





# Chapter 3

## Elucidating mechano-pathology of osteoarthritis: transcriptome-wide differences in mechanically stressed aged human cartilage explants

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## Abstract

### Background

Failing of intrinsic chondrocyte repair after mechanical stress is known as one of the most important initiators of osteoarthritis. Nonetheless, insight into these early mechano-pathophysiological processes in age related human articular cartilage is still lacking. Such insights are needed to advance clinical development. To highlight important molecular processes of osteoarthritis mechano-pathology, the transcriptome-wide changes following injurious mechanical stress on human aged osteochondral explants were characterized.

### Methods

Following mechanical stress at a strain of 65% (65%MS) on human osteochondral explants ( $n_{65\%MS}=14$  versus  $n_{control}=14$ ), RNA sequencing was performed. Differential expression analysis between control and 65%MS was performed to determine mechanical stress-specific changes. Enrichment for pathways and protein-protein interactions was analyzed with Enrichr and STRING.

### Results

We identified 156 genes significantly differentially expressed between control and 65%MS human osteochondral explants. Of note, *IGFBP5* (FC=6.01; FDR=7.81x10<sup>-3</sup>) and *MMP13* (FC=5.19; FDR=4.84x10<sup>-2</sup>) were the highest upregulated genes, while *IGFBP6* (FC=0.19; FDR=3.07x10<sup>-4</sup>) was the most downregulated gene. Protein-protein interactions were significantly higher than expected by chance ( $P=1.44x10^{-15}$  with connections between 116 out of 156 genes). Pathway analysis showed, among others, enrichment for cellular senescence, insulin-like growth factor (IGF) I & II binding, and focal adhesion.

### Conclusions

Our results faithfully represent transcriptomic wide consequences of mechanical stress in human aged articular cartilage with *MMP13*, IGF binding proteins, and cellular senescence as the most notable results. Acquired knowledge on the as such identified initial, osteoarthritis related, detrimental responses of chondrocytes may eventually contribute to the development of effective disease-modifying osteoarthritis treatments.

### Keywords

Osteoarthritis, cartilage, chondrocytes, mechanical stress, mechanopathology, RNA-sequencing, cellular senescence, IGF-1 signalling, MMP13

## Introduction

Osteoarthritis (OA) is an age-related joint disease, affecting diarthrodial joints [1,2]. Despite the fact that OA is the most prevalent and disabling disease among elderly, resulting in high social and economic burden, no effective treatment exists except for lifestyle changes, pain medication and eventually a joint replacement surgery at end stage-disease [3,4].

To characterize deregulated signalling pathways in OA cartilage, comprehensive differential expression analyses have been performed comparing preserved versus end-stage lesioned OA cartilage [5]. These studies revealed that OA pathology is marked by recuperation of growth plate signaling, wound healing, and skeletal system development, while also highlighting inherent differences in OA pathophysiology between patient subtypes based on gene expression changes [5-7]. Nonetheless, the preserved versus lesioned study design by definition captures end-stage pathophysiological OA disease processes and gives no information on early initial processes triggering cartilage to become diseased. In contrast, disease-modifying OA drugs should preferably target early OA disease triggers when irreversible damage of cartilage has not yet taken place. Therefore more knowledge on the initial response of chondrocytes to OA relevant stresses, such as mechanical trauma, should be investigated in an appropriate model.

In this regard, failing of intrinsic chondrocyte repair after mechanical stress is known to impact the integrity of articular cartilage via cell apoptosis [8], increased catabolic gene expression [9], and reduced matrix production [10] and is, as such, an important trigger to OA onset. Nonetheless, little knowledge exists on the inherent dysregulation of signaling pathways initiating repair responses in human aged articular cartilage upon mechanical stress. To gain some insight, several *in vivo* animal studies have investigated the effect of joint overuse or trauma on gene expression in cartilage [11-16]. Some examples of non-invasive *in vivo* mechanical loading studies are Bomer et al. [11], reporting on involvement of metabolic processes and skeletal system development pathways upon physiological forced running in 6-month-old mice, Chang et al. [12], reporting on involvement of cell proliferation and chondroitin sulfate proteoglycan metabolic process upon injurious tibial compression in 16-week-old mice and Sebastian et al. [13], reporting on single-Cell RNA-seq upon tibial compression in 10-week-old mice. Thus far, one study has investigated genome-wide expression consequences of an impact injury in porcine explants and identified involvement of genes associated with matrix molecules, protein biosynthesis, skeletal development, and cell proliferation [17]. Nevertheless, most studies were performed using relatively young animal tissues and likely do not cover the biological response to a trauma in adult (human) tissue [18]. More recently, global gene expression profiling in 14-month-old mice subjected to non-invasive injurious tibial compression identified genes involved in inflammation and matrix regeneration to be involved in the response of aged tissue [14].

A more appropriate model to identify which molecular processes are initiated in response to mechanical stress in humans would comprise of aged human *ex vivo* osteochondral explants. Injurious compression reaching strains above 50% induced catabolic processes in cartilage and eventually led to cell death [19]. In aged human osteochondral explants, injurious cyclic mechanical stress at a strain of 65% (65%MS), mimicking trauma, was previously shown to induce OA like damage [20]. In the current study we therefore exploited our previously established *ex vivo* osteochondral explant model by performing RNA sequencing on explants subjected to injurious mechanical stress in comparison to controls. The hypothesis free,

transcriptome wide approach presented here contributes to further understanding the debilitating response of aged chondrocytes to mechanical injury and how this affects their propensity to enter an OA disease state.

## **Material and Methods**

### ***Sample description***

To generate osteochondral explants, biopsies (diameter of 8 mm) were punched from the macroscopically preserved load-bearing area of femoral condyles of human knee joints obtained within the Research in Articular Osteoarthritis Cartilage (RAAK) biobank containing patients that undergo a joint replacement surgery as a consequence of OA [21]. For this study, a total of 60 osteochondral explants were investigated originating from nineteen independent donors in which multiple explants were taken from each donor. This difference between the amount of samples taken per donor was dependent on several factors. Among them were size of the knee condyle, size of the preserved area, surgical damage area, and other simultaneous experiments this donor was used for. RNA-sequencing was performed on samples from nine donors, while the remaining ten donors were used for replication purposes. All donor characteristics are given in **Table S1** and were equal between mechanical stressed and control explant donors.

