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# The impact of aging on achilles tendon ossification in mice

Hanhua Cai[1†], Yujian Lan[2,3†], Huan Liu[2] and Qi Hao[2,4*]

## Abstract

**Background** Heterotopic ossification is a frequent complication of soft tissue injuries, particularly in tendons. Although ossification in tendon tissue has been reported in a range of aging and disease models, the underlying biomarkers and mechanisms remain unknown. And the characterisation and sensitivity of previous diagnostic biomarkers for tendon ectopic ossification do not meet the demands of clinical use. The aim of this study was to characterise the effects of aging on ossification in the mouse Achilles tendon and to identify characteristic genes and therapeutic targets for tendon ossification in mice by using a machine learning approach.

**Methods** We retrieved the transcriptome profile of GSE126118 from the Gene Expression Omnibus (GEO) database. Following background correction and normalization using the transcripts per million (TPM) method, differentially expressed genes (DEGs) were identified with the limma R package ($p < 0.05$, $|\log2FC| > 1$). Subsequently, 468 senescence genes were downloaded from the Aging Atlas database, and senescence-associated DEGs (HO senescence genes) were identified. Gene ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment, and protein-protein interaction (PPI) network analyses were conducted on the identified DEGs. To further refine the HO aging signature, support vector machine (SVM) regression was employed. Additionally, we predicted transcription factors, miRNAs, and small molecule drugs potentially associated with the characterized genes.

**Results** Three characterised genes were identified as biomarkers associated with ectopic ossification and aging in the mouse Achilles tendon, Atp5o, Mmp2 and Mmp13. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses revealed significant enrichment in processes related to cartilage endochondral ossification, metalloendopeptidase activity, and mitochondrial proton transport ATP synthase complex. Additionally, HIF-1 and GnRH signaling pathways were prominently represented among the differentially expressed genes.

**Conclusion** Atp5o, Mmp2 and Mmp13 were identified as relevant signature genes for the effects of aging on Achilles tendon ossification in mice. Atp5o, Mmp2, and Mmp13 may influence tendon ossification by affecting mitochondrial function as well as extracellular matrix degradation to regulate senescence. This finding suggests a potential link between these processes, opening new avenues for research into diagnostic markers and therapeutic

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targets. These genes hold promise for the development of novel treatments for tendon ossification, a debilitating condition currently lacking effective therapeutic options.

**Keywords** Aging, Achilles tendon ossification, Biomarkers, GO, KEGG, PPI, SVM

## Introduction

Heterotopic ossification (HO), a painful disorder of unknown etiology, involves ectopic bone formation in extraskeletal tissues [1]. Acquired HO, the most common form, frequently arises after trauma like burns, surgeries, or fractures, culminating in disability and significant societal and familial burden [2]. Hereditary HO is associated with significant clinical severity, including progressive ossifying fibrous dysplasia (FOP), compared with non-genetic causes. Previously, an association between SARS-CoV-2 infection (COVID-19) and progression of HO or FOP has been reported. Recently it has been shown that Extensive progressive heterotopic ossification post-Covid-19 in a man [3]. Therefore avoidance of HO is crucial. While surgical resection remains the primary treatment, its limitations include long-term complications and high recurrence rates [4]. Preventive measures like radiotherapy or NSAIDs are often ineffective and carry substantial side effects [5]. Therefore, elucidating HO molecular mechanisms and identifying effective preventive strategies is crucial.

Approximately 28% of patients undergoing open Achilles tendon reconstruction experience HO [6]. This tendon's natural healing process involves inflammatory, proliferative, and remodeling phases, each modifying the local microenvironment [7]. Early dysregulation of the inflammatory response contributes to persistent fibrosis and tendon stem cell misdifferentiation, ultimately leading to HO [8, 9]. Histologically, HO progresses through four distinct phases: inflammation, chondrogenesis, osteogenesis, and maturation [10, 11]. Notably, nearly all acquired HO occurs via endochondral ossification, a process common to both bone development and fracture healing [12]. However, the mechanisms by which tendon stem cells differentiate into the chondrocyte/osteoblast system during HO, rather than promoting tissue regeneration, remain poorly understood.

Cellular senescence, a widespread phenomenon observed in human, primate, and rodent tissues, can be triggered by various factors like DNA damage, telomere dysfunction, or organelle stress [13]. In response, cells enter a non-replicative state, becoming senescent. These cells exhibit diverse biological functions and undergo distinct changes in gene expression, metabolism, and organization to maintain cell cycle arrest [14]. Although limited research exists on cellular senescence in HO, potential clues hint at its involvement. During aging, tendon stem cells show a marked decrease in self-renewal and colony formation and altered multidifferentiation capacity [15]. Tendon stem cells tend to differentiate into osteoblasts with excessive passaging, a common model of cellular senescence in vitro [16]. Ruzzini et al. [17] noted that aged tendon stem cells express higher levels of cartilage-associated gene expression. Although these changes were not definitively concluded, a potential role of altered differentiation capacity of tendon stem cells on age-related pathological changes in tendons was speculated [15]. Notably, features associated with premature senescence are shared by patients with progressive ossifying fibrous dysplasia, an inherited form of HO, suggesting a potential role for senescence in HO progression [18]. Additionally, single-cell analysis of HO revealed the involvement of damage-associated senescence in fibroblasts, potentially induced by PI3K/Akt-mediated SASP [19]. In addition, a previous study has shown that aging-related tendon disorders are strongly associated with the appearance of tendon heterotopic ossification [20]. However, most of the reports are related to age-related aging in humans, and no study has reported the effects of aging in a traumatic mouse model of Achilles tendon ossification, and more evidence is needed to elucidate the role of aging in mouse Achilles tendon HO as a target or a biological marker for better clinical use.

In this study, we employ a bioinformatics approach to analyze HO samples from a mouse burn/tendonotomy model, this mouse model of traumatic heterotopic ossification of the Achilles tendon is recognised by most scholars [21, 22]. Our aim is to evaluate the impact of cellular senescence on HO formation after tendon injury, identify critical points and potential therapeutic targets, and elucidate the mechanisms by which senescence may influence HO. We believe our findings can provide novel insights into the pathological mechanisms of tendon HO formation, unveil potential molecular drivers, and pave the way for future therapeutic strategies. The workflow of this study is outlined in Fig. 1.

