$) and HP-treated cells ($p = 0.7125$). **F** H3K9me foci counts in positive cells significantly increased in DOX stimulated chondrocytes ($p = 0.0044$) and non-significantly increased in DEX stimulated ($p = 0.5284$) and in HP cells ($p = 0.2459$) compared to LP chondrocytes. **G** Telomere length significantly decreased in HP chondrocytes (Primer A: $p < 0.0001$, Primer B: $p < 0.0001$) and DEX (Primer A: $p = 0.1979$, Primer B: $p = 0.0051$) stimulated cells and decreased non-significantly in DOX (Primer A: $p = 0.8181$, Primer B: $p = 0.0051$) stimulated cells compared to LP chondrocytes. **H** SA- β Gal activity (Fluorescence/ μ g total protein concentration) significantly increased in DEX stimulated ($p = 0.0373$) and HP ($p = 0.0009$) cells and non-significantly increased DOX stimulated cells ($p = 0.0696$) compared to LP chondrocytes. **I** Intracellular reactive oxygen species (ROS) levels (concentration of DCF (nM)) significantly increased in DOX treated cells ($p = 0.0046$) and in HP ($p = 0.0115$), but no alteration was observed in DEX treated cells ($p = 0.9974$) compared to LP cells. **J** TNF- α levels (pg/mL/ μ g total protein concentration) were significantly increased in DOX treated cells ($p < 0.0001$) and in HP ($p < 0.0001$), but no alteration was observed in DEX treated cells ($p = 0.5232$) compared to LP cells. **K** Caspase 3/7 activity (Fluorescence/ μ g total protein concentration) was only significantly increased in DOX treated cells ($p = 0.0051$) compared to LP cells, while no significant alteration was observed in DEX treated cells ($p = 0.7598$) or in HP cells ($p = 0.9844$) compared to LP cells. $n = 3$ biological replicates with three to six technical replicates per donor per condition. Data shown as mean $\pm$ SEM. LP Low Passage cells (P3), DEX low passage cells (P3) stimulated with DEX (1 μ M), DOX low passage cells (P3) stimulated with DOX (50 nM), HP High Passage cells (P40). Not significant (ns, $p \geq 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

senescence observed in OA. Thus, chondrocyte senescence in OA likely reflects the combined influence of lifelong replicative aging and accumulated damage from oxidative, mechanical and inflammatory stressors [12, 17, 20–23].

Despite the recognized role of senescent cells in OA, traditional in vitro and in vivo models often fail to capture this dimension of disease pathophysiology [16, 26–28]. While models based on mechanical injury or cytokine stimulation can induce cartilage damage, inflammation, and SIPS under intense exposure, they typically do not account for pre-existing senescent cells [16, 26–28]. Therefore, more physiologically relevant models incorporating senescent chondrocytes are crucial to better recapitulate the OA joint environment and support senescence-targeted research. In vitro, senescence can be induced either by serial passaging to model replicative senescence, or by exposure to noxious stimuli, such as oxidative stress or DNA-damaging agents, to induce SISP [17, 28–32]. Among SISP inducers, doxorubicin (DOX), a chemotherapeutic drug, induces senescence via DNA double-strand breaks, topoisomerase-II inhibition, p53/p21 pathways activation, and ROS generation [33–35]. In chondrocytes, DOX exposure yields a stable senescent phenotype with cell-cycle arrest, increased SA- β -gal activity, altered morphology, and robust SASP secretion [35]. Dexamethasone (DEX), a potent glucocorticoid widely used for its anti-inflammatory and analgesic efficacy in OA treatment, exerts context-dependent effects, attenuating inflammation and pain in diseased cartilage, while inducing oxidative stress, matrix degradation, and senescence-like changes in healthy chondrocytes or under prolonged or high-dose exposure [31, 36–40]. Thus, DOX and DEX serve as SISP models relevant for oxidative damage, DNA injury, or corticosteroid exposure in OA joints.

Given the central role of senescent cells in OA pathogenesis, this study compared three senescence induction methods: replicative senescence via prolonged serial passaging (HP) and chemically induced SISP via DOX and DEX with the aim to identify an in vitro model that reliably recapitulates the senescent phenotype characteristic of OA cartilage as a platform for mechanistic studies and the development of senescence-targeted therapies. We hypothesized that while all senescence induction strategies converge on shared hallmarks, stressor-specific mechanisms diverge in ways that critically impact each model's fidelity for osteoarthritis research.

RESULTS

Optimal senescence induction protocols for DOX, DEX, and HP cells were established in a pilot study (Supplementary Figs. 1 and 2, Supplementary Results).

Senescence hallmarks

All three senescence induction methods led to cell growth arrest, each associated with a distinct cell cycle arrest phase (Fig. 1A). DOX -treated cells showed a significant decrease in G1 phase ($p = 0.0227$), a non-significant accumulation in the S ($p = 0.3837$) and a non-significant decrease in the G2 ($p = 0.7515$) phase compared to LP cells. DEX led to a non-significant accumulation of cells in the G1 ($p = 0.8071$) phase, a significant decrease in the S phase ($p = 0.0057$) and no change in the G2 ($p > 0.999$) phase. HP cells showed non-significant decrease in the G1 phase ($p = 0.5716$), a significant decrease in the S ($p = 0.0437$) and non-significant decrease in the G2 phase ($p = 0.2911$) (Fig. 1A). Cell proliferation was significantly impaired after 144 h in DOX- ($p = 0.0076$) and HP-treated cells ($p = 0.0339$), while DEX showed a non-significant decrease ($p = 0.0880$, Fig. 1B).

The ratio of cytoplasm area to nucleus area was significantly increased in DEX stimulated cells (DEX: $p = 0.0129$) and showed non-significant increases in DOX ($p = 0.0520$) and HP cells ($p = 0.1736$) (Fig. 1C, D).

All three methods also induced other key senescence hallmarks, including p21 gene expression, heterochromatin modifications, telomere attrition and SA- β -galactosidase activity. p21 gene expression was significantly upregulated in in DOX-stimulated chondrocytes ($p = 0.0004$), with non-significant increases observed in DEX- ($p = 0.8291$) and HP-treated cells ($p = 0.7125$, Fig. 1E). H3K9me3 foci counts in positive cells significantly increased in DOX-stimulated chondrocytes ($p = 0.0044$), with non-significant increases observed in DEX- ($p = 0.5284$) and HP-treated cells ($p = 0.2459$, Fig. 1F, Supplementary Fig. 3). Relative telomere length significantly decreased in HP chondrocytes (Primer A: $p < 0.0001$; Primer B: $p < 0.0001$) and DEX-stimulated cells (Primer B: $p = 0.0051$), with a non-significant decrease in DOX-stimulated cells (Primer A: $p = 0.8181$; Primer B: $p = 0.0051$) (Fig. 1G). SA- β -galactosidase activity significantly increased in DEX- ($p = 0.0373$) and HP-treated cells ($p = 0.0009$), showing a non-significant increase in DOX-treated cells ($p = 0.0696$) (Fig. 1H).

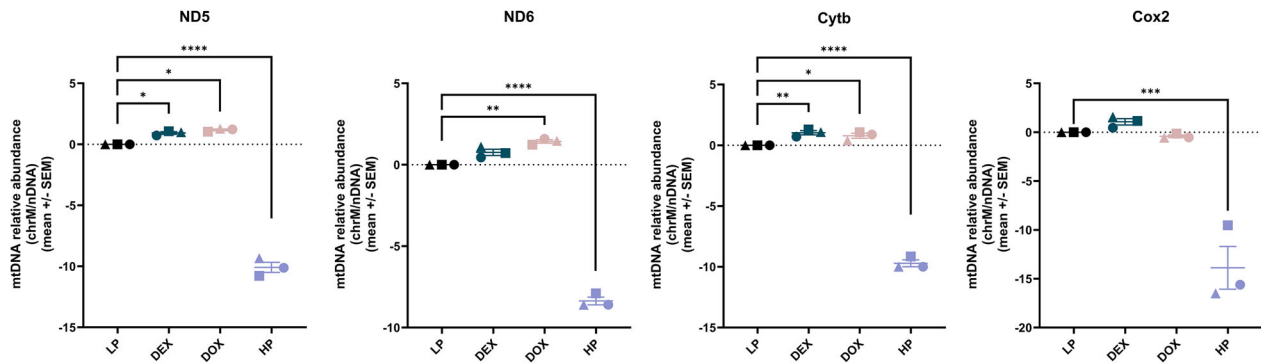
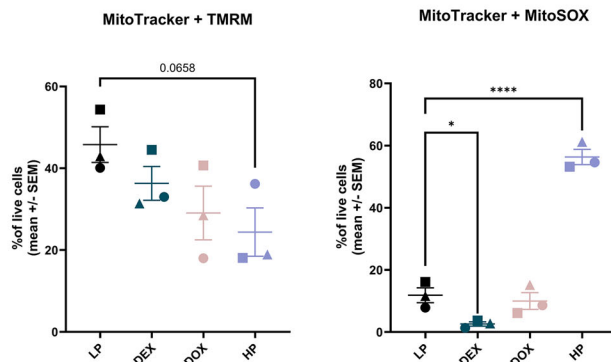
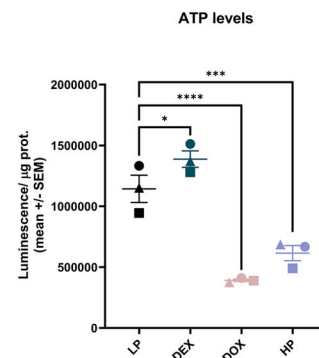
A**B****C**