### ***Application of mechanical stress***

Explants of nineteen donors were equilibrated in serum-free chondrogenic differentiation medium (DMEM, supplemented with Ascorbic acid (50 µg/ml; Sigma-Aldrich; Zwijndrecht, The Netherlands), L-proline (40 µg/ml; Sigma-Aldrich), sodium pyruvate (100 µg/ml; Sigma-Aldrich), dexamethasone (0.1 µM; Sigma-Aldrich), ITS+ and antibiotics (100 U/ml penicillin; 100 µg/ml streptomycin) in a 5% (v/v) CO<sub>2</sub> incubator at 37°C. As depicted in **Figure 1a**, after a six day period, dynamic unconfined compression was applied to explants (diameter of 8 mm) using the Mach-1 mechanical testing system on four subsequent days (Biomomentum Inc., Laval, QC, Canada). In short, osteochondral explants were placed under an indenter (diameter of 10 mm) attached to a 250N MACH-1 load cell (Figure 1a) and unconfined cyclic compression was applied at a strain of 65% of cartilage height at a frequency of 1 Hz (1 compression cycle per second), mimicking walking speed, during 10 minutes, long enough to be injurious and short enough for chondrocytes to survive, at strains suggested to be detrimental [22]. Dynamic (cyclic) compression means that a force was applied that varied over time to simulate a more cyclic compression such as walking. To investigate lasting effects of mechanical stress, four days after mechanical stress, the cartilage and bone were separated, snap-frozen in liquid nitrogen and stored at -80 °C.

### **Determining cartilage integrity**

#### ***Histology***

A sagittal section of the osteochondral explant was fixed in 4% formaldehyde for one week and

decalcified using EDTA (12.5%, pH=7.4) during two weeks, dehydrated with an automated tissue processing apparatus and embedded in paraffin. Tissue sections were cut at a thickness of 5  $\mu\text{m}$ , deparaffinized, rehydrated, subsequently stained for 1 minute in a toluidine blue solution with a pH of 2.5 (Sigma-Aldrich) and mounted with Pertex (Sigma-Aldrich) to investigate cartilage integrity as quantified by applying Mankin Score [23].

### ***Sulfated glycosaminoglycan (sGAG) measurement***

Sulfated glycosaminoglycan (sGAG) concentrations in conditioned media collected from osteochondral explants were measured with the photometric 1.9 dimethylene blue (DMMB; Sigma-Aldrich) dye method [24]. Shark chondroitin sulfate (Sigma-Aldrich) was used as the reference standard. The concentration of sGAG was determined in conditioned media collected on day 13, by measuring absorbance at 525nm and 595nm in a microplate reader (Synergy HT; BioTek, Winooski, USA).

### ***RNA sequencing***

RNA from cartilage was extracted by pulverizing the tissue and subsequently homogenizing the powder in TRIzol reagent (Invitrogen, San Diego, CA) using a Mixer mill 200 (Retsch, Germany). RNA was extracted using chloroform, followed by precipitation using ethanol, and purified with the RNeasy Mini Kit (Qiagen, Chatsworth, CA). Genomic DNA was removed by DNase digestion. Paired-end 2x150 base pair RNA sequencing (Illumina TruSeq mRNA Library Prep Kit, Illumina HiSeq X ten) was performed. Strand specific RNA-sequencing libraries were generated which yielded on average 14 million reads per sample. Data from the Illumina platform was analysed with an in-house pipeline as previously described [5]. The adapters were clipped using Cutadapt v1.1. RNA-seq reads were then aligned using GSNAP against GRCh38 [25]. Read abundances per sample were estimated using HTSeq count v0.11.1 [26] with Ensembl gene annotation version 94. Only uniquely mapping reads were used for estimating expression. The quality of the raw reads and initial processing for RNA-sequencing was checked using MulitQC v1.7 [27]. Samples containing >50% genes with zero values and average read count <10 were removed from further analysis. To identify outliers, principal component analysis (PCA) was applied. For further analysis, samples not in the main cluster were removed, resulting in n=28 samples from 9 unique donors. In total, 58735 unique genes were detected by RNA sequencing of which 6509 were protein-coding genes that were included in further analyses.

### ***Differential expression analysis, protein-protein interactions and pathway enrichment***

Differential expression analysis was performed in 65%MS cartilage compared to control cartilage obtained from osteochondral explants using DESeq2 R package version 1.24 [28] on 6509 protein-coding genes. A general linear model assuming a negative binominal distribution was applied and followed by a Wald-test between control and 65%MS samples in which donor number was added as a random effect to correct for inter-individual differences. In all analyses,

control samples were set as reference. To correct for multiple testing the Benjamini-Hochberg method was used, as indicated by the false discovery rate (FDR) in which a significant cutoff value of 0.05 was used. Furthermore, the comprehensive gene set enrichment analysis web tool Enrichr [29] was used to identify enrichment for gene ontologies (Cellular Component, Biological Process, Molecular Function) and pathways (KEGG and Reactome). For protein-protein interactions, analysis was performed using the online tool STRING version 11.0 [30].