## Materials and Methods

### Data sources

Transcriptome profiles for this study were retrieved from the Gene Expression Omnibus (GEO) database of the National Center for Biotechnology Information (NCBI) (GSE126118; https://www.ncbi.nlm.nih.gov/geo/). This dataset (GSE126118) employed the GPL13112 Illumina HiSeq 2000 platform (Mus musculus) and encompasses seven microarrays: two tendonotomy samples, three uninjured contralateral hindlimb tendon samples, and two normal tendon samples. The underlying study

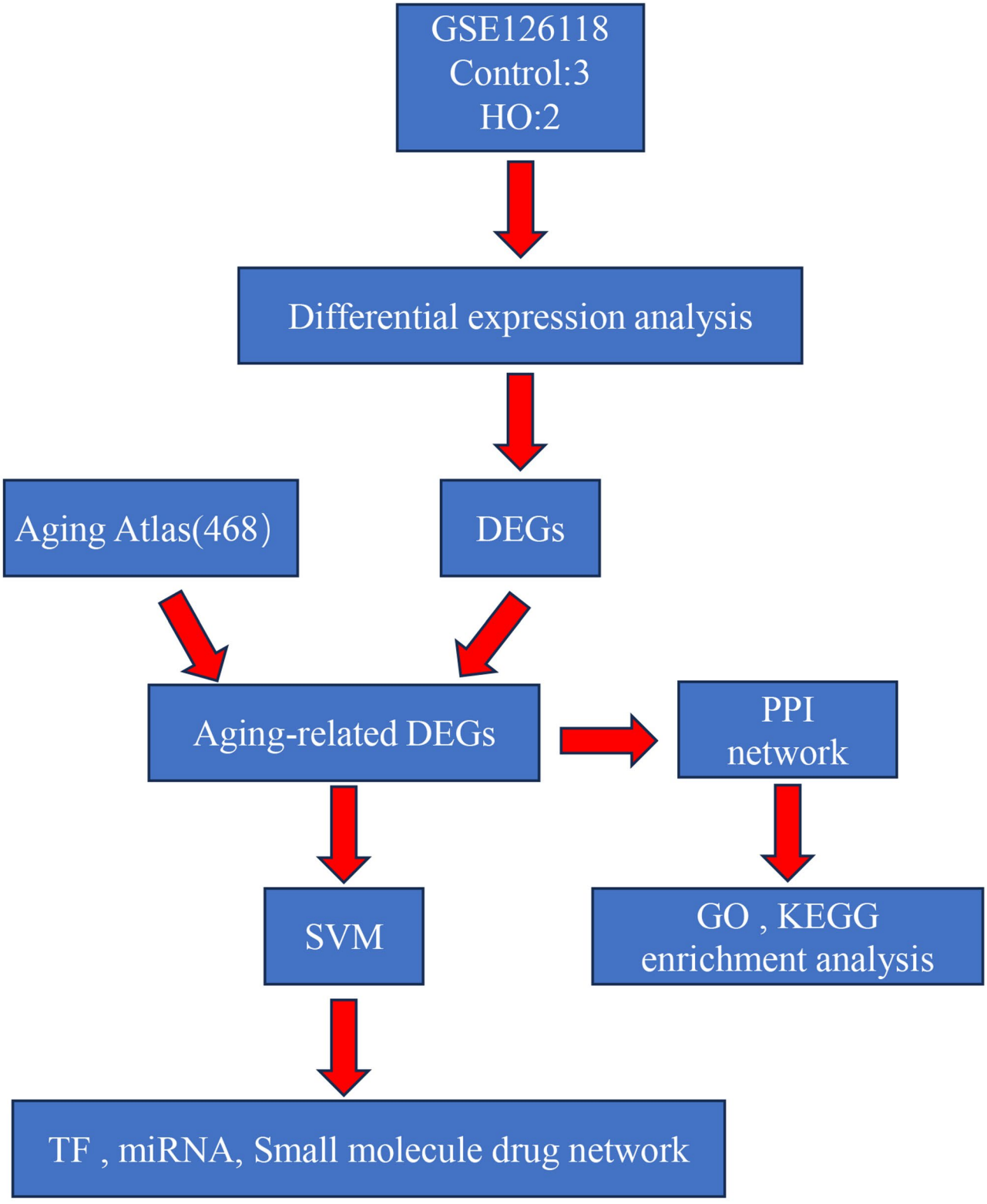


**Fig. 1** Workflow diagram for this study

utilized a mouse burn/tendonotomy-induced heterotopic ossification (HO) model, which involves a partial-thickness scalding burn and subsequent Achilles tendon transection at its midpoint. Notably, all mice originated from the same breed and batch and underwent simultaneous surgical procedures by the same individual. Tendon samples were collected and total RNA extracted three weeks post-injury. Additionally, 468 senescence-related genes were downloaded from the Aging Atlas database (Aging Atlas (cncb.ac.co.uk)) [23].

#### Data preprocessing and differential expression analysis

The raw gene expression data from GSE126118 underwent background correction and normalization using the transcripts per million (TPM) method to account for technical variations and ensure accurate comparisons. Subsequently, differentially expressed genes (DEGs) were identified with the limma R package. The Benjamini and Hochberg (BH) procedure was employed to control the false discovery rate (FDR) while identifying significant DEGs, with thresholds set at $p < 0.05$ and |log2 fold change (FC)| > 1.0. To facilitate downstream analysis, Ensembl transcript IDs were converted to gene symbols, and duplicate values originating from different probes mapping to the same gene were removed. Finally, the identified DEGs were intersected with 468 senescence-related genes downloaded from the Aging Atlas database, allowing us to focus on senescence-associated changes in expression within the context of heterotopic ossification.

#### Pathway and functional enrichment analyses

Functional enrichment analyses were conducted using the R package clusterProfiler [24] to explore the biological roles of differentially expressed genes (DEGs) associated with both senescence and heterotopic ossification (HO). At the molecular level, Gene Ontology (GO) [25] enrichment analysis identified significantly overrepresented categories in biological processes, cellular components, and molecular functions ($p < 0.05$). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) [26] pathway enrichment analysis revealed signaling pathways enriched within the DEGs, highlighting potentially relevant regulatory and functional networks ($p < 0.05$). This multi-layered approach provided insights into the biological mechanisms underlying the interplay between senescence and HO.

#### Protein-protein interaction PPI network construction

To further elucidate potential functional relationships among the identified differentially expressed genes, we constructed a protein-protein interaction (PPI) network using the STRING database (https://string-db.org/)(27). This interactive platform integrates diverse evidence channels, including text mining, genomic neighborhood analyses, co-expression data, protein co-occurrence, gene fusions, and published experimental findings. To ensure high confidence in predicted interactions, we restricted the analysis to those exceeding a medium confidence score (0.400). Visualizing the resulting PPI network could reveal novel functional modules and regulatory circuits associated with both senescence and HO, providing valuable insights into the underlying biological mechanisms.