Fig. 2 Effect of chemically induced senescence by Doxorubicin (DOX) and Dexamethasone (DEX) and serial passing (HP) on chondrocytes' mitochondrial function. **A** Serial passing led to significant downregulation of ND5 ($p < 0.0001$), ND6 ($p < 0.0001$), Cytb ($p < 0.0001$) and COX-2 ($p = 0.0003$) compared to LP chondrocytes, while DOX and DEX stimulation led to significant upregulation of ND5 (DOX: $p = 0.0181$ and DEX: $p = 0.0490$), ND6 (DOX: $p = 0.0031$ and DEX: $p = 0.0541$), Cytb (DOX: $p = 0.0296$ and DEX: $p = 0.0084$) but not COX-2 (DOX: $p = 0.9855$ and DEX: $p = 0.8306$) compared to LP chondrocytes. **B** Mitochondrial membrane potential (TMRM) was non-significantly decreased in DEX ($p = 0.4893$) and DOX ($p = 0.1472$) stimulated cells and in HP cells ($p = 0.0658$) compared to LP cells (Fig.2B). Mitochondrial superoxide (MitoSOX) levels were significantly increased in HP ($p < 0.0001$) and significantly decreased in DEX stimulated cells ($p = 0.0435$), while maintained unaltered in DOX stimulated cells ($p = 0.8545$) compared to LP cells. **C** ATP levels (Luminescence/ μ g total protein concentration) significantly decreased in HP chondrocytes ($p = 0.0006$) and in DOX stimulated cells ($p < 0.0001$), while significantly increased with DEX ($p = 0.0279$) compared to LP chondrocytes. $n = 3$ biological replicates with two to six technical replicates per donor per condition. Data shown as mean $\pm$ SEM. LP Low Passage cells (P3), DEX low passage cells (P3) stimulated with DEX (1μ M), DOX low passage cells (P3) stimulated with DOX (50 nM), HP High Passage cells (P40). Not significant (ns, $p \geq 0.05$), * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Oxidative stress and inflammatory markers showed model-specific patterns. Intracellular ROS levels significantly increased in DOX-treated ($p = 0.0046$) and HP ($p = 0.0115$) cells, but no alteration was observed in DEX-treated cells ($p = 0.9974$, Fig. 1I). Similarly, TNF- α levels, a prominent SASP component, were significantly increased in DOX- ($p < 0.0001$) and HP-treated cells ($p < 0.0001$), but remained unaltered in DEX-treated cells ($p = 0.5232$, Fig. 1J).

In contrast, apoptosis marker Caspase 3/7 activity was significantly increased only in DOX-treated cells ($p = 0.0051$), with no significant change in DEX- ($p = 0.7598$) or HP-treated cells ($p = 0.9844$, Fig. 1K).

Mitochondrial function

Mitochondrial gene expression varied distinctly (Fig. 2A). HP led to significant downregulation of ND5, ND6, Cytb (all $p < 0.0001$), and COX-2 ($p = 0.0003$). Conversely, DOX and DEX stimulation significantly upregulated ND5 (DOX: $p = 0.0181$; DEX: $p = 0.0490$), ND6 (DOX: $p = 0.0031$; DEX: $p = 0.0541$), and Cytb (DOX: $p = 0.0296$; DEX: $p = 0.0084$), though not COX-2 (DOX: $p = 0.9855$; DEX: $p = 0.8306$).

Mitochondrial membrane potential (TMRM) showed non-significant decreases across all groups (DEX: $p = 0.4893$; DOX: $p = 0.1472$; HP: $p = 0.0658$, Fig. 2B). Mitochondrial superoxide (MitoSOX) levels significantly increased in HP ($p < 0.0001$) but significantly decreased in DEX-stimulated cells ($p = 0.0435$), remaining unaltered in DOX-stimulated cells ($p = 0.8545$). ATP significantly decreased in HP ($p = 0.0006$) and DOX-stimulated chondrocytes ($p < 0.0001$), but significantly increased with DEX treatment ($p = 0.0279$, Fig. 2C).

Next generation sequencing

Serial passing induced the most extensive transcriptional remodeling, with 3356 differentially expressed genes (DEGs) compared to LP (496 up, 2860 down, Fig. 3A). DEX treatment yielded 538 DEGs (251 up, 287 down), while DOX yielded 651 DEGs (288 up, 363 down). A total of 136 DEGs were common across all three conditions. In addition, 192 DEGs were shared between HP and DOX, 116 between HP and DEX, and 115 between DOX and DEX (Fig. 3B). Furthermore, unique DEGs were identified for each condition: 2912 for HP, 208 for DOX and 171 for DEX.

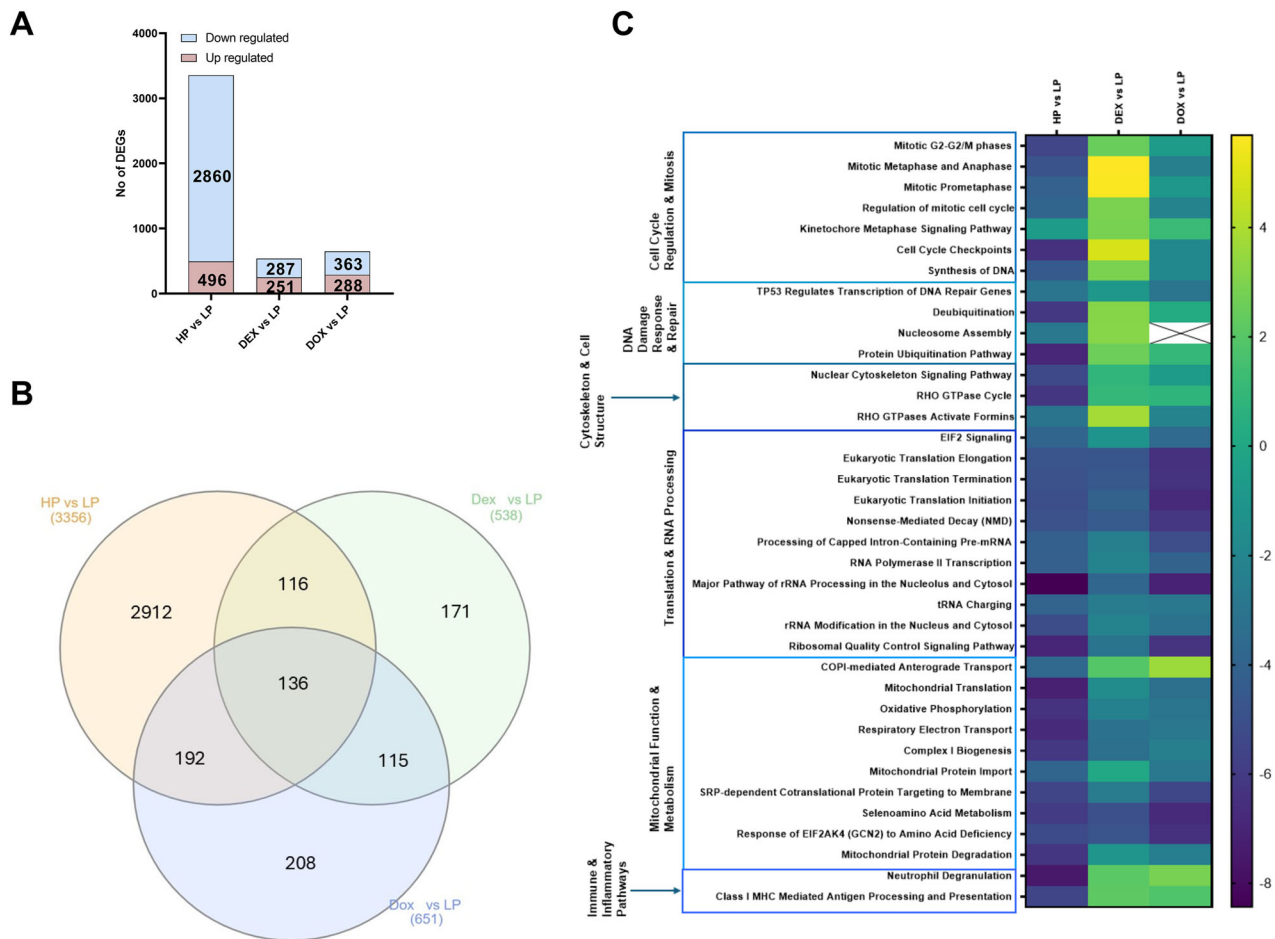


Fig. 3 Effect of chemically induced senescence by Doxorubicin (DOX) and Dexamethasone (DEX) and serial passaging (HP) on chondrocytes' gene expression (RNASeq analysis). **A** Serial passaging of chondrocytes (HP) resulted in a total of 3356 differentially expressed genes (DEGs) when compared to low-passage (LP) controls (496 up- and 2860 down-regulated). Chemical induction with DEX led to 538 DEGs (251 up- and 287 down-regulated), while DOX treatment yielded 651 DEGs (288 up- and 363 down-regulated). **B** Venn diagram of the genes differentially regulated (adjusted $p < 0.05$) between HP vs LP, DEX vs LP and DOX vs LP comparisons groups showing a total of 136 DEGs were commonly affected across all three experimental conditions and the highest number of shared DEGs was observed between HP and DOX treatments, with 192 genes in common. DEX and HP shared 116 DEGs in total, while DEX and DOX stimulation shared 115 DEGs in total. Furthermore, 2912 unique DEGs were identified between HP vs LP chondrocytes, 171 unique DEGs in DEX vs LP and 208 unique DEGs between DOX vs LP. **C** Heat map with canonical pathways enriched in genes differentially expressed in ovine adult articular chondrocytes between HP vs LP, DEX vs LP and DOX vs LP comparisons groups showing that all three senescence induction models (HP, DEX and DOX vs LP) activate of core senescence-associated pathways, but with distinct patterns reflecting their triggers. When comparing the top 20 Ingenuity Canonical Pathways shared across conditions, we observed a consistent downregulation of pathways related to mitochondrial function and protein synthesis. Oxidative Phosphorylation (HP vs LP, z-score: 6.429; DEX vs LP, z-score: -2.333; DOX vs LP, z-score: -3), Ribosomal Quality Control Signaling Pathway (HP vs LP, z-score: -6.94; DEX vs LP, z-score: -3.024; DOX vs LP, z-score: -6.351) and Selenoamino acid metabolism (HP vs LP, z-score: -5.986; DEX vs LP, z-score: -5; DOX vs LP, z-score: -6.788) are suppressed in all three models. Both HP and DOX-induced senescence exhibited strong inhibition of mitotic and checkpoint-associated signaling, showed by the suppression of Cell Cycle Checkpoints pathway (HP vs LP, z-score: -6.47; DOX vs LP, z-score: -1.877) and Mitotic Metaphase and Anaphase (HP vs LP, z-score: -4.882; DOX vs LP, z-score: -2.4), whereas DEX-treated cells show activation of these same cell cycle progression pathways (DEX vs LP, z-score: 4.841; z-score: 5.692 respectively). DEX and DOX led to pathways like neutrophil degranulation (DEX, z-score: 2.082; DOX, z-score: 2.828) and Class I MHC mediated antigen processing and presentation (DEX, z-score: 2.2; DOX, z-score: 1.8) to be activated. $n = 3$ biological replicates with one technical replicate per donor per condition. LP Low Passage cells (P3), DEX low passage cells (P3) stimulated with DEX (1 μ M), DOX low passage cells (P3) stimulated with DOX (50 nM), HP High Passage cells (P40).