### ***Real-time quantitative PCR (RT-qPCR) validation***

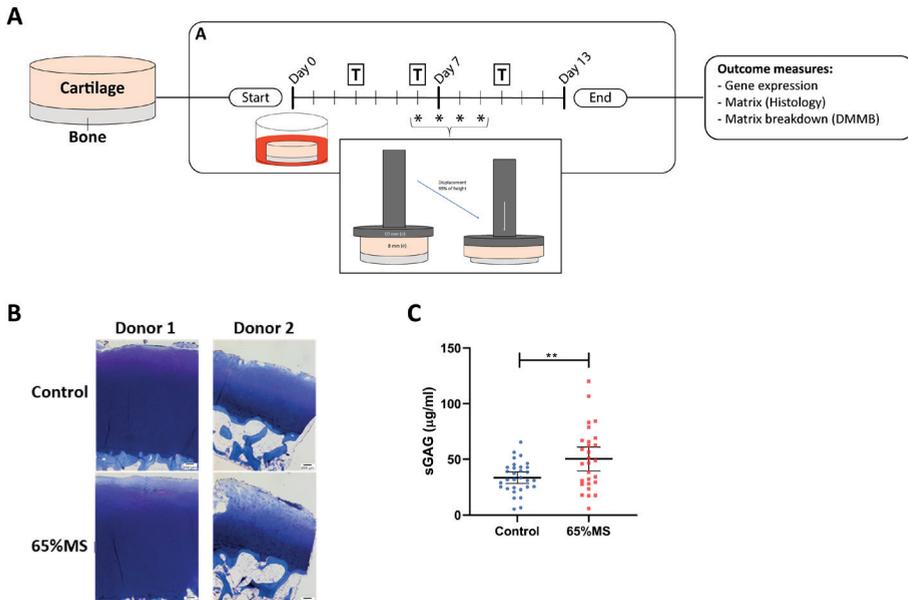
250 ng of RNA was processed into cDNA using the First Strand cDNA Synthesis Kit (Roche Applied Science, Almere, The Netherlands). RT-qPCR was performed on 10 paired 65%MS samples with matched controls included in the RNA-sequencing (Technical validation) and 10 novel paired 65%MS samples with matched controls (Biological validation) to determine the expression of six downregulated (*IGFBP6*, *CNTFR*, *WISP2*, *FRZB*, *COL9A3*, and *GADD45A*) and four upregulated genes (*IGFBP5*, *PTGES*, *TNC*, and *IGFBP4*). Primer sequences are listed in **Table S2**. The relative gene expression was normalized for two endogenous reference genes, *SDHA* and *YWHAZ*, to determine  $-\Delta\Delta CT$  values. To determine effect sizes, fold changes (FC) were calculated according to the  $2^{-\Delta\Delta CT}$  method, in which expression of 65%MS was extracted from controls ( $-\Delta\Delta CT$ ). These two endogenous reference genes were chosen based on literature stating the stability of these genes in response to mechanical stress, which was confirmed by our RNA-sequencing [31,32].

### ***Statistical analysis***

Analysis on RNA-sequencing data was performed in R as described above. Statistical analysis for RT-qPCR and sGAG concentrations were performed using IBM SPSS statistics 25. The P-values were determined by applying a linear generalized estimating equation (GEE) to effectively adjust for dependencies among donors of the explants by adding a random effect for the sample donor as we did not have perfect pairs for each analysis [33]. The following GEE was fitted in which gene expression was the dependent variable and treatment the covariate: *Gene expression*  $\sim$  *Treatment* + (1|*donor*). To determine differences in sGAG concentration on day 13, another linear GEE model was fitted with sGAG concentration as dependent variable and treatment as covariate: *sGAG concentration*  $\sim$  *Treatment* + (1|*donor*).

## Results

Prior to RNA-sequencing, cartilage tissue integrity of human osteochondral explants was characterized by performing histology and measuring sGAG concentrations in conditioned media. Mechanical strains at 65% cause detrimental changes to cartilage integrity as previously shown [20] (**Figure 1B**) and these effects were further explored in a larger samples size ( $n_{\text{control}}=31$ ;  $n_{65\%MS}=28$ ), where an increased sGAG release was measured in 65%MS cartilage when compared to controls (**Figure 1C**).

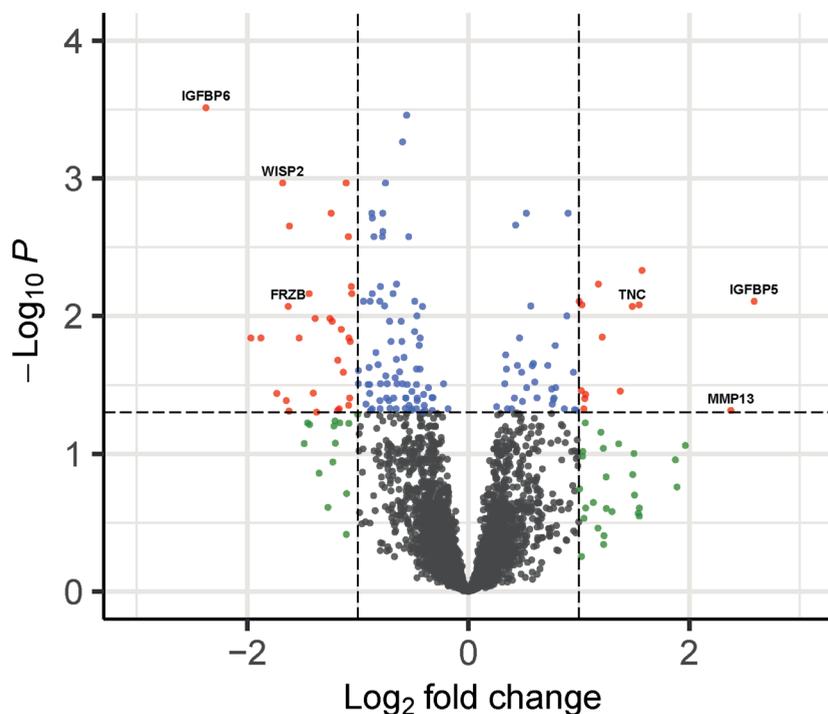


**Figure 1 | Study setup of human osteochondral explants receiving 65% MS.** [A] osteochondral explants were punched from preserved areas of knee joints and medium is refreshed on indicated days (T). [B] Damage in our mechanical stress model was confirmed by degradation of sGAG in cartilage by toluidine blue staining (histology of two independent donors) and measuring [C] sGAG release in conditioned media on day 13 ( $n_{\text{control}}=31$  versus  $n_{65\%MS}=28$ ). Figure 1C shows the average  $\pm$  95%CI and each dot represents a sample. To adjust for donor variation, P-values were estimated by performing logistic generalized estimation equations, with sGAG concentration as dependent variable and treatment as covariate:  $\text{sGAG concentration} \sim \text{Treatment} + (1|\text{Donor})$ .  $^{**}P \leq 0.01$ . Legend: 65%MS= 65% mechanical stress, DMMB= dimethylmethylene blue, sGAG= sulphated glycosaminoglycans.