#### Screening feature genes by support vector machine (SVM) regression

To further refine the identified differentially expressed genes (DEGs) and pinpoint specific features associated with aging in heterotopic ossification (HO), we employed support vector machine (SVM) regression. This machine learning technique excels at variable selection in complex datasets, making it ideal for our study. To ensure the reproducibility of the SVM algorithm, we set the seed to 12,345 in each disease group.First, the 10 DEGs previously obtained were fed into the SVM-RFE algorithm for each disease group to eliminate recursive features. SVM modelling was performed using the "e1071" and "MSVM-RFE" software packages, and SVM-RFE applied sequential backward feature elimination to identify the best hub genes. All 10 DEGs were used in our SVM model. The SVM-RFE results were visualised and validated by five cross validations. Subsequently, we fit the model by four kernel functions of SVM: linear, polynomial, radial basis function (RBF), and sigmoid. and plotted the ROC curves of the model.

#### Signature gene transcription factor (TF) network prediction

To elucidate the regulatory mechanisms controlling the identified signature genes, we utilized NetworkAnalyst 3.0 to predict their potential transcription factors (TFs). TF targets derived from the JASPAR TF binding site profile database. This analysis enabled us to map a network of putative upstream regulators, providing insights into the transcriptional control of the signature gene expression in heterotopic ossification.

#### miRNA prediction related to Signature genes

To further understand the post-transcriptional regulation of the identified key genes in heterotopic ossification, we employed NetworkAnalyst 3.0 (NetworkAnalyst) [27] to construct a miRNA-gene interaction network. Comprehensive experimentally validated miRNA-gene interaction data collected from TarBase v8.0. This network visualizes the predicted interactions between miRNAs and the key genes, offering insights into potential regulatory pathways that operate beyond the transcriptional level.

### Small molecule drug prediction

To explore potential therapeutic interventions based on the identified signature genes, we utilized the HERB database to retrieve small molecule compounds targeting these genes. Subsequently, we mapped these compounds onto the established gene network using Cytoscape software. This analysis allows us to visualize the potential therapeutic landscape associated with the identified key regulators of heterotopic ossification, highlighting potential drug targets for future development.

## Results

### Data preprocessing and screening of HO senescence-related genes

Gene expression data from GSE126118 were normalized using the TPM method. Differentially expressed genes (DEGs) were identified by comparing tendonectomy samples with uninjured contralateral tendon controls. After filtering for redundant entries and converting Ensembl transcript IDs to gene symbols, a total of 337 DEGs were found: 267 upregulated and 71 downregulated with $p < 0.05$ and $|\log2FC| > 1.0$. The statistical metrics for key DEGs was shown in Supplemental Table 1. These DEGs were visualized using a volcano plot (Fig. 2A). Further analysis revealed 10 genes overlapping with the 468 senescence-related genes from the Aging Atlas database (Fig. 2B and C). Notably, five of these (Atp5o, Ldhb, Hspa9, Gapdh, Ldha) were upregulated, while five (Mmp2, Mmp13, Timp2, Mmp14, Eef1a1) were downregulated in the tendonectomy group(Table 1). Gene expression patterns for the identified aging-related genes were further explored through a heatmap visualization (Fig. 2C).

### HO Functional annotation of candidate senescence-related genes

To elucidate the potential roles of the identified aging-related genes in HO, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses. The top 10 most significantly enriched GO terms highlight a focus on extracellular matrix remodeling, with collagen catabolic and metabolic processes prominently featured (Fig. 3A). Additionally, genes associated with key metabolic pathways such as pyruvate, lactate, and NAD metabolism were enriched. Notably, endochondral ossification, the primary mode of HO formation, emerged as a significantly enriched process. Cellular components enriched included myelin, mitochondrial ATP synthase complexes, and proton-transporting machinery, suggesting possible functional alterations in these structures. Notably, the molecular functions enriched focused on proteolytic activities (metalloendopeptidase, endopeptidase) and protein binding (fibronectin), potentially highlighting cellular remodeling processes. KEGG pathway analysis revealed enrichment in glycolysis/glycogenesis, propionate metabolism, and cysteine and methionine metabolism, suggesting altered metabolic landscapes in HO (Fig. 3B). Notably, central carbon metabolism, known for its upregulation in cancer, was also over-represented. Moreover, HIF-1 and GnRH were identified as central signaling pathways within the enriched KEGG pathways, hinting at potential regulatory mechanisms involved in HO development.

### PPI network analysis

To elucidate potential functional relationships among the identified HO candidate senescence-related genes, we constructed a protein-protein interaction (PPI) network using the STRING database. The network visualizes predicted physical and functional interactions between protein products of these genes, offering insights into potential protein complexes and signaling pathways associated with HO and aging. STRING assigns confidence scores to predicted interactions based on various evidence channels, including text mining, gene co-expression, and experimental data. We focused on high-confidence interactions, excluding genes with a STRING score below 0.4 (Fig. 4A). This filtering resulted in a core network of interacting proteins that may play key roles in the interplay between senescence and HO.

### SVM regression screening to identify characterized genes

To further refine the identified HO candidate senescence-related genes, we employed SVM regression analysis. This approach revealed three genes with the strongest association with HO senescence: Atp5o, Mmp2, and Mmp13 (Fig. 4B). The accuracy of the SVM model was rigorously evaluated through four-fold cross-validation, achieving a perfect score of 1.0 for each function (Fig. 4C, D and E). This high accuracy suggests that the identified genes are highly predictive of HO senescence and warrant further investigation.

### TF and miRNA prediction of characterized genes

To gain insights into the regulatory landscape governing the identified key genes, we predicted their potential upstream transcription factors (TFs) and post-transcriptional regulators via miRNAs. This analysis led to the construction of two comprehensive regulatory networks: a gene-TF network (Fig. 5A) and a gene-miRNA network (Fig. 5B). Notably, the predicted TF network revealed one key nodes: FOXC1, suggesting their potential roles in controlling the expression of the characterized genes. The miRNA network identified 16 prominent nodes highlighting their potential regulatory impact (Tables 2 and 3).