Canonical mitochondrial function and protein synthesis pathways, including "Oxidative Phosphorylation", "Ribosomal Quality Control Signaling", and "Selenoamino Acid Metabolism pathways, were consistently downregulated across all three models (Table 1, Fig. 3C).

However, transcriptomic analysis also highlighted key divergences (Table 1, Supplementary Tables 1, 2, and 3, Fig. 3C). HP and DOX-induced senescence strongly inhibited mitotic and cell cycle checkpoint signaling, including Cell Cycle Checkpoints pathway and Mitotic Metaphase and Anaphase. Conversely, DEX-treated

cells showed activation of these pathways. These differences align with telomere erosion and DNA damage observed in HP and DOX, suggesting DEX induces a stress that doesn't immediately silence checkpoint genes.

Although p16 could not be directly detected, CDK4, a key target inhibited by p16 to enforce cell cycle arrest, was significantly downregulated in HP vs LP chondrocytes (logFC: -3.81; adj $p = 0.0010$), and non-significantly downregulated in DEX vs LP (logFC: -0.23; adj $p = 0.94$) and DOX vs LP (logFC: -0.58; adj $p = 0.18$). The observed suppression of CDK4 expression may

Table 1. Commonly or uniquely top 20 Ingenuity Canonical Pathways found among all sets of variables in Chondrocytes (sorted by descending absolute z-score).

Comparable Set of Variables	Ingenuity Canonical Pathways	z-score HP vs LP	z-score Dexa vs LP	z-score Doxo vs LP
[HP vs LP]	Neutrophil degranulation	−7.553	2.082	2.828
	Protein Ubiquitination Pathway	−6.935	2.556	0.962
	Mitochondrial protein degradation	−6.252	−1.069	−2.496
	RHO GTPase cycle	−6.248	0.98	0.729
	Asparagine N-linked glycosylation	−6.112	0.905	1.508
	Class I MHC mediated antigen processing and presentation	−5.584	2.2	1.8
	Mitotic G2-G2/M phases	−5.5	2.449	−0.688
	Granzyme A Signaling	5.385	1.667	N/A
	Nuclear Cytoskeleton Signaling Pathway	−5.345	0.816	−0.688
[Dexa vs LP]	Mitotic Metaphase and Anaphase	−4.882	5.692	−2.4
	Mitotic Prometaphase	−4.16	5.604	−0.943
	RHO GTPases Activate Formins	−3.042	3.78	−2.138
	Nucleosome assembly	−2.828	3.162	
	Protein Sorting Signaling Pathway	−3.883	3.051	2
	Regulation of mitotic cell cycle	−3.893	2.887	−2.121
	Synthesis of DNA	−4.523	2.887	−1.89
	Kinetochore Metaphase Signaling Pathway	−0.728	2.837	1.134
[Doxo vs LP]	Processing of Capped Intron-Containing Pre-mRNA	−4.21	−2.414	−5.154
	RNA Polymerase II Transcription	−4.258	−2.138	−4.025
	EIF2 Signaling	−3.938	−1.147	−3.656
	COPI-mediated anterograde transport	−3.773	1.941	3.606
	rRNA modification in the nucleus and cytosol	−5.209	−2.121	−3.162
	TP53 Regulates Transcription of DNA Repair Genes	−2.985	−1	−3
	tRNA Charging	−4	−2.646	−2.828
	Mitochondrial protein import	−3.9	0	−2.828
[HP vs LP] and [Doxo vs LP]	Mitochondrial translation	−7.211	−1.667	−3.317
	Oxidative Phosphorylation	−6.429	−2.333	−3
	SRP-dependent cotranslational protein targeting to membrane	−5.578	−2.646	−5.485
[Dexa vs LP] and [Doxo vs LP]	Eukaryotic Translation Elongation	−4.811	−4.69	−6.487
	Eukaryotic Translation Termination	−4.901	−4.583	−6.41
	Nonsense-Mediated Decay (NMD)	−4.906	−4.491	−6.223
	Eukaryotic Translation Initiation	−5.093	−4.041	−6.804
[HP vs LP] and [Dexa vs LP] and [Doxo vs LP]	Major pathway of rRNA processing in the nucleolus and cytosol	−8.433	−3.8	−7.147
	Ribosomal Quality Control Signaling Pathway	−6.94	−3.024	−6.351
	Respiratory electron transport	−6.782	−3.162	−2.828
	Selenoamino acid metabolism	−5.986	−5	−6.788
	Response of EIF2AK4 (GCN2) to amino acid deficiency	−5.252	−4.796	−6.487
[HP vs LP] and [Dexa vs LP]	Cell Cycle Checkpoints	−6.47	4.841	−1.877
	Deubiquitination	−6.183	3.128	0.2
	Complex I biogenesis	−6.164	−3.317	−2.449