### Differential expression of genes responsive to injurious mechanical stress

To characterize the response of cartilage to mechanical stress at a strain of 65% indentation in aged articular cartilage, we performed RNA-sequencing on control ( $n=14$  samples) and 65% mechanically stressed ( $n=14$  samples) articular cartilage samples obtained from macroscopically preserved osteochondral explants of human patients that underwent a knee replacement surgery due to OA. Baseline characteristics of donors of the RNA-sequencing dataset are depicted in **Table S1a**. We found 156 genes to be significantly differentially expressed (DE) ( $\text{FDR} < 0.05$ ) with absolute fold changes (FC) ranging between 1.1 and 6.0 (**Figure 2, Table S3**). Among these 156 DE genes, 46 (29%) were upregulated and 110 (71%) were downregulated. The 20 genes with the highest absolute FC, and their respective direction of effect previously identified in OA cartilage [5], are shown in **Table 1**. Notable among the upregulated genes were *IGFBP5* ( $\text{FC}=6.01$ ;  $\text{FDR}=7.81 \times 10^{-3}$ ), *MMP13* ( $\text{FC}=5.19$ ;

FDR=4.84x10<sup>-2</sup>), *TNC* (FC=2.80; FDR=8.51x10<sup>-3</sup>), and *PTGES* (FC=2.92; FDR=8.29x10<sup>-3</sup>). Notable genes among the downregulated genes were *IGFBP6* (FC=0.19; FDR=3.07x10<sup>-4</sup>), *CNTFR* (FC=0.27; FDR=1.44x10<sup>-2</sup>), *WISP2* (FC=0.31; FDR=1.08x10<sup>-3</sup>), and *FRZB* (FC=0.32; FDR=8.51x10<sup>-3</sup>).



**Figure 2 | Volcano plot of differentially expressed genes.** Dots represent genes expressed in mechanically stressed cartilage in comparison to control osteochondral explant cartilage. Red dots represent significantly differentially expressed (DE) genes that have an absolute fold change (FC) of  $\geq 2$ , blue dots represent significantly DE genes, green dots represent genes that have an absolute FC of  $\geq 2$  but are not significantly DE and grey dots represent genes not DE expressed between controls and 65% mechanically stressed cartilage. The FC presented here is the gene expression of 65% mechanically stressed relative to control cartilage.

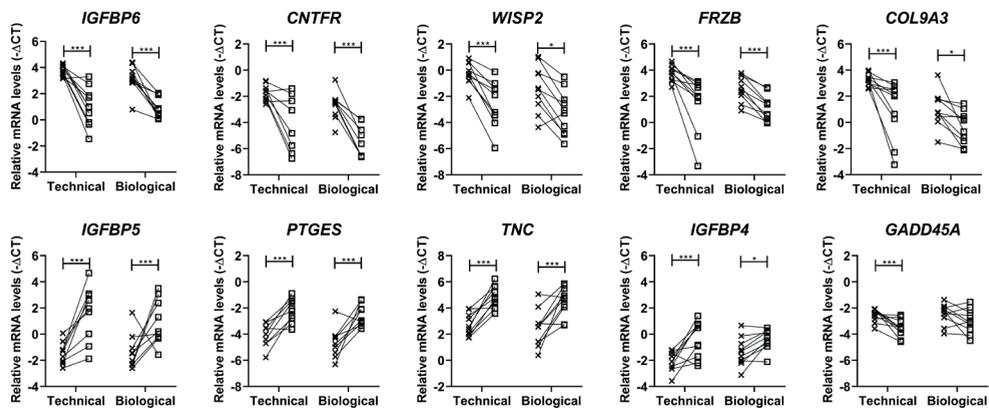
### Validation of differentially expressed genes with mechanical stress

For validation and replication of the differentially expressed genes identified, a set of samples for technical (n=10 pairs) and biological (n=10 pairs) replication was selected for RT-qPCR. Baseline characteristics of donors in the replication dataset are depicted in **Table S1b**. Replication was performed for ten genes (**Figure 3**), of which six were upregulated (*IGFBP6*, *CNTFR*, *WISP2*, *FRZB*, *COL9A3*, and *GADD45A*) and four were downregulated (*IGFBP5*, *PTGES*, *TNC*, and *IGFBP4*). Technical replication showed a significant difference for all ten genes between controls and 65%MS cartilage, with similar direction and size of effects. Biological replication also showed the same direction of effects and similar effect sizes as identified in the RNA-sequencing data. For *GADD45A*, however, the difference was not significant (P-value=0.12). Taken together, technical and biological replication confirmed the robustness of our RNA-sequencing results.

**Table 1 | Top 20 genes with the highest absolute FC in 65% mechanically stressed cartilage compared to controls.**

Ensemble ID	Gene name	FC	FDR <sup>a</sup>	Differential expression in OA cartilage[5] <sup>b</sup>
ENSG00000115461	<i>IGFBP5</i>	6.01	7.81x10 <sup>-3</sup>	
ENSG00000137745	<i>MMP13</i>	5.19	4.84x10 <sup>-2</sup>	
ENSG00000204103	<i>MAFB</i>	2.97	4.66x10 <sup>-3</sup>	↓
ENSG00000148344	<i>PTGES</i>	2.92	8.29x10 <sup>-3</sup>	↑
ENSG00000041982	<i>TNC</i>	2.80	8.51x10 <sup>-3</sup>	↑
ENSG00000141753	<i>IGFBP4</i>	2.59	3.50x10 <sup>-2</sup>	↑
ENSG00000160111	<i>CPAMD8</i>	0.39	4.98x10 <sup>-2</sup>	↓
ENSG00000166165	<i>CKB</i>	0.38	1.04x10 <sup>-2</sup>	↑
ENSG00000106258	<i>CYP3A5</i>	0.38	3.62x10 <sup>-2</sup>	
ENSG00000107736	<i>CDH23</i>	0.37	6.88x10 <sup>-3</sup>	
ENSG00000187720	<i>THSD4</i>	0.35	1.44x10 <sup>-2</sup>	
ENSG00000144908	<i>ALDH1L1</i>	0.33	2.22x10 <sup>-3</sup>	↓
ENSG00000092758	<i>COL9A3</i>	0.32	4.89x10 <sup>-2</sup>	
ENSG00000162998	<i>FRZB</i>	0.32	8.51x10 <sup>-3</sup>	↓
ENSG00000170891	<i>CYTL1</i>	0.32	4.11x10 <sup>-2</sup>	
ENSG00000064205	<i>WISP2</i>	0.31	1.08x10 <sup>-3</sup>	↓
ENSG00000082196	<i>C1QTNF3</i>	0.30	3.64x10 <sup>-2</sup>	↑
ENSG00000122756	<i>CNTFR</i>	0.27	1.44x10 <sup>-2</sup>	↓
ENSG00000165966	<i>PDZRN4</i>	0.26	1.44x10 <sup>-2</sup>	↓
ENSG00000167779	<i>IGFBP6</i>	0.19	3.07x10 <sup>-4</sup>	

<sup>a</sup> To correct for multiple testing, the Benjamini-Hochberg method was applied to p-values and reported as the false discovery rate (FDR). <sup>b</sup> Gene expression changes measured in RNA-sequencing data between preserved and lesioned OA articular cartilage, with preserved as reference [5]. Legend: FC=fold change; FDR= false discovery rate.