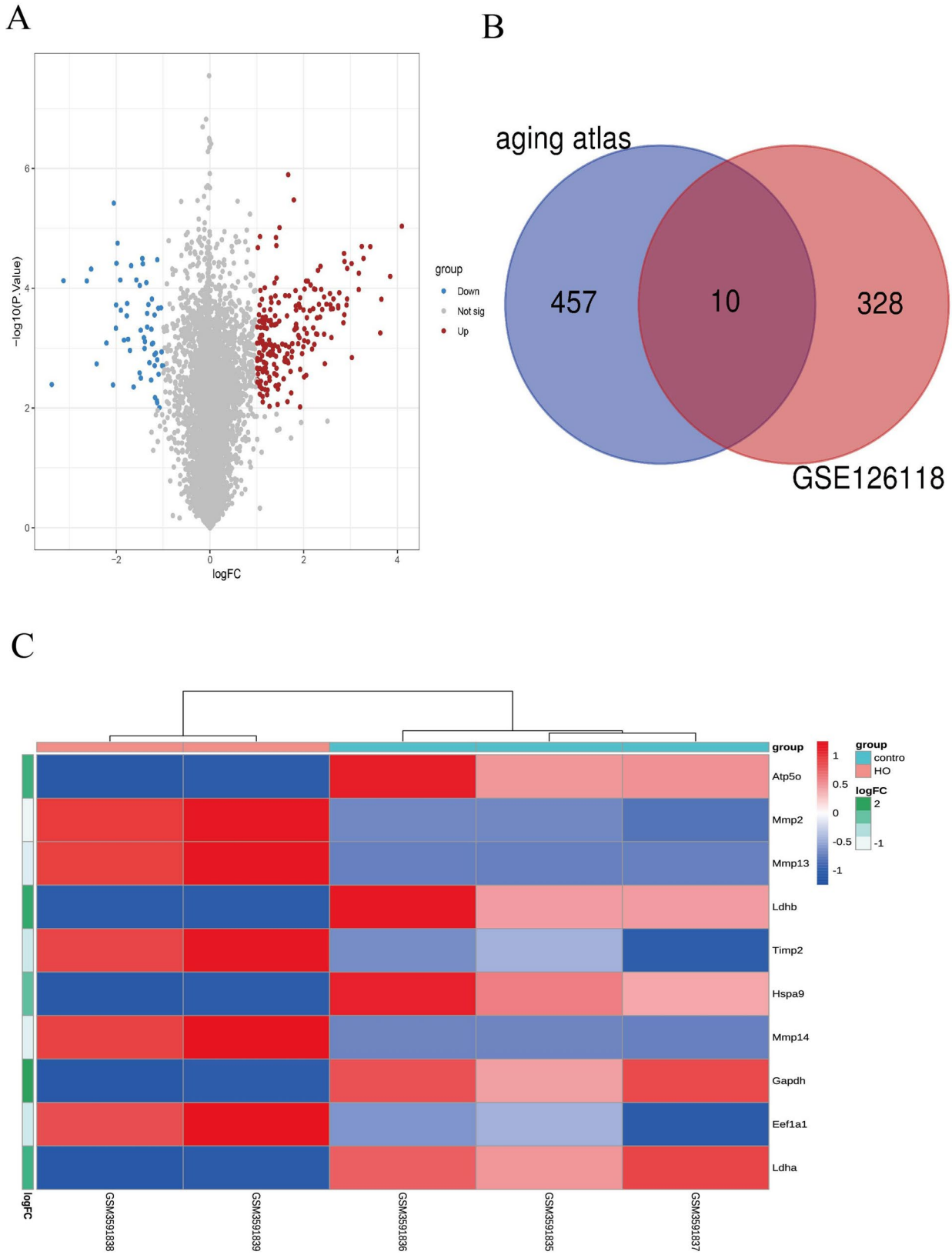


**Fig. 2** Screening of differential genes associated with tendon ossification and aging. (**A**) Volcano plot of DEGs for GSE126118 with adjusted $P < 0.05$ and a threshold of |log2 FC| > 1. Red and blue dots indicate significantly up- and down-regulated genes, respectively. (**B**) Venn diagram of differentially expressed genes for tendon ossification versus aging-related genes. (**C**) Heatmap of the expression of 10 genes associated with aging and tendon ossification

**Table 1** Expression of 10 genes associated with tendon ossification and aging

| Gene | logFC | AveExpr | t | *P*.Value | adj.*P*.Val | B |
|---|---|---|---|---|---|---|
| Atp5o | 1.685859 | 2.099794 | 11.3829 | 0.000876 | 0.062597 | -2.46071 |
| Mmp2 | -1.90204 | 1.604209 | -17.5773 | 0.00021 | 0.034992 | -0.60978 |
| Mmp13 | -1.44346 | 0.580435 | -32.2621 | 2.80E-05 | 0.014952 | 2.00281 |
| Ldhb | 1.992265 | 1.59944 | 11.41263 | 0.000869 | 0.062412 | -2.44968 |
| Timp2 | -1.1261 | 1.777354 | -5.76922 | 0.007687 | 0.166185 | -5.2545 |
| Hspa9 | 1.020984 | 1.155264 | 9.702225 | 0.001474 | 0.076283 | -3.13274 |
| Mmp14 | -1.57188 | 0.843622 | -25.1037 | 6.44E-05 | 0.021743 | 0.921846 |
| Gapdh | 2.358495 | 3.937682 | 17.35345 | 0.000219 | 0.035625 | -0.66469 |
| Eef1a1 | -1.15297 | 3.898104 | -4.93288 | 0.012391 | 0.21832 | -5.86201 |
| Ldha | 1.549691 | 2.742921 | 18.97906 | 0.000163 | 0.031874 | -0.28069 |

### Characterization gene-related small molecule drug prediction

To explore potential therapeutic interventions based on the identified key genes, we employed the HERB database to predict small molecule drugs targeting these genes. This analysis revealed a landscape of potential drug candidates for the treatment of tendon HO (Fig. 5C). Notably, these identified compounds offer new avenues for therapeutic development and hold promise for improving clinical outcomes in patients suffering from this debilitating condition.

## Discussion

The aim of this study was to describe the effects of aging on Achilles tendon ossification in mice. We analyzed publicly available transcriptome data (GSE126118) from the GEO database, employing a multi-pronged approach.

Initially, raw data were normalized and differentially expressed genes (DEGs) were identified. We then intersected these DEGs with a curated set of aging-related genes, yielding a focused pool of candidate genes potentially involved in HO pathogenesis. Subsequent pathway and gene ontology enrichment analyses using KEGG and GO revealed key processes associated with both aging and HO, including collagen metabolism, mitochondrial dysfunction, and inflammatory responses. Notably, HIF-1 signaling emerged as a potential driver of HO, aligning with its established roles in chondrogenesis and aging-related diseases [28, 29].

Machine learning analysis using SVM algorithms further pinpointed three key genes: Atp5o, Mmp2, and Mmp13. These genes warrant further investigation due to their involvement in mitochondrial energy metabolism, extracellular matrix remodeling, and cellular senescence, all processes implicated in HO development.