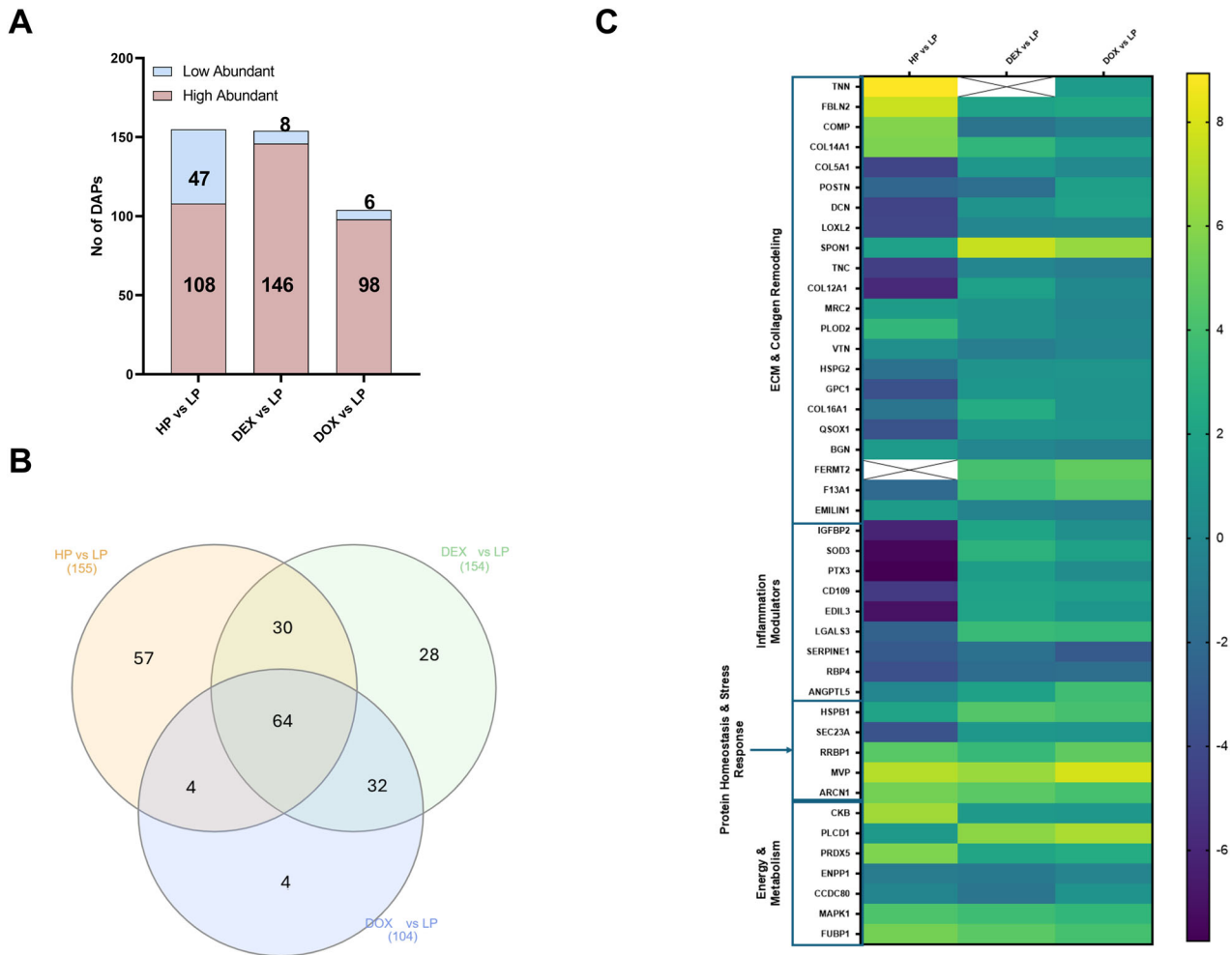


Fig. 4 Effect of chemically induced senescence by Doxorubicin (DOX) and Dexamethasone (DEX) and serial passaging (HP) on chondrocytes' proteomics. **A** Serial passaging of chondrocytes (HP) resulted in 155 abundant DAPs (108 high and 47 low abundant) between HP and LP chondrocytes, 154 DAPs (146 high and 8 low abundant) between DEX vs LP and 104 (98 high and 6 low abundant) DAPs between DOX vs LP. **B** Venn diagram of the differentially abundant proteins (adjusted $p < 0.05$) between HP vs LP, DEX vs LP and DOX vs LP comparisons groups showing that chemically induced and replicative induced cells shared 64 DAPs between each other. Chondrocytes stimulated with DEX shared more DAPs with DOX induced cells (32 DAPs) than with HP cells (30 DAPs); in contrast DOX stimulated cells shared the least proteins with HP chondrocytes (4 DAPs). Furthermore, 57 unique DAPs were identified between HP vs LP chondrocytes, 28 unique DAPs DEX vs LP and 4 unique DAPs DOX vs LP. **C** Heat map with top 10 low and high regulated proteins (ranked by Log₂ fold change) revealed that replicative senescence (HP) uniquely show a substantial increase in structural ECM proteins, including Tenascin-N (TNN, Log₂FC: 8.93), Fibulin-2 (FBLN2, Log₂FC: 7.61), Cartilage oligomeric matrix protein (COMP, Log₂FC: 5.82), and Collagen XIV (COL14A1, Log₂FC: 5.68); and the reduced expression of Spondin-1 (SPON1, Log₂FC: 1.65225). DEX and DOX stimulated cells do not exhibit this level of ECM protein induction; key matrix components such as COMP are less expressed (DEX Log₂FC: -1.45096; DOX Log₂FC: 6.3222) and extracellular matrix glycoproteins like SPON1 are highly expressed (DEX Log₂FC: 7.49653; DOX Log₂FC: -0.613366). Several secreted proteins associated with the SASP are strongly decreased in HP vs LP, including pentraxin-3 (PTX3 Log₂FC: -7.74642), the extracellular antioxidant enzyme superoxide dismutase 3 (SOD3 Log₂FC: -7.54732), an angiogenic glycoprotein (EDIL3 Log₂FC: -7.07963), and IGF-binding protein 2 (IGFBP2 Log₂FC: -6.11824). These same proteins are strongly highly expressed in DEX-treated cells (PTX3 Log₂FC: 1.46653; SOD3 Log₂FC: 2.96889; EDIL3 Log₂FC: 1.88753; IGFBP2 Log₂FC: 1.98434) and to a lesser extent in the DOX condition (PTX3 Log₂FC: 0.299594; SOD3 Log₂FC: 1.73983; EDIL3 Log₂FC: 1.00945; IGFBP2 Log₂FC: 0.549595). $n = 3$ biological replicates with one technical replicate per donor per condition. LP Low Passage cells (P3), DEX low passage cells (P3) stimulated with DEX (1 μ M), DOX low passage cells (P3) stimulated with DOX (50 nM), HP High Passage cells (P40).

indirectly suggest p16 pathway activation in the different senescence induction models. Additionally, CDKN2A (p16) interacting protein (CDKN2AIP), which regulates the DNA damage response by influencing pathways like the p53-HDM2-p21 pathway [41], was upregulated in HP vs LP (logFC = 0.956; adj. $p = 0.353$), but not regulated in DEX vs LP (logFC = 0.012; adj. $p = 1$) and DOX vs LP (logFC = 0.081; adj. $p = 1$).

Furthermore, DEX and DOX activated pro-inflammatory and immune-activating programs, such as neutrophil degranulation and Class I MHC-mediated antigen processing and presentation, which were, conversely, inhibited in HP cells. This suggests that DEX and DOX-induced senescence is driven by canonical SASP

components with a more active immune signature, while HP-induced senescence presents a more attenuated immune profile (Table 3).

Proteomics

High-resolution mass spectrometry of the chondrocyte secretome identified 571 proteins, of which 185 were annotated and secreted (Supplementary Table 4).

Venn analysis of differentially abundant proteins (DAPs) identified 155 DAPs (108 high, 47 low) between HP and LP chondrocytes, 154 DAPs (146 high, 8 low) between DEX vs LP and 104 (98 high, 6 low) DAPs between DOX vs LP (Fig. 4A).

Table 2. Commonly or uniquely top 10 up and down Differentially abundant Proteins found among all sets of variables in Chondrocytes, sorted by descending logFC.

Comparable Set of Variables	Gene Symbol	LOG FC HP vs LP	LOG FC Dexa vs LP	LOG FC Doxo vs LP
[HP vs LP]	TNN	8.93487	0	1.28831
	FBLN2	7.60916	1.81018	2.17614
	LOC101111669	7.39552	0	2.0469
	CKB	6.60706	1.23089	1.03836
	COMP	5.82252	−1.45096	−0.613366
	PRDX5	5.69171	2.05213	2.52071
	COL14A1	5.67957	3.17315	1.51943
	SEC23A	5.51315	3.30076	3.40171
	LOXL2	−4.30531	−0.106401	−0.0519409
	COL5A1	−4.34614	1.07169	0.0837688
	DCN	−4.44267	0.820508	1.83648
	TNC	−4.7323	−0.101554	−0.784161
	CD109	−5.04904	1.83798	1.49548
	COL12A1	−5.89748	1.61587	0.000428518
	IGFBP2	−6.11824	1.98434	0.549595
	EDIL3	−7.07963	1.88753	1.00945
	SOD3	−7.54732	2.96889	1.73983
	PTX3	−7.74642	1.46653	0.299594
[Dex vs LP]	MAPK1	4.23177	3.74785	3.26995
	LGALS3	−2.55877	3.53249	3.31746
	MRC2	1.3605	0.58038	−0.218061
	PLOD2	3.26088	0.568567	0.00794792
	VTN	0.540558	−0.810243	−0.110742
	ENPP1	−0.829931	−1.0456	−0.26598
	CCDC80	−0.188419	−1.28869	0.772641
	POSTN	−2.27068	−1.85607	1.5701
[Dox vs LP]	RRBP1	4.61151	3.43837	4.85925
	ANGPTL5	−0.0987027	1.79267	3.81808
	QSOX1	−3.52953	1.12325	0.952967
	HSPG2	−1.5812	0.816158	0.914053
	GPC1	−3.65928	0.969566	0.814065
	COL16A1	−1.29796	2.47281	0.799962
	BGN	1.34528	−0.149156	−0.593702
	LOC114114921	−1.07612	0.772727	−1.15538
[Dex vs LP] and [Dox vs LP]	SPON1	1.65225	7.49653	6.3222
	PLCD1	1.16092	6.06344	6.8285
	HSPB1	1.9135	4.47272	4.00182
	FERMT2	0	4.02472	4.89491
	ARCN1	4.72982	4.00793	3.77874
	F13A1	−2.09206	3.60202	4.54532
	EMILIN1	1.35788	−0.348153	−0.804801
	LOC101102413	0.102794	−0.920633	−0.343047
	SERPINE1	−3.12593	−1.58794	−3.12957
	RBP4	−3.69731	−1.86536	−1.78199
[HP vs LP] and [Dex vs LP] and [Dox vs LP]	MVP	7.09993	6.40629	7.89294
	FUBP1	5.43331	4.65852	3.96437

A total of 64 DAPs were common across all three conditions. DEX and DOX shared more DAPs (32) than DEX and HP (30), while DOX and HP shared the fewest (4, Fig. 4B). Furthermore, unique DAPs were identified for each condition: 57 for HP, 28 for DEX and 4 for DOX.

Proteomic analysis demonstrated functional distinctions (Fig. 4C, Table 2, Supplementary Table 4). HP uniquely showed increased abundance of structural ECM proteins, including Tenascin-N, Fibulin-2, Cartilage oligomeric matrix protein (COMP),

Table 3. The senescence-associated secretory phenotype (SASP) factors found among all sets of variables in Chondrocytes.

Gene / Protein name	Gene expression/ Regulation				Protein abundance/ regulation					
	Log FC HP_LP	padj HP vs LP	Log FC DEX_LP	padj DEX vs LP	Log FC DOX_LP	padj DOX vs LP	Log FC DEX_LP	q-value HP_LP	Log FC DEX_LP	q-value DOX_LP
Interleukins										
interleukin 6(IL6)	− 3.4622	0.0028	− 4.9148	0.0000	− 2.0590	0.0014				
interleukin 15(IL15)	0.0000	1.0000	0.0157	1.0000	0.0256	1.0000				
Chemokines										
chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)(CXCL1)	0.1563	1.0000	− 0.0245	1.0000	− 0.0203	1.0000				
Other inflammatory markers										
C-X-C motif chemokine ligand 8(CXCL8)	0.0000	1.0000	0.0000	1.0000	0.0256	1.0000				
granulocyte-macrophage colony-stimulating factor receptor subunit alpha(LOC101115509)	0.3120	1.0000	− 0.0129	1.0000	0.0253	1.0000				
complement C1s(C1S)	− 4.7106	0.0000	− 0.8793	0.0191	0.1627	0.6948	− 1.9943	0.0038	2.3822	0.0139
complement C3(LOC443475)	0.0162	1.0000	− 0.0383	1.0000	− 0.0352	1.0000	− 1.0761	0.0855	0.7727	0.0163
Growth factors and regulators										
vascular endothelial growth factor B(VEGFB)	0.2599	0.6120	0.1523	1.0000	0.0107	1.0000				
vascular endothelial growth factor C(VEGFC)	− 0.0834	1.0000	− 0.0853	1.0000	− 0.1870	0.7033				
vascular endothelial growth factor D(VEGFD)	0.0000	1.0000	0.0267	1.0000	0.0396	1.0000				
epidermal growth factor receptor(EGFR)	0.3120	1.0000	0.0014	1.0000	− 0.0071	1.0000				
amphiregulin(AREG)	0.0000	1.0000	0.0000	1.0000	0.0255	1.0000				
nerve growth factor(NGF)	− 1.3422	1.0000	− 0.3154	1.0000	− 0.3092	1.0000				
insulin like growth factor binding protein 2(IGFBP2)							− 6.1182	0.0038	1.9843	0.0152
insulin like growth factor binding protein 3(IGFBP3)	1.0000	0.3409	0.1319	1.0000	0.5565	1.0000				
insulin like growth factor binding protein 4(IGFBP4)	0.1566	1.0000	− 0.0080	1.0000	− 0.0203	1.0000	1.7262	0.0728	0.6648	0.2763
insulin like growth factor binding protein 5(IGFBP5)							− 2.4970	0.0000	0.1516	0.4618
insulin like growth factor binding protein 6(IGFBP6)	− 6.0580	0.0000	− 0.4773	0.1326	1.8387	0.0000	− 1.8265	0.0693	1.4104	0.0315
insulin like growth factor binding protein 7(IGFBP7)	− 0.9227	0.4167	− 0.7161	0.0416	0.1087	0.8031				
hepatocyte growth factor(HGF)	− 0.3474	0.6241	0.1961	1.0000	0.1126	1.0000				
angiogenin-1- like(LOC10113335)	− 0.2233	1.0000	0.0030	1.0000	− 0.1080	1.0000				