**Figure 3 | Technical and biological validation of the highest up- and downregulated genes was performed using RT-qPCR.** Expression of six downregulated (*IGFBP6*, *CNTFR*, *WISP2*, *FRZB* and *GADD45A*) and four upregulated (*IGFBP5*, *PTGES*, *TNC* and *IGFBP4*) genes was measured in n=10 paired technical and n=10 paired biological osteochondral explants. Figures show connected paired samples and  $-\Delta CT$  of each independent sample is depicted by crosses (control) or open squares (65%MS) in the graphs. Statistical differences between gene expression in control and 65% mechanically stressed was determined with a linear generalized estimation equation (GEE) with mRNA level as dependent variable. \*P $\leq$ 0.05; \*\*\*P $\leq$ 0.001. Legend: 65%MS: 65% mechanical stress, RT-qPCR: reverse transcriptase-quantitative PCR.

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### *In silico exploration of differentially expressed genes*

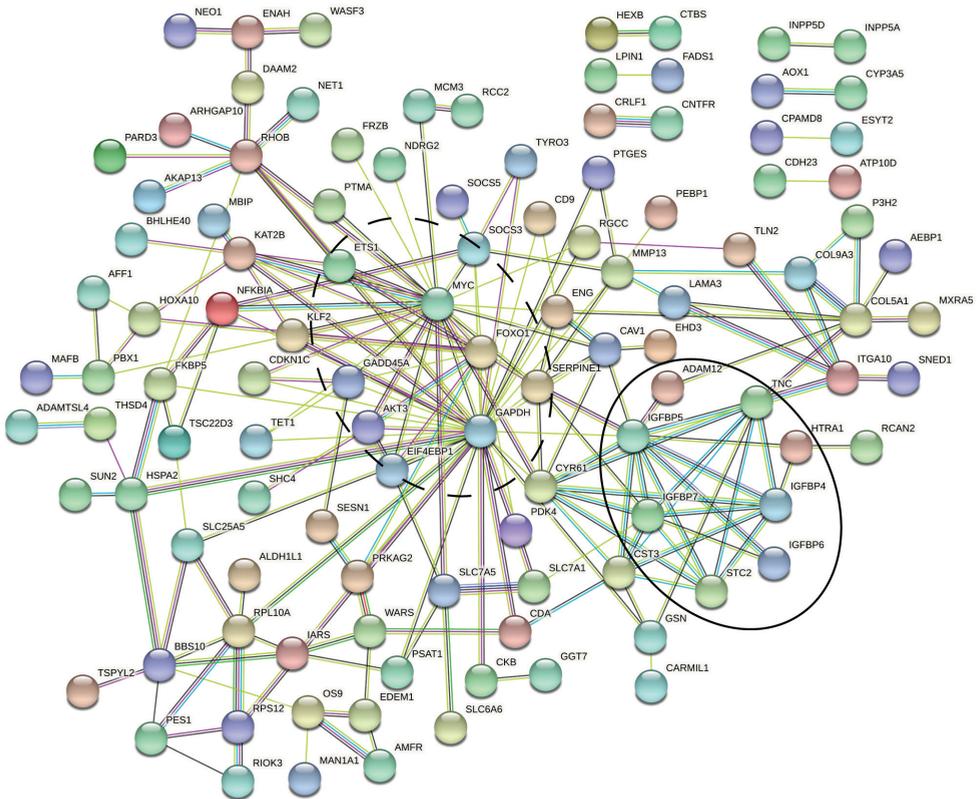
To explore whether significant DE genes (N=156 genes) were involved in particular pathways, they were further analyzed using Enrichr. Gene enrichment was observed, among others, for insulin-like growth factor I & II binding (GO:0031995; GO:0031994,  $\text{P}_{\text{adj}}=1.83 \times 10^{-2}$ ;  $\text{P}_{\text{adj}}=2.89 \times 10^{-2}$ , involving *IGFBP4*, *IGFBP5*, and *IGFBP6*), cellular senescence (hsa04218,  $\text{P}_{\text{adj}}=1.15 \times 10^{-2}$ , involving 8 genes, e.g. *GADD45A*, *MYC*, *SERPINE1*, and *FOXO1*) and focal adhesion (GO:0005925; hsa04510,  $\text{P}_{\text{adj}}=2.54 \times 10^{-2}$ ;  $\text{P}_{\text{adj}}=1.33 \times 10^{-2}$ , involving 11 and 6 genes, respectively, e.g. *TNC*, *CAV1*, and *TLN2*) (**Table 2**; **Table S4a**).

**Table 2 | Gene ontology and pathway enrichment analysis of differentially expressed genes in mechanically stressed cartilage.**

Term	Entry	Overlap	Adj P-value <sup>a</sup>	Odds Ratio	Genes
Cellular senescence	hsa04218	8/160	$1.15 \times 10^{-2}$	6.41	GADD45A, MYC, SERPINE1, AKT3, EIF4EBP1, SLC25A5, ETS1, FOXO1
Focal adhesion	hsa04510	8/199	$1.33 \times 10^{-2}$	5.15	SHC4, CAV1, ITGA10, AKT3, LAMA3, TNC, COL9A3, TLN2
Insulin-like growth factor II binding	GO:0031995	3/7	$1.83 \times 10^{-2}$	54.95	IGFBP5, IGFBP4, IGFBP6
Focal adhesion	GO:0005925	11/356	$2.54 \times 10^{-2}$	3.96	ENAH, EHD3, GSN, CAV1, TNC, CD9, TLN2, RPL10A, DCAF6, RHOB, ENG
Insulin-like growth factor I binding	GO:0031994	3/13	$2.89 \times 10^{-2}$	29.59	IGFBP5, IGFBP4, IGFBP6

<sup>a</sup> Enrichr uses a modified Fishers exact test to compute enrichment and this is reported as the adjusted p-value [29]. Legend: Adj P-value= adjusted P-value.