Atp5o, also known as the ATP synthase O subunit, is a crucial component of the mitochondrial ATP synthase complex, primarily responsible for intracellular ATP synthesis [30]. It plays a pivotal role in maintaining the structural stability and catalytic activity of the ATP synthase complex, essential for cellular energy metabolism. Atp5o has implications in various age-related physiological changes. Age-related alterations in Atp5o activity can impede intracellular ATP synthesis, impacting cellular energy metabolism and function, potentially accelerating the aging process and associated pathological changes [31, 32]. Therefore, investigating the relationship between Atp5o and aging may yield new strategies for anti-aging treatment and related diseases. Additionally, the specific relationship and mechanism of Atp5o and HO are not fully elucidated, but some studies suggest that Atp5o may influence the HO process. Abnormal expression or dysfunction of Atp5o may disrupt cellular energy metabolism, affecting the normal function and metabolism of tendon tissues, potentially leading to HO. Moreover, Atp5o may interact with other factors or signaling pathways to co-regulate the process of HO.

Investigating the role of Atp5o in cellular senescence during HO can yield novel insights and methodologies for comprehending the pathogenesis of HO and developing therapeutic strategies. Further in-depth studies are required to enhance our understanding of its role, mechanism, and contribution to future medical research and practice.

Mmp2 and Mmp13, members of the matrix metalloproteinase (MMP) family, are associated with cellular senescence. These proteases are implicated in extracellular matrix degradation, influencing cell growth and differentiation. Aberrant expression or activity of MMP2 and MMP13 may promote cellular senescence. Additionally, they may affect the tendon ossification process by degrading tendon tissues, making them susceptible to ossification. Notably, in an HO model, Mmp2 and Mmp13 were highly expressed, with Mmp2 showing significant elevation at all time points and Mmp13 at days 5 and 8 [33]. In the early stage of HO, inflammatory cells infiltrate Achilles tendon tissue, and MMPs secreted by macrophages and neutrophils mediate cell migration, ECM degradation, and remodeling, creating a conducive environment for inducing osteoblast precursor cells. Moreover, sustained and increased expression of specific growth factors in Achilles tendon tissues leads to the

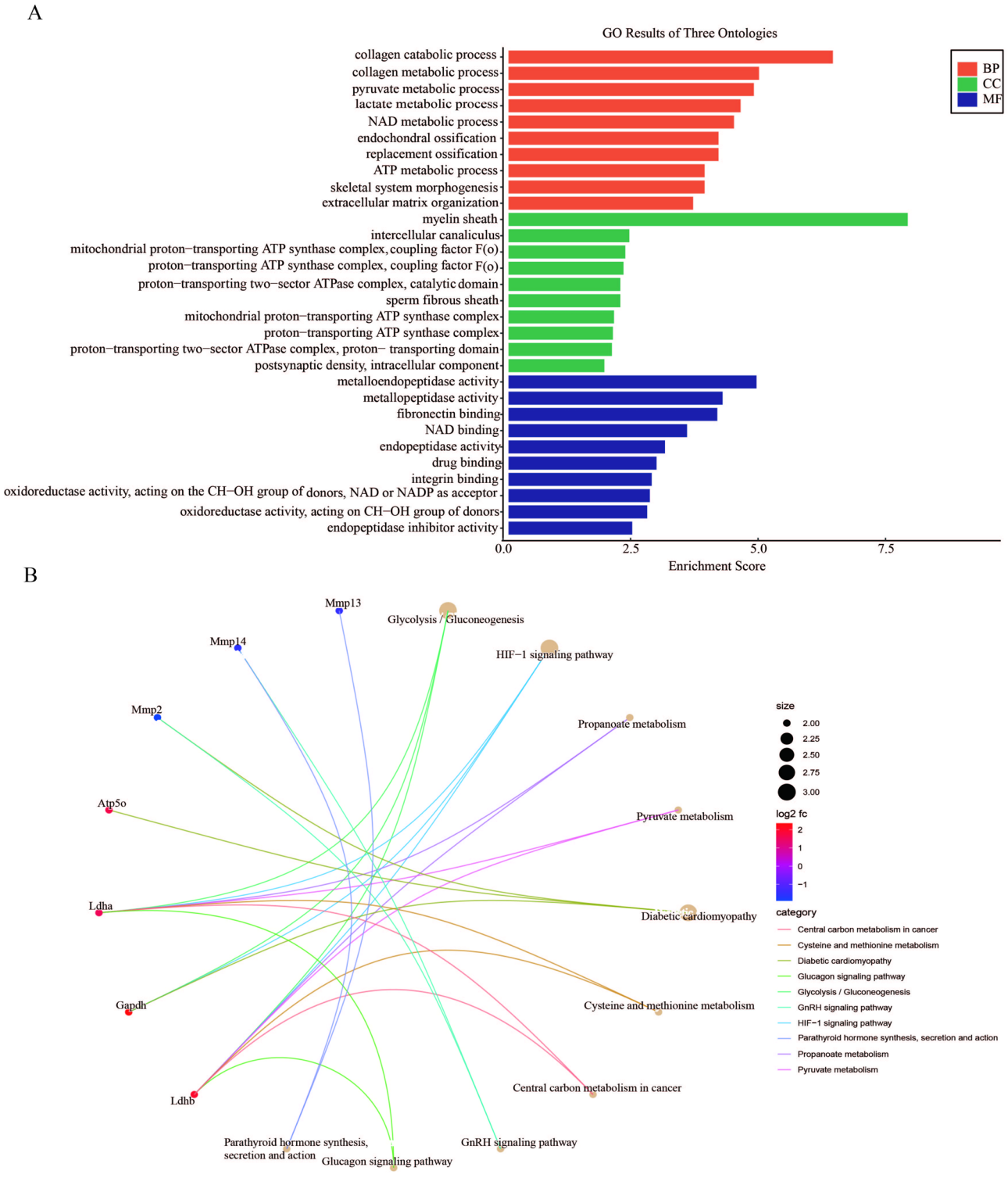


**Fig. 3** Functional enrichment analysis of 10 genes associated with aging and tendon ossification. (**A**) GO enrichment analysis; top 10 significantly enriched biological processes ($P < 0.05$), molecular functions and cellular components. (**B**) KEGG analysis; top 10 significant signalling pathways listed ($P < 0.05$)