Table 3. continued

Gene / Protein name	Gene expression/ Regulation				Protein abundance/ regulation					
	Log FC HP_LP	padj HP vs LP	Log FC DEX_LP	padj DEX vs LP	Log FC DOX_LP	padj DOX vs LP	Log FC HP_LP	q-value HP_LP	Log FC DEX_LP	q-value DEX_LP
angiogenin-2-like(LOC101114959)	-0.8386	0.3769	0.0583	1.0000	0.4211	1.0000				
Proteases and regulators										
matrix metalloproteinase 1(MMP1)	-0.1389	1.0000	-0.0810	1.0000	0.0210	1.0000				
matrix metalloproteinase 2(MMP2)	-1.3391	0.2479	0.4093	0.4529	1.3826	0.0028	-1.0839	0.0195	2.0037	0.0106
matrix metalloproteinase 13(MMP13)	0.0000	1.0000	0.0472	1.0000	0.0000	1.0000				
TIMP metalloproteinase inhibitor 1(TIMP1)	-4.8544	0.0000	-0.9051	0.0003	0.1899	0.5103	-1.7375	0.0317	-0.2063	0.7851
TIMP metalloproteinase inhibitor 2(TIMP2)	-1.4483	0.0124	-0.3468	0.2414	0.8870	0.0068	2.0238	0.0045	2.3465	0.0086
TIMP metalloproteinase inhibitor 3(TIMP3)	-7.2245	0.0000	0.0183	0.8073	0.1583	0.6610				
cathepsin B(CTSB)	-1.9834	0.0702	-0.1446	1.0000	0.1823	1.0000	-4.0543	0.0035	2.1710	0.0093
cathepsin F(CTSF)	-1.2728	0.2472	-0.1009	1.0000	0.0966	1.0000				
ADAM metalloproteinase with thrombospondin type 1 motif 5(ADAMTS5)	-5.2868	0.0000	-0.0414	0.8094	-0.0335	0.9001	-0.3924	0.5875	1.2322	0.0546
plasminogen activator, tissue type(PLAT)	0.3120	1.0000	-0.0129	1.0000	-0.0071	1.0000				
plasminogen activator, urokinase(PLAU)	-5.3510	0.0000	-1.2482	0.0037	-0.6560	0.0984				
EGF containing fibulin extracellular matrix protein 1(EFEMP1)	-4.0266	0.0010	-1.8014	0.0062	0.1858	0.7792	-0.0347	0.8890	0.5210	0.0989
lysyl oxidase like 3(LOXL3)	-5.1840	0.0000	-1.0898	0.0040	-0.7597	0.0485	2.7952	0.0032	1.4534	0.0121
perostin(POSTN)	-4.1142	0.0043	0.2267	0.4113	1.4711	0.0074	-2.2707	0.0075	-1.8561	0.0199
protein disulfide isomerase family A member 3(PDIA3)	-0.4976	0.9167	0.4639	0.1289	0.6245	0.0845	1.0648	0.0266	2.0144	0.0101
quiescin sulphydryl oxidase 1(QSOX1)	-0.3725	1.0000	-0.0873	1.0000	-0.0805	1.0000	-3.5295	0.0043	1.1233	0.0233
major vault protein(MVP)	-4.3029	0.0003	0.1326	0.6036	0.3235	0.3591	7.0999	0.0067	6.4063	0.0116
secreted frizzled related protein 2(SFRP2)	0.7429	0.4405	0.0529	1.0000	0.3738	1.0000	0.8337	0.5205	3.3796	0.0164
inter-alpha-trypsin inhibitor heavy chain 2(IH2)							0.2357	0.1176	-0.2799	0.1460
ectonucleotide pyrophosphatase/	-1.3778	0.2186	-0.1372	1.0000	0.0675	1.0000				

Table 3. continued

Gene / Protein name	Gene expression/ Regulation				Protein abundance/ regulation					
	Log FC HP_LP	padj HP vs LP	Log FC DEX_LP	padj DEX vs LP	Log FC DOX_LP	padj DOX vs LP	Log FC HP_LP	q-value HP_LP	Log FC DEX_LP	q-value DEX_LP
phosphodiesterase family member 5(ENPP5)										
Soluble or shed receptors or ligands	intercellular adhesion molecule 1(ICAM1)	0.3179	1.0000	0.0132	1.0000	0.0096	1.0000			
	plasminogen activator, urokinase receptor(PLAUR)	-2.1435	0.0616	-0.1594	1.0000	-0.0389	1.0000			
	Fas cell surface death receptor(FAS)	-1.7300	0.0987	0.4231	0.7266	0.7412	0.2100			
	thrombospondin 1(THBS1)	-0.1800	1.0000	0.2620	0.4074	0.2910	0.4222	0.3719	1.6400	0.0422
	thrombospondin 2(THBS2)							3.9507	2.0558	0.0432
	serpin family E member 1(SERPINE1)	-2.5859	0.0253	-0.3581	0.8542	-0.6359	0.1967	-3.1259	-1.5879	0.0233
	serpin family B member 1(SERPINB1)	-4.5161	0.0001	0.3125	0.2535	1.8926	0.0000	-3.5016	2.0906	0.0225
	serpin family F member 1(SERPINF1)	-2.3370	0.0410	-1.1553	0.0311	-0.8898	0.0429	3.3045	0.2590	0.4865
	serpin family G member 1(SERPING1)	-2.3458	0.0348	-0.1682	1.0000	0.0474	1.0000	-3.1116	2.3203	0.0141
	S100P binding protein(S100PBP)	-0.5137	1.0000	-0.1411	1.0000	-0.1272	1.0000			
	S100 calcium binding protein B(S100B)	0.4229	0.5577	0.4718	1.0000	0.4926	1.0000			
	S100 calcium binding protein A4(S100A4)	-6.1516	0.0000	0.0012	0.8466	-1.0843	0.0101	-0.7606	1.6923	0.0190
	HtrA serine peptidase 1(HTRA1)	-3.0685	0.0096	-0.1397	1.0000	0.2578	0.5713	1.8632	1.6023	0.0104
	secreted protein acidic and cysteine rich(SPARC)	-2.6339	0.0000	-0.2060	0.4747	1.3120	0.0002	1.1569	1.4877	0.0152
	proteasome 20S subunit alpha 7(PDMA7)	-6.3170	0.0000	0.9222	0.0000	0.4038	0.0874	1.9219	1.4686	0.0200
	proteasome 20S subunit beta 4(PSMB4)	-2.5862	0.0000	-0.3453	0.2514	-0.3409	0.3424			
	folliculin like 1(FSTL1)	0.1174	1.0000	-0.0989	0.6832	0.0799	0.8191	0.8309	1.5800	0.0160
	cystatin C(CST3)	-5.6486	0.0000	-0.5704	0.0851	0.3242	0.4153	-3.2859	1.7115	0.0098
	cellular communication network factor 2(CCN2)	-1.9736	0.0006	-1.8771	0.0000	-0.7820	0.0140	-0.7490	-0.2718	0.6775
	cadherin 13(CDH13)	-4.6027	0.0001	-0.6045	0.0324	-0.4972	0.1267	-1.7728	1.7785	0.0103
	clusterin(LOC101113728)	-6.5598	0.0000	-1.0080	0.0006	0.7473	0.0188	2.7319	0.0000	1.0000

Table 3. continued

Gene / Protein name	Gene expression/ Regulation				Protein abundance/ regulation			
	Log FC HP_LP	padj HP vs LP	Log FC DEX_LP	padj DEX vs LP	Log FC HP_LP	q-value HP_LP	Log FC DEX_LP	q-value DEX_LP
pentraxin 3(PTX3)	-3.6839	0.0034	2.5648	0.0000	3.5607	0.0000	-7.7464	0.0023
dipeptidyl peptidase 4(DPP4)	0.0000	0.6760	3.8513	0.0007	3.9655	0.0005		
laminin subunit beta 1(LAMB1)	-2.8349	0.0122	0.3033	0.6529	0.4465	0.2339		
fibronectin 1(FN1)	-2.8517	0.0041	-0.1548	0.5666	0.1908	0.6397	-9.1643	0.0071
HMG box domain-containing protein (W5N72_SHEEP)	0.7494	0.4482	0.1323	1.0000	-0.2151	1.0000		
high mobility group box 2(HMGB2)	-1.4367	0.2694	0.9680	0.0401	-0.2009	0.6378		
high mobility group box 3(HMGB3)	-1.3985	0.1715	0.5228	0.4844	-0.3261	0.7019		

and Collagen XIV, alongside reduced Spondin-1 (SPON1). In contrast, in DEX and DOX key matrix components such as COMP were less abundant, and extracellular matrix glycoproteins like SPON1 were highly abundant, indicating potential impairment in matrix synthesis. Notably, several SASP factors (pentraxin-3, superoxide dismutase 3, EDIL3, IGF-binding protein 2) were less abundant in HP compared to LP cells, but highly abundant in DEX-treated cells and, to a lesser extent, in DOX-stimulated cells (Table 3).

DISCUSSION

This study systematically compared three senescence induction methods - serial passaging (HP), doxorubicin (DOX), and dexamethasone (DEX)—to assess their biological fidelity and utility for modeling OA-associated chondrocyte senescence. All three protocols induced classical senescence features, albeit to varying extents. DOX and HP significantly reduced cellular proliferation, while DEX caused a non-significant decrease. DEX and HP significantly increased SA- β -Gal activity, whereas DOX induced a non-significant rise in SA- β -Gal activity. DOX significantly elevated H3K9me3 levels, while DEX and HP showed a non-significant increase. DEX significantly increased the cytoplasm-to-nucleus area ratio, with DOX and HP showing a non-significant trend. These findings indicate that each method activates distinct molecular pathways and stress responses, underscoring their differing relevance and applicability for senescence modeling and senotherapeutic research.

Mitochondrial dysfunction emerged as a converging hallmark across all models. Mitochondria play a central role in the regulation and execution of cellular senescence, serving as both the primary site of ATP production and the major source of ROS during oxidative phosphorylation (OXPHOS) [42–44]. When mitochondrial function is compromised, this dual role shifts from supporting cellular energy demands to mediating oxidative damage [42–44]. Reflecting this functional disruption, transcriptomic analyses in this study revealed downregulation of gene sets involved in OXPHOS, electron transport, and Complex I biogenesis across all models, most prominently in HP chondrocytes. However, the functional impact of mitochondrial impairment was model-specific. In both HP and DOX-treated cells, mitochondrial dysfunction coincided with elevated ROS, reduced ATP, and broad suppression of biosynthetic and proliferative pathways, including those governing DNA replication, cell cycle progression, ribosomal function, and translation. DEX produced a similar, though milder, transcriptomic profile. Intriguingly, DEX-treated chondrocytes paradoxically exhibited reduced mitochondrial superoxide, increased ATP levels, and upregulated mitochondrial gene expression. These findings may reflect a dose- and time-dependent compensatory metabolic adaptation, consistent with glucocorticoids' known cell type-specific and context-dependent effects on mitochondrial mass, metabolism and biogenesis via mitochondrial-localized glucocorticoid receptor signaling [45–49]. Such responses may transiently elevate ATP despite declining mitochondrial efficiency, particularly when biosynthetic demand is reduced due to senescence. DEX may also promote a shift from glycolysis to OXPHOS, contributing to altered metabolic profiles [45–47].