To visualize interacting proteins, the online tool STRING was used. Among the 156 genes, 116 of the encoded proteins showed significant protein-protein interactions (PPI) ( $P=1.44 \times 10^{-15}$ ; **Figure 4**). Among these proteins we found several that have many connections with other proteins in the DE gene network, such as *GAPDH* with 35 connections, *IGFBP5* with 12 connections and the in cellular senescence involved genes *MYC* and *FOXO1* with respectively 26 and 13 connections to other DE genes. Moreover, two clusters of genes are observed that correspond with two of the pathways identified. One cluster corresponds with genes found mainly in the cellular senescence pathway (**Figure 4**, dotted circle), while the other cluster consists of proteins that are involved in IGF-1 signaling (**Figure 4**, black circle).



**Figure 4 | Protein-protein interaction network in STRING of proteins encoded by differentially expressed genes.** Only connected (N=116 genes) genes that were identified to be differentially expressed between mechanically stressed and control cartilage of osteochondral explants are shown. Two clusters with high interactions were identified upon studying connections within String. One cluster corresponds with genes found in the cellular senescence pathway (dotted circle), while the other cluster consists of proteins that are involved in IGF-1 signaling (black circle).

### **Comparison between mechanical stress genes and OA responsive genes.**

To investigate to what extent the genes DE with mechanical stress ( $DE_{MS}$ ) coincide with OA pathophysiology, we next compared the  $DE_{MS}$  genes (**Table S3**) to previously identified genes DE between preserved and lesioned OA cartilage ( $DE_{OA}$ ) [5]. Of the 156  $DE_{MS}$  genes, 64 were previously identified with OA pathophysiology and their majority (48 genes, 75%) had the same direction of effect (**Table 1** and **Table S5a, Figure S1**). Notable genes coinciding with OA pathophysiology and showing the same direction of effect are the highly downregulated *FRZB*, *WISP2*, and *CNTFR* and the upregulated *PTGES* and *CRLF1*.

Next, we selected for exclusive mechanical stress responsive genes i.e.  $DE_{MS}$  genes, not overlapping with previously identified  $DE_{OA}$  genes [5]. This resulted in 92 genes that were differentially expressed exclusively in response to mechanical stress ( $DE_{ExclusiveMS}$ ; **Table S6; Figure S1**). Notable  $DE_{ExclusiveMS}$  genes are the downregulated *IGFBP6*, *ITGA10*, and *COL9A3* and the upregulated *IGFBP5*, *MMP13*, and *GAPDH*. Subsequent pathway analyses showed gene enrichment among genes involved in focal adhesion (GO:0005925,  $P_{adj}=0.02$ , 9 genes, e.g. *CD9*, *RPL10A*, and *ENAH*) and kinase inhibitor activity (GO:0019210,  $P_{adj}=0.01$ , 5 genes,

e.g. *CDKN1C*, *SOCS3*, and *SOCS5*) (**Table S4b**). Upon exploring protein-protein interactions between the 92 DE<sub>ExclusiveMS</sub> genes using STRING, a highly significant enrichment for PPI was identified ( $P=1.07 \times 10^{-4}$ ; **Figure S2**), indicating that these genes act together or respond in concert to detrimental mechanical stress.

### **OA risk genes responding to mechanical stress**

Finally, to investigate which OA risk genes are represented among the mechanically stress responsive genes in cartilage, we checked  $N=90$  genes previously recognized as strong OA risk genes [34] identified in recent genome-wide association studies (GWAS) [35,36]. As shown in **Table S7**, two of our identified DE<sub>MS</sub> genes were also shown to be an OA risk gene in previous studies. These genes were *TNC*, encoding for tenascin C, which was highly increased ( $FC=2.80$ ;  $FDR=8.5 \times 10^{-3}$ ) upon 65%MS and *SCUBE1*, encoding for signal peptide, CUB domain and EGF like domain containing 1, which was decreased ( $FC=0.53$ ;  $FDR=0.04$ ) upon 65%MS.

## **Discussion**

To our knowledge we are the first to report genome-wide differentially expressed mRNAs in articular cartilage following repeated exposure to 65% mechanical stress using a human *ex vivo* osteochondral explant model. Since injurious loading is considered a major trigger in the initiation of OA onset, the results presented in our manuscript contribute important insight into how injurious stress affects the propensity of aged human articular chondrocytes to lose their steady state towards a debilitating OA disease state. Notable genes identified were different members of the insulin-like growth factor I & II binding family (*IGFBP6*, *IGFBP5*, and *IGFBP4*) and the catabolic gene *MMP13*. Gene enrichment analyses showed that cellular senescence (*GADD45A*, *MYC*, *SERPINE1*, and *FOXO1*) and focal adhesion (*ITGA10*, *TLN2*, and *CAV1*) processes are significantly changing in articular cartilage with injurious loading. Together, identified genes and pathways facilitate clinical development by exploring ways to counteract these initial unbeneficial responses to injurious loading by supplementing or inhibiting of key genes. Moreover, we advocate that here identified specific responsive genes to injurious loading can function as sensitive markers facilitating the development of scientifically founded strategies with respect to preventive or curative exercise OA therapy among elderly.

Among the highest FDR significantly upregulated genes with 65% mechanical stress we identified *MMP13*, encoding matrix metalloproteinase 13 ( $FC=5.19$ ;  $FDR=4.84 \times 10^{-2}$ ) [20]. *MMP13* is involved in the detrimental breakdown of extracellular matrix in articular cartilage by cleaving, among others, collagen type II. Despite the well-known role of *MMP13* in collagen type II breakdown, it should be noted that the *MMP13* gene is not found to be responsive with end-stage OA pathophysiology, i.e., not consistent and not among the genes highest differentially expressed between preserved and lesioned OA cartilage (**Table 1**) [5,21,37]. We therefore advocate that *MMP13* expression could specifically mark initial responses to cartilage damage and not that of a chronic degenerative OA disease state. Henceforth, abrogating the *MMP13* signaling shortly after an injurious cartilage event could prevent the detrimental downstream enzymatic breakdown of extracellular matrix proteins. Moreover, and as indicated above, *MMP13* may be a suitable candidate sensitively marking injurious

loading of aged human articular cartilage independent of other physiological factors such as OA disease state.