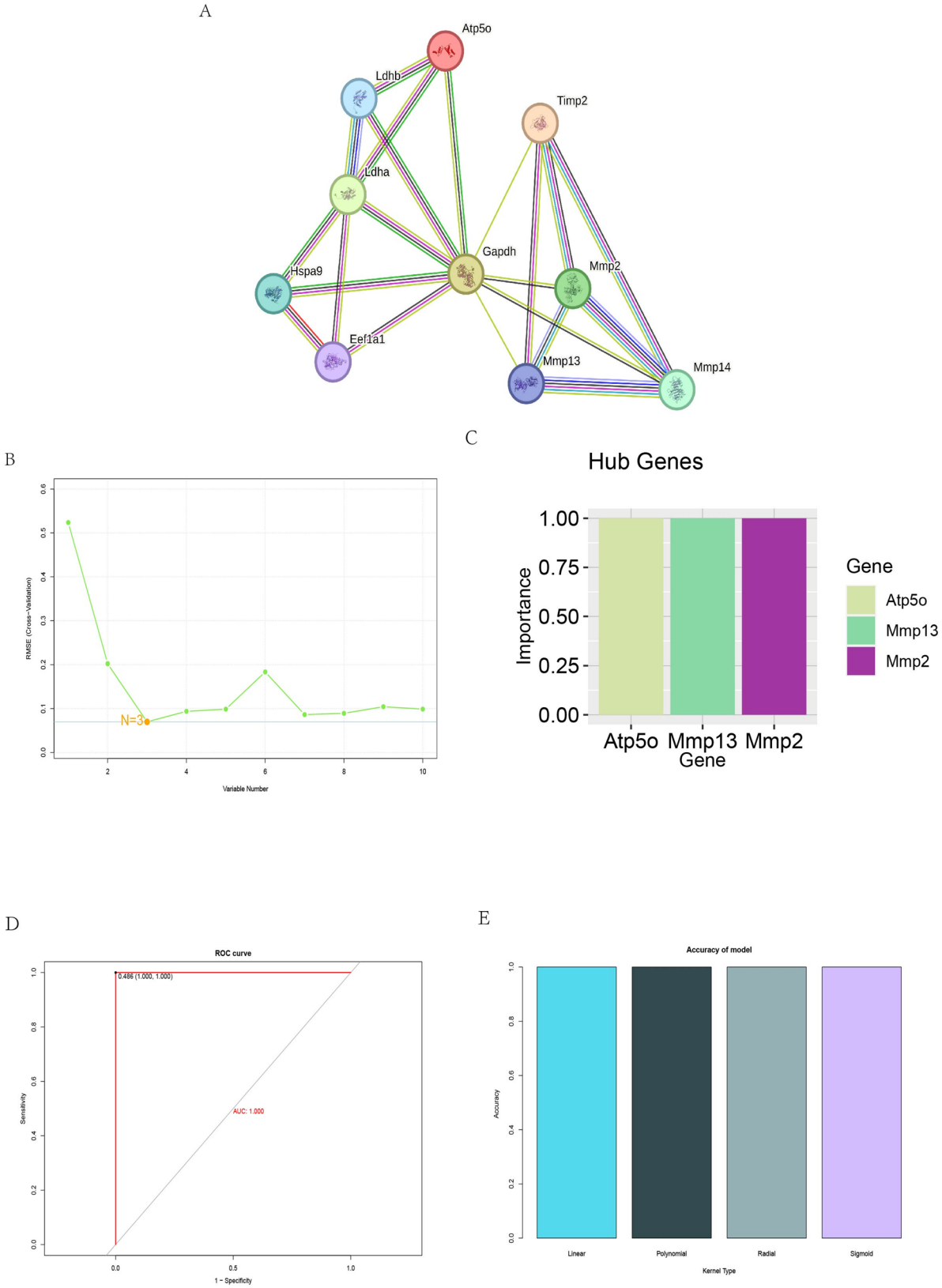


**Fig. 4** PPI protein interaction network as well as SVM machine learning screening of HO candidate senescence signature genes. (**A**) PPI protein interactions network. (**B**, C) HO candidate senescence-related genes screened by SVM algorithm. (**D**) SVM model ROC curves. (**E**) Accuracy prediction of four function models in SVM