All models also exhibited downregulation of the “Selenoamino Acid Metabolism” pathway, suggesting impaired antioxidant defense. Selenium and selenoproteins are essential for redox homeostasis, and their depletion accelerates senescence via increased ROS and SASP expression [50–52]. A ~72% proteomic overlap between selenium deficiency and senescence further supports this link [50], suggesting that oxidative stress in all models is exacerbated by compromised redox buffering capacity.

Together, these findings underscore mitochondrial dysfunction and oxidative stress as core, reciprocally reinforcing hallmarks of

cellular senescence, acting both as initiators and amplifiers of senescence across diverse stimuli. Aging and cellular stress promote mtDNA damage, which impairs electron transport, elevates ROS and activates p53/p21 signaling and SASP expression [53]. This feed-forward loop contributes to cellular dysfunction and OA progression. Notably, mitochondrial disturbances are exacerbated in the osteoarthritic joint, where mechanical stress and inflammation synergistically increase oxidative stress, which impairs matrix homeostasis, reduces proteoglycan synthesis, and disrupts antioxidant defenses [2, 4–8, 10, 54–57]. Redox-sensitive pathways like NF- κ B and PKC δ /IKK α /p53 further amplify MMP production and SASP release, linking oxidative stress to cartilage degradation [2, 4–8, 10, 54–57].

Despite these shared features, the three models differed in immune-related transcriptional and proteomic responses and cellular stress pathways. Notably, the neutrophil degranulation pathway was uniquely downregulated in HP chondrocytes, consistent with an immune-evasive phenotype that promotes age-related senescent cells accumulation despite a pro-inflammatory SASP [58]. In contrast, DOX and DEX induced SIPS, typically associated with active inflammatory signaling. Both maintained neutrophil degranulation-related gene expression, likely reflecting ongoing SASP activity, fueled by ROS and DNA damage responses, especially in DOX-treated cells. DOX is known to promote neutrophil-driven inflammation [59], while glucocorticoids like DEX, despite their immunosuppressive effects, exert complex and contradictory actions on neutrophils, including enhanced survival and delayed apoptosis [60].

DOX treatment triggered a robust DNA damage response (DDR), marked by increased p53, CDKN1A (p21), and H3K9me3 expression, consistent with its genotoxic mode of action [30, 33, 35, 61–64]. Concurrent increases in ROS and SASP reinforced DDR-induced senescence. However, prominent activation of cleaved caspase-3/7 indicated early apoptosis, raising concerns about its fidelity as a model for chronic OA-associated senescence, where senescent chondrocytes typically are long-lived and resistant to apoptosis [15]. DOX-induced mitochondrial dysfunction likely contributed to this apoptotic priming via cytochrome c release, caspase activation and oxidative stress [44, 64, 65]. The overlap between senescence and apoptotic pathways could confound interpretations of SASP dynamics or senolytic efficacy.

In contrast, DEX-induced senescence developed more gradually and lacked strong DDR activation or apoptotic priming. Although DEX-stimulated chondrocytes exhibited classical senescence markers including SA- β -gal activity, cell cycle and proliferation arrest, SASP and altered morphology, they did not show elevated ROS. This supports a mechanistically distinct SIPS phenotype, consistent with clinical observations of glucocorticoid-induced cartilage degeneration and prior studies showing that DEX induces mitochondrial dysfunction and p53/p21 signaling independent of DNA damage [32, 48, 49, 66].

Serial passaging induced the most robust and consistent senescence phenotype, with marked reductions in mitochondrial gene expression and telomere length and increased ROS and SASP - hallmarks of replicative senescence observed in aging and OA cartilage [20, 67]. However, repeated trypsinization during passaging may introduce confounding stress. Trypsin-mediated detachment reduces intracellular glutathione levels and upregulates stress-response and apoptosis-related proteins, such as p53 and p21 [68–71]. Proteomic studies have shown that trypsinisation decreases proteins associated with cell metabolism, growth, mitochondrial electron transportation and cell adhesion, while increasing proteins involved in cell apoptosis and cell cycle arrest [68–71]. This makes it critical to distinguish between replicative exhaustion and trypsin-induced damage when interpreting results from this model.

In summary, this study demonstrates that mitochondrial dysregulation is a central and unifying feature across diverse

senescence models. While all models converged on core senescence hallmarks, they diverged in their molecular mechanisms, immune responses, and apoptotic profiles. These differences emphasize the need for careful model selection guided by mechanistic alignment with *in vivo* pathology, particularly for translational studies seeking to evaluate senotherapeutic strategies or dissect disease-specific senescence mechanisms.

MATERIALS AND METHODS

All experiments were carried out with 3 biological replicates. All experiments adhered to ethical guidelines and were performed with appropriate institutional approval (details: see declarations). For comprehensive descriptions of all experimental procedures, please refer to the Supplementary Material.

Chondrocyte isolation and culture

Primary ovine articular chondrocytes ($n = 3$ biological replicates), which had been previously isolated and biobanked from female, musculoskeletally mature Merino-cross sheep (2–5 years old, body weight 95 ± 12 kg) without orthopedic disease euthanized for reasons unrelated to this study [72], were cultured in StemMACS[®] medium (Miltenyi Biotec, Cologne, Germany) supplemented with StemMACS[®] XF plus 1% Pen/Strep.

Senescence induction

Optimal doses and administration periods for doxorubicin (DOX) and dexamethasone (DEX) to induce senescence in chondrocytes were established through a pilot study. This preliminary work compared concentrations of 25, 50, 100, and 200 nM for DOX, and 1, 5, 10, and 20 μ M for DEX (Supplementary Figs. 1 and 2, Supplementary Results) [32, 73, 74]. Based on cell viability and gene expression levels of senescence-associated genes (Suppl. Results), a dose of 1 μ M DEX and 50 nM DOX was selected for subsequent experiments. Cell seeding densities were adjusted to accommodate the varying durations of senescence induction for each protocol.

For DOX-induced senescence, cells in passage 3 (P3) were seeded at 9091 cells/cm² and treated with 50 nM DOX on days 1, 4, and 7 post-seeding. A recovery phase was initiated on day 10 by switching to DOX-free medium for 3 days [73]. For DEX-induced senescence, cells (P3) were seeded at 455 cells/cm² and treated with 1 μ M every 72 h over a total period of 9 days [32, 74]. Replicative senescence (HP) was established by serial passaging of chondrocytes up to P40. Low passage cells (LP, P3) served as controls. Both LP and HP cells were seeded at 3030 cells/cm² for all assays.

Cell cycle analysis by flow cytometry

Cell cycle distribution was determined by propidium iodide (PI) staining. Cells were stained with a PI buffer containing sodium citrate, Triton X-100, RNase (Invitrogen, USA), and 20 μ g/mL PI (Sigma-Aldrich, USA). Following a 30-min incubation at 37 °C, samples were analyzed using a flow cytometer, and data were processed using FlowJo (v10) with the Watson Pragmatic model.

Cell viability and proliferation assays

Cell viability was quantitatively assessed using the MTT assay (Promega, USA). Absorbance was measured at 595 nm using a Varioskan LUX plate reader (Thermo Fisher, USA). Cell proliferation was continuously monitored by confluence measurements utilizing the IncuCyte[®] S3 live-cell analysis system (Sartorius, Germany).

Cell morphology by Phalloidin staining

Cells were fixed, permeabilized, and stained with Atto 488 Phalloidin (Sigma-Aldrich, USA) and DAPI (250 ng/mL). Images were acquired with the EVOS FL Auto microscope (Thermo Fisher) and quantified using ImageJ software (NIH, USA).

Relative telomere length by RT-qPCR

Genomic DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Germany). Telomere and single-copy gene (B2M) levels were quantified using EvaGreen[®] mix (Bio&Sell, Germany) on a Viia7 qPCR system (Thermo Fisher, USA), and relative telomere length was calculated using the $\Delta\Delta$ Ct method.

Immunofluorescence staining

Cells were fixed, permeabilized, and blocked with buffer containing FCS, BSA, and Triton X-100. H3K9me3 was detected using rabbit anti-H3K9me3 (nsJ Bioreagents, USA) and Alexa Fluor 488 secondary antibody (Thermo Fisher). Samples were mounted with DAPI mounting medium (ibidi, Germany) and images acquired with a Zeiss Observer microscope.

SA- β -Gal activity

Senescence-associated β -galactosidase activity was measured using a fluorometric assay kit (Cell Biolabs, USA) and the Varioskan LUX plate reader. Fluorescence was normalized to protein content, which was determined using the Qubit Protein Assay Kit (Thermo Fisher, USA).

Intracellular ROS measurement

ROS levels were assessed using the OxiSelect™ ROS assay kit (Cell Biolabs, USA). Fluorescence was measured at 480/530 nm with the Varioskan LUX plate reader.

Enzyme-Linked Immunosorbent Assay (ELISA)

TNF- α concentrations in cell culture supernatants were measured using a sandwich ELISA kit (MBS2701341, MyBiosource, USA). Absorbance was recorded at 450 nm using the Varioskan LUX plate reader and normalized to protein content measured with Bradford assay (Bio-Rad, USA).

Caspase 3/7 activity

Apoptotic activity was evaluated by measuring Caspase 3/7 activity using the Caspase-Glo® 3/7 Assay System (Promega, USA). Luminescence was measured using the Varioskan LUX plate reader and normalized to protein content measured with the Qubit Protein Assay Kit.

Gene expression by RT-qPCR

RNA was extracted with TRIzol (Invitrogen, USA) and purified with chloroform and isopropanol precipitation. RT-qPCR was performed using the RevTrans EvaGreen One-Step kit (Bio&Sell, Germany), and gene expression was normalized to GAPDH and RPLP0.

Mitochondrial DNA extraction and qPCR

Mitochondrial DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Germany), and qPCR was performed using EvaGreen qPCR Mix II (Bio&Sell, Germany) with specific primers for mitochondrial genes ND5, ND6, Cytb, and COX2.