Four out of seven members of the insulin growth factor binding proteins (*IGFBP4*, *IGFBP5*, *IGFBP6*, and *IGFBP7*; **Table S8**), were found to be FDR DE. *IGFBP1-6* have an equal or greater affinity for binding IGF-1 when compared to IGF-1R; hence most of IGF-1 in the body is bound to IGFBPs, antagonizing IGF-1 signaling [38-41]. On the other hand, *IGFBP7* has a low affinity for IGF and therefore more likely affects cell metabolism via binding to activin A, influencing the growth-suppressing effects of TGF- $\beta$ , and antagonizing bone morphogenetic protein (BMP) signaling [42,43]. *IGFBP4* and *IGFBP5* can also function as transporter and bring IGF-1 close to its receptor, where IGF-1 is released via cleavage by proteins such as pregnancy-associated plasma protein-A (PAPPA), HtrA Serine Peptidase 1 (*HTRA1*) and disintegrin and metalloproteinase domain-containing protein 12 (*ADAM12*) [44-46]. Additionally notable in this respect is that three genes, *HTRA1*, *ADAM12* and *STC2* [47], involved in IGF-1 cleavage were found among the FDR significant upregulated genes in our dataset (**Table S3**). IGFBPs can also affect cells via IGF-independent mechanisms. The most noteworthy IGF-independent mechanism is observed for the highly upregulated *IGFBP5*, being induction of cell proliferation and apoptosis [48,49]. In summary our data showed that, despite the fact that the mechanical stress applied affected cartilage integrity (**Figure 1**), the upregulation of *IGFBP4* and *IGFBP5* in combination with the upregulation of its cleaving proteins might reflect an anabolic response of chondrocytes to initiate repair by increasing bio-availability of IGF-1. Two studies support our suggestion that IGF-1 signaling might be a beneficial anabolic response to mechanical stress. In an OA dog model, increasing intact *IGFBP5* proteins resulted in increased IGF-1 levels and reduced destruction of cartilage [50]. While in a human explant model, addition of IGF-1 after mechanical stress increased *COL2A1* gene expression and slightly increased cell viability [51]. Our results in combination with those previously found, suggest that addition of *IGFBP4* and/or *IGFBP5* would be an interesting therapy to further explore in combatting the catabolic response.

To identify upstream processes and to put our results in a broader perspective, we investigated connections between genes on the protein level in STRING (**Figure 4**) and determined pathway enrichment (**Table 2**) of the differentially expressed genes. Based on this pathway analysis, we identified enrichment for proteins involved in cellular senescence. DE genes with mechanical stress in this pathway have already been linked to aging and OA, such as *GADD45A*, *SERPINE1*, *MYC*, and *FOXO1*. Notable are the two transcription factors, *MYC* and *FOXO1*, showing many connections to other proteins (**Figure 4**) and previously shown to be dysregulated in OA chondrocytes [52,53]. *FOXO1* is an essential mediator of cartilage growth and homeostasis and its expression is decreased in aged and OA cartilage [52]. In addition, *FOXO1* was shown to be an antagonist of *MYC* and prevents, among others, ROS production [54,55]. Our results suggest that reduced expression of *FOXO1* could be one of the reasons for increased expression of *MYC*. As one of the known responses of chondrocytes to mechanical stress is ROS production, this would be a promising target to follow up on in future research. Next to genes in this pathway, lookup of our DE<sub>MS</sub> genes in a proteomic atlas of senescence-associated secretory phenotype (SASP) identified 35 of our DE<sub>MS</sub> genes to have previously been found in different senescent cells (**Figure S3**)[56]. Taken together, the upregulation of *MYC* in combination with upregulation of several important SASP protein markers suggests increased cellular damage is occurring upon mechanical stress likely driving cells to go into senescence. As cellular senescence is a factor that is thought to play a significant role in the OA

pathophysiology, our model could provide more knowledge on how this pathway is involved in the onset of OA and how therapeutics could be used to minimize this response [57].

To investigate whether OA risk loci could confer risk via modifying response to mechanical stress, we compared  $DE_{MS}$  genes to strong OA risk genes identified in the most recent GWAS [35,36]. This resulted in the identification of two OA risk genes, *TNC* and *SCUBE1* present in our dataset (**Table S7**). Based on allelic imbalanced expression and linkage disequilibrium, the *TNC* OA risk allele rs1330349-C, in high linkage disequilibrium with the transcript SNP rs2274836-T, appeared to act via decreasing expression of *TNC* [58]. For that matter, the observed high upregulation of *TNC* expression with mechanical stress ( $FC=2.80$ ;  $FDR=8.51 \times 10^{-3}$ ) as well as, the previously observed upregulation with OA pathophysiology ( $FC=1.41$ ;  $FDR=1.09 \times 10^{-2}$ ) [5] is likely a beneficial response to rescue or maintain articular cartilage integrity. This is further confirmed by animal studies showing that addition of exogenous TNC reduced cartilage degeneration and repaired cartilage [59,60]. In contrast, for the intronic OA risk SNP located in the vicinity of *SCUBE1* (rs528981060) we were not able to determine a transcript proxy SNP, hence potential AEI of *SCUBE1* could not be explored.

With regard to overlap with *in vivo* animal models, we compare our DE genes to those found in physiological [11], surgical destabilization of the medial meniscus (DMM) [18] and non-invasive tibial compression (TC) models [12,14]. The most striking overlap in DE genes (46 genes) was found between our model and the non-invasive TC model using gene expression data of 14 month old mice 1 week after injury. Among the overlapping genes we confirmed involvement of all *IGFBPs*, *HTRA1*, *ADAM12* and of OA associated genes such as, *FRZB*, *TNC* and *SCUBE1* in both models [14]. As also shown by other studies [14,18], age of animals used in these models can greatly influence results. This could also, next to a difference in species, partially explain why there is little overlap with other injurious mechanical stress studies.