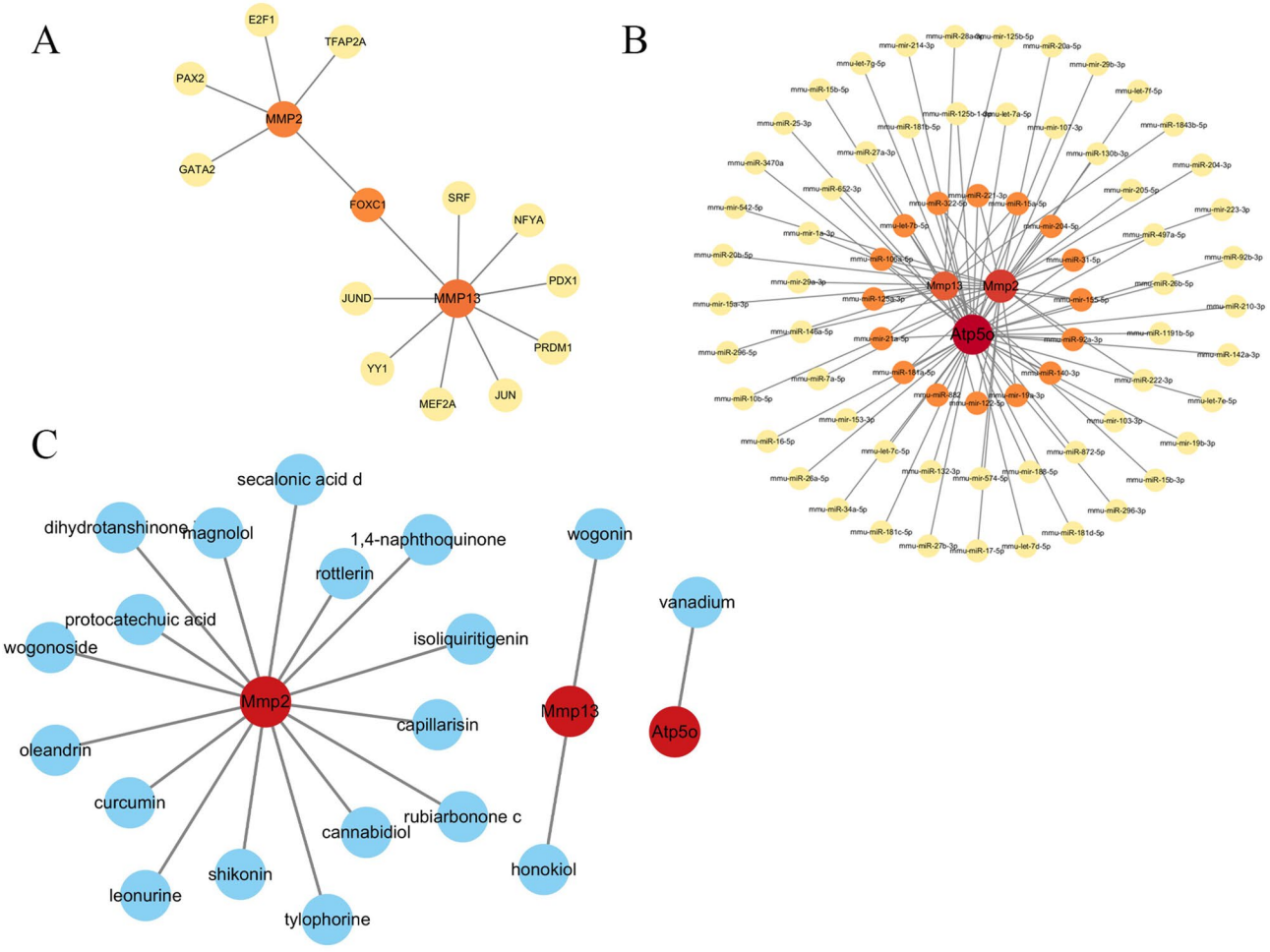


**Fig. 5** HO and aging-related trait genes TF, miRNA, and small fraction drug prediction. (**A**) TF prediction of characteristic genes associated with aging in HO. (**B**) miRNA prediction of characteristic genes associated with aging in HO. (**C**) Small molecule drug prediction of HO aging-related signature genes

**Table 2** The gene-TF regulatory network was constructed including 14 interaction pairs among 2 genes and 13 TFs

| Gene | connection | Transcriptional factors |
|---|---|---|
| MMP2 | interacts with | E2F1 |
| MMP2 | interacts with | FOXC1 |
| MMP2 | interacts with | GATA2 |
| MMP2 | interacts with | PAX2 |
| MMP2 | interacts with | TFAP2A |
| MMP13 | interacts with | FOXC1 |
| MMP13 | interacts with | JUN |
| MMP13 | interacts with | JUND |
| MMP13 | interacts with | MEF2A |
| MMP13 | interacts with | NFYA |
| MMP13 | interacts with | PDX1 |
| MMP13 | interacts with | PRDM1 |
| MMP13 | interacts with | SRF |
| MMP13 | interacts with | YY1 |

transformation of MSCs into chondrocytes, osteoblasts, and osteoclasts, resulting in pathologic bone tissue in localized areas [34].

While this study sheds light on the cellular senescence link in HO, limitations necessitate further research. The small sample size restricts the generalizability of our findings. Additionally, pinpointing the definitive protein(s) or pathway(s) solely responsible for HO suppression and senescence remains elusive. Future studies with larger datasets and in vivo models are crucial to validate these findings and elucidate the precise molecular mechanisms underlying the complex interplay between cellular senescence and HO.

## Conclusion

This study used a multi-pronged approach combining bioinformatics and machine learning to decipher the genomic landscape of mouse tendon heterotopic ossification (HO) and its intricate relationship with cellular senescence. We identified three key genes - Atp5o,

**Table 3** The 16 prominent nodes in the constructed gene-miRNA regulatory network include 32 pairs of interactions between 3 genes and 16 MiRNAs

| Gene | connection | miRNA |
|---|---|---|
| Atp5o | interacts with | mmu-mir-19a-3p |
| Atp5o | interacts with | mmu-mir-204-5p |
| Atp5o | interacts with | mmu-let-7b-5p |
| Atp5o | interacts with | mmu-mir-21a-5p |
| Atp5o | interacts with | mmu-miR-106a-5p |
| Atp5o | interacts with | mmu-miR-140-3p |
| Atp5o | interacts with | mmu-miR-15a-5p |
| Atp5o | interacts with | mmu-miR-181a-5p |
| Atp5o | interacts with | mmu-miR-221-3p |
| Atp5o | interacts with | mmu-miR-31-5p |
| Atp5o | interacts with | mmu-miR-322-5p |
| Atp5o | interacts with | mmu-miR-882 |
| Atp5o | interacts with | mmu-miR-92a-3p |
| Mmp2 | interacts with | mmu-mir-19a-3p |
| Mmp2 | interacts with | mmu-mir-204-5p |
| Mmp2 | interacts with | mmu-mir-122-5p |
| Mmp2 | interacts with | mmu-mir-155-5p |
| Mmp2 | interacts with | mmu-miR-106a-5p |
| Mmp2 | interacts with | mmu-miR-125a-3p |
| Mmp2 | interacts with | mmu-miR-181a-5p |
| Mmp2 | interacts with | mmu-miR-221-3p |
| Mmp2 | interacts with | mmu-miR-31-5p |
| Mmp2 | interacts with | mmu-miR-322-5p |
| Mmp2 | interacts with | mmu-miR-882 |
| Mmp2 | interacts with | mmu-miR-92a-3p |
| Mmp13 | interacts with | mmu-mir-122-5p |
| Mmp13 | interacts with | mmu-mir-155-5p |
| Mmp13 | interacts with | mmu-let-7b-5p |
| Mmp13 | interacts with | mmu-mir-21a-5p |
| Mmp13 | interacts with | mmu-miR-125a-3p |
| Mmp13 | interacts with | mmu-miR-140-3p |
| Mmp13 | interacts with | mmu-miR-15a-5p |

Mmp2 and Mmp13 - that play multidirectional roles in the pathogenesis of mouse tendon HO, particularly in the context of cellular senescence. These central genes are involved in key processes such as mitochondrial energy metabolism, extracellular matrix remodelling and senescence itself, and are potential therapeutic targets for further investigation.

To solidify our findings and refine our understanding of these pivotal genes, further investigations leveraging retrospective datasets and clinical specimens are warranted. Such rigorous validation efforts will pave the way for translating these insights into tangible therapeutic strategies for tackling HO, potentially through interventions targeting these identified genes or their associated pathways.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12891-025-08788-5.

Supplementary Material 1

### Author contributions

HC and YL contributed equally to this work. HC and YL analysed and collated the data. HC, YL, HL and QH reviewed the literature and wrote the manuscript. HC, YL, HL and QH conceived the idea of the article and revised the manuscript. All authors approved the final manuscript for publication.

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### Data availability

The datasets analysed during the current study can be found in the GEO repository [https://www.ncbi.nlm.nih.gov/geo/].

## Declarations

### Ethics approval and consent to participate

(Not applicable)

### Consent for publication

(Not applicable)

### Competing interests

The authors declare no competing interests.