Mitochondrial staining and flow cytometry

Cells were stained with MitoTracker™ Green FM and TMRM or MitoSOX™ Red (Invitrogen, USA), and analyzed by flow cytometry (BD FACS Canto). DAPI (Thermo Fisher, USA) was used to stain dead cells.

ATP quantification

Intracellular ATP levels were measured using the CellTiter-Glo® 2.0 Assay (Promega, USA). Luminescence was read with the Varioskan LUX plate reader and normalized to total protein measured with the Qubit assay.

Transcriptomic analysis (3' RNA-Seq)

RNA-seq libraries were prepared using QuantSeq 3' mRNA-Seq FWD protocol. Reads were mapped using STAR and analyzed with Bioconductor (DESeq2, IHW, EnhancedVolcano). Reference genomes were obtained from Ensembl and GENCODE.

Proteomics by mass spectrometry

Proteins from supernatants were digested using S-Trap microcolumns (Protifi), separated via nano-HPLC (Thermo), and analyzed on a Q-Exactive HF Orbitrap (Thermo Fisher). Data were processed using Proteome Discoverer and Perseus software.

Statistical analysis

Data (except NGS and Mass-Spec data) were analysed using Graphpad Prism (version 10.2.3). Statistical analysis was performed by paired t-test and paired one-way analysis of variance with Dunnett's multiple comparison test. $P < 0.05$ were considered statistically significant. The

variation within each group is presented in the form of standard error of the mean (SEM).

Ethical approval

No human or animal participants were involved in this study. Ovine chondrocytes had been obtained and biobanked from sheep euthanised for reasons unrelated to this study. Based on the "Good Scientific Practice. Ethics in Science und Research" regulation implemented at the University of Veterinary Medicine Vienna, the Institutional Ethics Committee ("Ethics and Animal Welfare Committee") of the University of Veterinary Medicine Vienna does not require approval of in vitro cell culture studies, if the cells were isolated from tissue, which was obtained either solely for diagnostic or therapeutic purposes or in the course of institutionally and nationally approved experiments. The sheep from which the cells were obtained had been euthanised in the course of a study for which approval of the national ("Commission for Animal Research" of the Austrian Federal Ministry of Science, Research and Economy) and institutional ("Ethics and Animal Welfare Committee" of the University of Veterinary Medicine Vienna) animal welfare committees (ethical approval number: 68.205/0100-V/3b/2018, 13.7.2018) had been granted and which had been reported according to ARRIVE guidelines 2.0. Euthanasia had been conducted following sedation with detomidine and butorphanol, placement of a catheter in the jugular vein, and induction of general anesthesia with thiopental, by the administration of T61, a veterinary euthanasia drug containing tetracaine hydrochloride, mebezonium iodide, and embutramide. All methods were carried out in accordance with the relevant guidelines and regulations.

DATA AVAILABILITY

The datasets generated and analysed during the current study are included in the paper and its supplementary materials. Please use the following link for the deposited RNA-Seq data: <https://dataview.ncbi.nlm.nih.gov/object/PRJNA1347060?reviewer=cunkk64deujh7e6igfgprgkje0>.

REFERENCES

- Cui A, Li H, Wang D, Zhong J, Chen Y, Lu H. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EclinicalMedicine*. 2020;29:100587.
- Arra M, Abu-Amer Y. Cross-talk of inflammation and chondrocyte intracellular metabolism in osteoarthritis. *Osteoarthritis Cartil*. 2023;31:1012–21.
- Thomas E, Peat G, Croft P. Defining and mapping the person with osteoarthritis for population studies and public health. *Rheumatology*. 2014;53:338–45.
- Liu Y, Zhang Z, Li J, Chang B, Lin Q, Wang F, et al. Piezo1 transforms mechanical stress into pro senescence signals and promotes osteoarthritis severity. *Mech Ageing Dev*. 2023;216:111880.
- Coryell PR, Diekmann BO, Loeser RF. Mechanisms and therapeutic implications of cellular senescence in osteoarthritis. *Nat Rev Rheumatol*. 2021;17:47–57.
- Bolduc JA, Collins JA, Loeser RF. Reactive oxygen species, aging and articular cartilage homeostasis. *Free Radic Biol Med*. 2019;132:73–82.
- Mobasheri A, Matta C, Zákány R, Musumeci G. Chondrosenescence: definition, hallmarks and potential role in the pathogenesis of osteoarthritis. *Maturitas*. 2015;80:237–44.
- Jiang W, Chen H, Lin Y, Cheng K, Zhou D, Chen R, et al. Mechanical stress abnormalities promote chondrocyte senescence - The pathogenesis of knee osteoarthritis. *Biomed Pharmacother*. 2023;167:115552.
- Xu M, Bradley EW, Weivoda MM, Hwang SM, Pirtskhalava T, Decklever T, et al. Transplanted senescent cells induce an osteoarthritis-like condition in mice. *J Gerontol Ser A: Biomed Sci Med Sci*. 2017;72:780–5.
- Minguzzi M, Cetrullo S, D'Adamo S, Silvestri Y, Flamigni F, Borzi RM. Emerging players at the intersection of chondrocyte loss of maturational arrest, oxidative stress, senescence and low-grade inflammation in osteoarthritis. *Oxid Med Cell Longev*. 2018;2018:3075293.
- Han Z, Wang K, Ding S, Zhang M. Cross-talk of inflammation and cellular senescence: a new insight into the occurrence and progression of osteoarthritis. *Bone Res*. 2024;12:69.
- Ramasamy TS, Yee YM, Khan IM. Chondrocyte aging: the molecular determinants and therapeutic opportunities. *Front Cell Dev Biol*. 2021;9:625497.
- Vidal-Bralo L, Lopez-Golan Y, Mera-Varela A, Rego-Perez I, Horvath S, Zhang Y, et al. Specific premature epigenetic aging of cartilage in osteoarthritis. *Aging*. 2016;8:2222–30.
- Harbo M, Delaisse JM, Kjaersgaard-Andersen P, Soerensen FB, Koelvraa S, Bendix L. The relationship between ultra-short telomeres, aging of articular cartilage and