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### References

1. Wang X, Li F, Xie L, Crane J, Zhen G, Mishina Y et al. Inhibition of overactive TGF-β attenuates progression of heterotopic ossification in mice. Nat Commun. 2018;9(1).
2. Luo G, Sun Z, Liu H, Yuan Z, Wang W, Tu B et al. Verteporfin attenuates trauma-induced heterotopic ossification of Achilles tendon by inhibiting osteogenesis and angiogenesis involvingYAP/β-catenin signaling. FASEB J. 2023;37(7).
3. Brance ML, Cóccaro NM, Casalongue AN, Durán A, Brun LR. Extensive progressive heterotopic ossification post-Covid-19 in a man. Bone. 2022;155(116287):8.
4. Hoyt BW, Pavey GJ, Potter BK, Forsberg JA. Heterotopic ossification and lessons learned from fifteen years at war: A review of therapy, novel research, and future directions for military and civilian orthopaedic trauma. Bone. 2018;109:3–11.
5. Hwang CD, Pagani CA, Nunez JH, Cherief M, Qin Q, Gomez-Salazar M et al. Contemporary perspectives on heterotopic ossification. JCI Insight. 2022;7(14).
6. Delgado Caceres M, Angerpointner K, Galler M, Lin D, Michel PA, Brochhausen C et al. Tenomodulin knockout mice exhibit worse late healing outcomes

with augmented trauma-induced heterotopic ossification of Achilles tendon. Cell Death Dis. 2021;12(11).
7. Thomopoulos S, Parks WC, Rifkin DB, Derwin KA. Mechanisms of tendon injury and repair. J Orthop Res. 2015;33(6):832–9.
8. Zhou W, Lin X, Chu J, Jiang T, Zhao H, Yan B, et al. Magnolol prevents ossified tendinopathy by inhibiting PGE2-induced osteogenic differentiation of TDSCs. Int Immunopharmacol. 2019;70:117–24.
9. Rui YF, Lui PPY, Wong YM, Tan Q, Chan KM. Altered fate of Tendon-Derived stem cells isolated from a failed Tendon-Healing animal model of tendinopathy. Stem Cells Dev. 2013;22(7):1076–85.
10. Vanden Bossche L, Vanderstraeten G. Heterotopic ossification: a review. J Rehabil Med. 2005;37(3):129–36.
11. Cholok D, Chung MT, Ranganathan K, Ucer S, Day D, Davis TA, et al. Heterotopic ossification and the Elucidation of pathologic differentiation. Bone. 2018;109:12–21.
12. Zhang Q, Zhang Y, Yan M, Zhu K, Zhou D, Tan J. Bioinformatics analysis of the molecular mechanism of Late-Stage heterotopic ossification. Biomed Res Int. 2020;2020:1–8.
13. Kudlova N, De Sanctis JB, Hajduch M. Cellular senescence: molecular targets, biomarkers, and senolytic drugs. Int J Mol Sci. 2022;23(8).
14. Roger L, Tomas F, Gire V. Mechanisms and regulation of cellular senescence. Int J Mol Sci. 2021;22(23).
15. Dai GC, Li YJ, Chen MH, Lu PP, Rui YF. Tendon stem/progenitor cell ageing: modulation and rejuvenation. World J Stem Cells. 2019;11(9):677–92.
16. Tan Q, Lui PP, Rui YF. Effect of in vitro passaging on the stem cell-related properties of tendon-derived stem cells-implications in tissue engineering. Stem Cells Dev. 2012;21(5):790–800.
17. Ruzzini L, Abbruzzese F, Rainer A, Longo UG, Trombetta M, Maffulli N, et al. Characterization of age-related changes of tendon stem cells from adult human tendons. Knee Surg Sports Traumatol Arthrosc. 2014;22(11):2856–66.
18. Pignolo RJ, Wang H, Kaplan FS. Fibrodysplasia ossificans progressiva (FOP): A segmental progeroid syndrome. Front Endocrinol. 2020;10.
19. Zhang Q, Zhou D, Liang Y. Single-Cell analyses of heterotopic ossification: characteristics of Injury-Related senescent fibroblasts. J Inflamm Res. 2022;15:5579–93.
20. Agabalyan NA, Evans DJ, Stanley RL. Investigating tendon mineralisation in the avian hindlimb: a model for tendon ageing, injury and disease. J Anat. 2013;223(3):262–77.
21. Peterson JR, Agarwal S, Brownley RC, Loder SJ, Ranganathan K, Cederna PS, et al. Direct mouse trauma/burn model of heterotopic ossification. J Vis Exp. 2015;6(102):52880.
22. Li R, Lai C, Luo H, Lan Y, Duan X, Bao D, et al. Animal models of tendon calcification: past, present, and future. Anim Model Exp Med. 2024;17(10):12439.
23. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44(D1):D457–62.
24. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res. 2021;49(D1):D605–12.
25. Yu G, Wang L-G, Han Y, He Q-Y. ClusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012;16(5):284–7.
26. The Gene Ontology Resource. 20 Years and still going strong. Nucleic Acids Res. 2019;47(D1):D330–8.
27. Zhou G, Soufan O, Ewald J, Hancock REW, Basu N, Xia J. NetworkAnalyst 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis. Nucleic Acids Res. 2019;47(W1):W234–41.
28. Lin L, Shen Q, Xue T, Yu C. Heterotopic ossification induced by Achilles tenotomy via endochondral bone formation: expression of bone and cartilage related genes. Bone. 2010;46(2):425–31.
29. Alique M, Sánchez-López E, Bodega G, Giannarelli C, Carracedo J, Ramírez R. Hypoxia-Inducible Factor-1α: the master regulator of endothelial cell senescence in vascular aging. Cells. 2020;9(1).
30. Yang W, Zhang K, Zhang Z, Zhou J, Li L, Xu Y, et al. Claudin-10 overexpression suppresses human clear cell renal cell carcinoma growth and metastasis by regulating ATP5O and causing mitochondrial dysfunction. Int J Biol Sci. 2022;18(6):2329–44.
31. Rivera-Torres J, Acín-Perez R, Cabezas-Sánchez P, Osorio FG, Gonzalez-Gómez C, Megias D, et al. Identification of mitochondrial dysfunction in Hutchinson-Gilford Progeria syndrome through use of stable isotope labeling with amino acids in cell culture. J Proteom. 2013;91:466–77.
32. Ling C, Poulsen P, Simonsson S, Rönn T, Holmkvist J, Almgren P, et al. Genetic and epigenetic factors are associated with expression of respiratory chain component NDUFB6 in human skeletal muscle. J Clin Investig. 2007;117(11):3427–35.
33. Yu D, Ju J, Xue F, Zhao Y, Shi W, Xiao H. Expression and significance of related genes in the early stage of post-traumatic heterotopic ossification in a rat model of Achilles tenotomy. Acta Orthop Traumatol Turc. 2021;55(2):94–101.
34. Shi WZ, Ju JY, Xiao HJ, Xue F, Wu J, Pan MM, et al. Dynamics of MMP–9, MMP–2 and TIMP–1 in a rat model of brain injury combined with traumatic heterotopic ossification. Mol Med Rep. 2017;15(4):2129–35.

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