- the development of human hip osteoarthritis. *Mech Ageing Dev.* 2013;134:367–72.
15. Jeon OH, Kim C, Laberge R-M, Demaria M, Rathod S, Vasserot AP, et al. Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat Med.* 2017;23:775–81.
 16. Jeon OH, David N, Campisi J, Elisseff JH. Senescent cells and osteoarthritis: a painful connection. *J Clin Investig.* 2018;128:1229–37.
 17. Martin JA, Buckwalter JA. Telomere erosion and senescence in human articular cartilage chondrocytes. *J Gerontol Ser A: Biol Sci Méd Sci.* 2001;56:B172–B179.
 18. Ogrodnik, Acosta M, Adams JC, Fagagna PD, d'Adda di F, Baker DJ, et al. Guidelines for minimal information on cellular senescence experimentation in vivo. *Cell.* 2024;187:4150–75.
 19. Ajoalabady A, Pratico D, Bahijri S, Tuomilehto J, Uversky VN, Ren J. Hallmarks of cellular senescence: biology, mechanisms, regulations. *Exp Mol Med* 2025;57:1482–1491.
 20. Rossiello F, Jurk D, Passos JF, Fagagna F, d'Adda di. Telomere dysfunction in ageing and age-related diseases. *Nat Cell Biol.* 2022;24:135–47.
 21. Atasoy-Zeybek A, Hawse GP, Nagelli CV, Padilla CMLD, Abdel MP, Evans CH. Transcriptomic changes during the replicative senescence of human articular chondrocytes. *Int J Mol Sci.* 2024;25:12130.
 22. Fragkiadaki P, Nikitovic D, Kalliantasi K, Sarandi E, Thanasoula M, Stivaktakis PD, et al. Telomere length and telomerase activity in osteoporosis and osteoarthritis. *Exp Ther Med.* 2020;19:1626–32.
 23. Zhang X-X, He S-H, Liang X, Li W, Li T-F, Li D-F. Aging, cell senescence, the pathogenesis and targeted therapies of osteoarthritis. *Front Pharm.* 2021;12:728100.
 24. Goldring MB, Otero M, Tsuchimochi K, Ijiri K, Li Y. Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. *Ann Rheum Dis.* 2008;67:iii75.
 25. Roach H, Aigner T, Soder S, Haag J, Welkerling H. Pathobiology of osteoarthritis: pathomechanisms and potential therapeutic targets. *Curr Drug Targets.* 2007;8:271–82.
 26. Liu Y, Zhang Z, Li T, Xu H, Zhang H. Senescence in osteoarthritis: from mechanism to potential treatment. *Arthritis Res Ther.* 2022;24:174.
 27. Georget M, Defois A, Guiho R, Bon N, Allain S, Boyer C, et al. Development of a DNA damage-induced senescence model in osteoarthritic chondrocytes. *Aging* 2023;15:8576–93.
 28. Wang N, He Y, Liu S, Makarczyk MJ, Lei G, Chang A, et al. Engineering osteoarthritic cartilage model through differentiating senescent human mesenchymal stem cells for testing disease-modifying drugs. *Sci China Life Sci.* 2022;65:309–27.
 29. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res.* 1961;25:585–621.
 30. Casella G, Munk R, Kim KM, Piao Y, De S, Abdelmohsen K, et al. Transcriptome signature of cellular senescence. *Nucleic Acids Res.* 2019;47:7294–305.
 31. Tarasova K, Arteaga MB, Kiditwong A, Gueltekin S, Bileck A, Gerner C, et al. Dexamethasone: a double-edged sword in the treatment of osteoarthritis. *Sci Rep.* 2025;15:11832.
 32. Poulsen RC, Watts AC, Murphy RJ, Snelling SJ, Carr AJ, Hulley PA. Glucocorticoids induce senescence in primary human tenocytes by inhibition of siirtuin 1 and activation of the p53/p21 pathway: in vivo and in vitro evidence. *Ann Rheum Dis.* 2014;73:1405.
 33. Bielak-Zmijewska A, Wnuk M, Przybylska D, Grabowska W, Lewinska A, Alster O, et al. A comparison of replicative senescence and doxorubicin-induced premature senescence of vascular smooth muscle cells isolated from human aorta. *Biogerontology.* 2014;15:47–64.
 34. Hernandez-Segura A, Rubingh R, Demaria M. Identification of stable senescence-associated reference genes. *Aging Cell.* 2019;18:e12911.
 35. Kirsch V, Ramge J-M, Schoppa A, Ignatius A, Riegger J. In vitro characterization of Doxorubicin-mediated stress-induced premature senescence in human chondrocytes. *Cells.* 2022;11:1106.
 36. Black R, Grodzinsky A. Dexamethasone: chondroprotective corticosteroid or catabolic killer? *Eur cells Mater.* 2019;38:246–63.
 37. Sabatini FM, Cohen-Rosenblum A, Eason TB, Hannon CP, Mounce SD, Krueger CA, et al. Incidence of rapidly progressive osteoarthritis following intra-articular hip corticosteroid injection: a systematic review and meta-analysis. *Arthroplast Today.* 2023;24:101242.
 38. McAlindon TE, LaValley MP, Harvey WF, Price LL, Driban JB, Zhang M, et al. Effect of Intra-articular triamcinolone vs saline on knee cartilage volume and pain in patients with knee osteoarthritis: a randomized clinical trial. *JAMA.* 2017;317:1967–75.
 39. Zeng C, Lane NE, Hunter DJ, Wei J, Choi HK, McAlindon TE, et al. Intra-articular corticosteroids and the risk of knee osteoarthritis progression: results from the Osteoarthritis Initiative. *Osteoarthr Cartil.* 2019;27:855–62.
 40. Samuels J, Pillinger MH, Jevsevar D, Felson D, Simon LS. Critical appraisal of intra-articular glucocorticoid injections for symptomatic osteoarthritis of the knee. *Osteoarthr Cartil.* 2021;29:8–16.
 41. Cao Y, Chen Z, Qin Z, Qian K, Liu T, Zhang Y. CDKN2AIP-induced cell senescence and apoptosis of testicular seminoma are associated with CARM1 and eIF4 β . *Acta Biochim Biophys Sin.* 2022;54:604–14.
 42. Passos JF, Saretzki G, Ahmed S, Nelson G, Richter T, Peters H, et al. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol.* 2007;5:e110.
 43. Miwa S, Kashyap S, Chini E, Zglinicki T, von. Mitochondrial dysfunction in cell senescence and aging. *J Clin Investig.* 2022;132:e158447.
 44. Martini H, Passos JF. Cellular senescence: all roads lead to mitochondria. *FEBS J.* 2023;290:1186–202.
 45. Hunter RG, Seligsohn M, Rubin TG, Griffiths BB, Ozdemir Y, Pfaff DW, et al. Stress and corticosteroids regulate rat hippocampal mitochondrial DNA gene expression via the glucocorticoid receptor. *Proc Natl Acad Sci USA.* 2016;113:9099–104.
 46. Aoki S, Morita M, Hirao T, Yamaguchi M, Shiratori R, Kikuya M, et al. Shift in energy metabolism caused by glucocorticoids enhances the effect of cytotoxic anticancer drugs against acute lymphoblastic leukemia cells. *Oncotarget.* 2017;8:94271–85.
 47. Weber K, Brück P, Mikes Z, Küpper J-H, Klingenspor M, Wiesner RJ. Glucocorticoid hormone stimulates mitochondrial biogenesis specifically in skeletal muscle. *Endocrinology.* 2002;143:177–84.
 48. Luan G, Li G, Ma X, Jin Y, Hu N, Li J, et al. Dexamethasone-induced mitochondrial dysfunction and insulin resistance-study in 3T3-L1 adipocytes and mitochondria isolated from mouse liver. *Molecules.* 2019;24:1982.
 49. Graybeal K, Sanchez L, Zhang C, Stiles L, Zheng JJ. Characterizing the metabolic profile of dexamethasone treated human trabecular meshwork cells. *Exp Eye Res.* 2022;214:108888.
 50. Hammad G, Legrain Y, Touat-Hamici Z, Duhieu S, Cornu D, Bulteau A-L, et al. Interplay between selenium levels and replicative senescence in WI-38 human fibroblasts: a proteomic approach. *Antioxidants.* 2018;7:19.
 51. Björklund G, Shanaida M, Lysiuk R, Antonyak H, Klishch I, Shanaida V, et al. Selenium: an antioxidant with a critical role in anti-aging. *Molecules.* 2022;27:6613.
 52. Lee MY, Ojeda-Britez S, Ehrbar D, Samwer A, Begley TJ, Melendez JA. Selenoproteins and the senescence-associated epitranscriptome. *Exp Biol Med.* 2022;247:2090–102.
 53. Blanco FJ, Rego I, Ruiz-Romero C. The role of mitochondria in osteoarthritis. *Nat Rev Rheumatol.* 2011;7:161–9.
 54. Koike M, Nohji H, Ozawa Y, Watanabe K, Muramatsu Y, Kaneko H, et al. Mechanical overloading causes mitochondrial superoxide and SOD2 imbalance in chondrocytes resulting in cartilage degeneration. *Sci Rep.* 2015;5:11722.
 55. Toh WS, Brittberg M, Farr J, Foldager CB, Gomoll AH, Hui JHP, et al. Cellular senescence in aging and osteoarthritis. *Acta Orthopaedica.* 2016;87:6–14.
 56. Diekmann BO, Loeser RF. Aging and the emerging role of cellular senescence in osteoarthritis. *Osteoarthr Cartil.* 2024;32:365–71.
 57. Mobasheri A, Rayman MP, Gualillo O, Sellam J, Kraan P, van der, et al. The role of metabolism in the pathogenesis of osteoarthritis. *Nat Rev Rheumatol.* 2017;13:302–11.
 58. Iltis C, Moskalevska I, Debiesse A, Seguin L, Fissoun C, Cervera L, et al. A ganglioside-based immune checkpoint enables senescent cells to evade immunosurveillance during aging. *Nat Aging.* 2025;5:219–36.
 59. Wang C, Kaur K, Xu C, Abu-Amer Y, Mbalaviele G. Chemotherapy activates inflammasomes to cause inflammation-associated bone loss. *eLife.* 2024;13:RP92885.
 60. Ronchetti S, Ricci E, Migliorati G, Gentili M, Riccardi C. How glucocorticoids affect the neutrophil life. *Int J Mol Sci.* 2018;19:4090.
 61. Kozhukharova I, Zemelko V, Kovaleva Z, Alekseenko L, Lyublinskaya O, Nikolsky N. Therapeutic doses of doxorubicin induce premature senescence of human mesenchymal stem cells derived from menstrual blood, bone marrow and adipose tissue. *Int J Hematol.* 2018;107:286–96.
 62. Zhang X, Xiang S, Zhang Y, Liu S, Lei G, Hines S, et al. In vitro study to identify ligand-independent function of estrogen receptor- α in suppressing DNA damage-induced chondrocyte senescence. *FASEB J.* 2023;37:e22746.
 63. Gilliam LAA, Fisher-Wellman KH, Lin C-T, Maples JM, Cathey BL, Neuffer PD. The anticancer agent doxorubicin disrupts mitochondrial energy metabolism and redox balance in skeletal muscle. *Free Radic Biol Med.* 2013;65:988–96.
 64. Ueno M, Kakinuma Y, Yuhki K, Murakoshi N, Iemitsu M, Miyauchi T, et al. Doxorubicin induces apoptosis by activation of Caspase-3 in cultured cardiomyocytes in vitro and rat cardiac ventricles in vivo. *J Pharm Sci.* 2006;101:151–8.
 65. Kuznetsov AV, Margreiter R, Amberger A, Saks V, Grimm M. Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death. *Biochim Biophys Acta (BBA) - Mol Cell Res.* 2011;1813:1144–52.

66. Ma W, Brenmoehl J, Trakooljul N, Wimmers K, Murani E. Dexamethasone has profound influence on the energy metabolism of porcine blood leukocytes and prevents the LPS-induced glycolytic switch. *Front Immunol.* 2025;16:1514061.
67. Vaiserman A, Krasnienkov D. Telomere length as a marker of biological age: state-of-the-art, open issues, and future perspectives. *Front Genet.* 2021;11:630186.
68. Huang H-L, Hsing H-W, Lai T-C, Chen Y-W, Lee T-R, Chan H-T, et al. Trypsin-induced proteome alteration during cell subculture in mammalian cells. *J Biomed Sci.* 2010;17:36.
69. Halliwell B. Oxidative stress in cell culture: an under-appreciated problem? *FEBS Lett.* 2003;540:3–6.
70. Lordon B, Campion T, Gibot L, Gallot G. Impact of trypsin on cell cytoplasm during detachment of cells studied by terahertz sensing. *Biophys J.* 2024;123:2476–83.
71. Kurashina Y, Imashiro C, Hirano M, Kuribara T, Totani K, Ohnuma K, et al. Enzyme-free release of adhered cells from standard culture dishes using intermittent ultrasonic traveling waves. *Commun Biol.* 2019;2:393.
72. Ribitsch I, Bileck A, Egerbacher M, Gabner S, Mayer RL, Janker L, et al. Fetal immunomodulatory environment following cartilage injury—the key to CARTILAGE regeneration? *Int J Mol Sci.* 2021;22:12969.
73. Wahlmueller M, Narzt M-S, Missfeldt K, Arminger V, Krasensky A, Lämmermann I, et al. Establishment of in vitro models by stress-induced premature senescence for characterizing the stromal vascular niche in human adipose tissue. *Life.* 2022;12:1459.
74. Xue E, Zhang Y, Song B, Xiao J, Shi Z. Effect of autophagy induced by dexamethasone on senescence in chondrocytes. *Mol Med Rep.* 2016;14:3037–44.

ACKNOWLEDGEMENTS

The authors acknowledge the Vetcore facility of the University of Veterinary Medicine Vienna, especially Dr. Ursula Reichart and Dr. Stephan Handschuh for their support with Immunofluorescence assays by Zeiss Observer. The authors acknowledge the use of ChatGPT (OpenAI) for linguistic refinement of the manuscript. All intellectual content, data interpretation, and conclusions remain the sole responsibility of the authors.

AUTHOR CONTRIBUTIONS

MBA and KT: study design, data acquisition, analysis and interpretation, manuscript preparation; AK and SG: data acquisition; IG: study design, data analysis and

interpretation; FJ: study conception and design, data analysis and interpretation, manuscript preparation. All authors reviewed the manuscript.

FUNDING

Funded by wings4innovation and the KHAN-I technology transfer fund.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41420-026-02961-y>.

Correspondence and requests for materials should be addressed to Florian Jenner.

Reprints and permission information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026