A strength of our aged human *ex vivo* osteochondral model is that it allowed us to investigate the chondrocyte response to an OA-relevant trigger in its natural environment. In addition, our model comprises aged cartilage, which is likely more vulnerable to OA onset, and hence, results are relevant to the population at risk. Another strong point in our model is that we measure the changes in gene expression that are measured 4 days post-injury as such reflecting representative lasting changes in chondrocyte signaling rather than acute stress responses only. On the other hand, our data could facilitate treatment strategies, prior to irreversible damage of OA affected cartilage. Some limitations of our study are the relatively low sample size of 14 explants per condition, hence limiting our power. As a result we may have missed subtle gene expression changes in response to detrimental mechanical stress. Another point of our study to address is the heterogeneity of preserved cartilage collected from OA patients with Mankin scores ranging from 0 to 7. Although such heterogeneity may also have affected the power of our study, hence the total number of differentially expressed genes with injurious loading, we want to highlight that despite the differences in Mankin scores we were able to consistently detect (at the genome-wide significant level) 156 differentially expressed genes reflecting strong and/or very consistent mechano-pathological processes triggered after mechanical stress. Moreover, due to the heterogeneity in eligible waste articular cartilage after joint replacement surgery (i.e., osteochondral explants) we were not able to generate a RNA-sequencing dataset of perfect control – mechanically stressed sample pairs. Henceforth, to adjust for dependencies among control and/or mechanically stressed samples we added donor as random effect during differential expression analyses. Adding to the validity of this

approach was the fact that we successfully replicated expression changes for ten genes in ten novel independent perfectly paired samples. A final limitation of our study is that we have focused on exploring gene expression changes following mechanical stress and have not studied changes at the protein level. However, we advocate that chondrocyte signaling at the gene expression level is a more sensitive measure of underlying ongoing processes.

### **Conclusions**

To conclude, our results faithfully represent transcriptomic wide consequences of injurious loading in human aged articular cartilage with *MMP13*, IGF binding proteins, and cellular senescence as the most notable results. Since injurious loading is considered a major trigger of OA onset, these findings provide important insight into how injurious stress affects the propensity of aged human articular chondrocytes to lose their steady state towards a debilitating OA disease state. Exploring ways to counteract the initial unbeneficial responses to injurious loading may facilitate clinical development prior to the onset of irreversible damage. Moreover, we advocate that the here identified unique responsive genes to injurious loading, such as *MMP13*, can function as a sensitive marker to strategically develop preventive and/or curative exercise therapy for OA independent of other physiological factors. Preferably such an endeavor would exploit our established *ex vivo* osteochondral model while applying variable mechanical loading regimes.

### **Declarations**

#### ***Ethics approval and consent to participate***

Informed consent was obtained from participants of the RAAK biobank and ethical approval was given by the medical ethics committee of the Leiden University Medical Center (P08.239/P19.013).

#### ***Consent for publication***

Not applicable

#### ***Availability of data and materials***

The list of all significantly affected genes is included in the Supplementary data (Table S3).

#### ***Competing interests***

The authors declare that they have no competing interests

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### ***Authors' contributions***

All authors have made contributions to the completion of this study. Study concept and design: EH, YFM, RCA, IM. Acquisition of material and data: EH, MT, JR, HED, RJPvdW, RGHHN, HM. Data analysis: EH, MT, RCA, IM. Preparation of the manuscript: EH, IM. Critical reviewing and approval of the manuscript: All authors.

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## REFERENCES

1. Englund M, Guermazi A, Gale D, et al. Incidental meniscal findings on knee MRI in middle-aged and elderly persons. *N Engl J Med*. 2008;359(11):1108-15.
2. Jordan JM, Helmick CG, Renner JB, et al. Prevalence of knee symptoms and radiographic and symptomatic knee osteoarthritis in African Americans and Caucasians: the Johnston County Osteoarthritis Project. *J Rheumatol*. 2007;34(1):172-80.
3. Woolf AD, Erwin J, March L. The need to address the burden of musculoskeletal conditions. *Best Pract Res Clin Rheumatol*. 2012;26(2):183-224.
4. Tonge DP, Pearson MJ, Jones SW. The hallmarks of osteoarthritis and the potential to develop personalised disease-modifying pharmacological therapeutics. *Osteoarthritis Cartilage*. 2014;22(5):609-21.
5. Coutinho de Almeida R, Ramos YFM, Mahfouz A, et al. RNA sequencing data integration reveals an miRNA interactome of osteoarthritis cartilage. *Ann Rheum Dis*. 2019;78(2):270-7.
6. Soul J, Dunn SL, Anand S, et al. Stratification of knee osteoarthritis: two major patient subgroups identified by genome-wide expression analysis of articular cartilage. *Ann Rheum Dis*. 2018;77(3):423.
7. Coutinho de Almeida R, Mahfouz A, Mei H, et al. Identification and characterization of two consistent osteoarthritis subtypes by transcriptome and clinical data integration. *Rheumatology (Oxford)*. 2021;60(3):1166-75.
8. Chen CT, Burton-Wurster N, Borden C, Hueffer K, Bloom SE, Lust G. Chondrocyte necrosis and apoptosis in impact damaged articular cartilage. *J Orthop Res*. 2001;19(4):703-11.
9. Lee JH, Fitzgerald JB, Dimicco MA, Grodzinsky AJ. Mechanical injury of cartilage explants causes specific time-dependent changes in chondrocyte gene expression. *Arthritis Rheum*. 2005;52(8):2386-95.
10. Kurz B, Jin M, Patwari P, Cheng DM, Lark MW, Grodzinsky AJ. Biosynthetic response and mechanical properties of articular cartilage after injurious compression. *J Orthop Res*. 2001;19(6):1140-6.
11. Bomer N, Cornelis FM, Ramos YF, et al. The effect of forced exercise on knee joints in *Dio2(-/-)* mice: type II iodothyronine deiodinase-deficient mice are less prone to develop OA-like cartilage damage upon excessive mechanical stress. *Ann Rheum Dis*. 2016;75(3):571-7.
12. Chang JC, Sebastian A, Muruges DK, et al. Global molecular changes in a tibial compression induced ACL rupture model of post-traumatic osteoarthritis. *J Orthop Res*. 2017;35(3):474-85.
13. Sebastian A, McCol JL, Hum NR, et al. Single-Cell RNA-Seq Reveals Transcriptomic Heterogeneity and Post-Traumatic Osteoarthritis-Associated Early Molecular Changes in Mouse Articular Chondrocytes. *Cells*. 2021;10(6).
14. Sebastian A, Muruges DK, Mendez ME, et al. Global Gene Expression Analysis Identifies Age-Related Differences in Knee Joint Transcriptome during the Development of Post-Traumatic Osteoarthritis in Mice. *Int J Mol Sci*. 2020;21(1).
15. Gardiner MD, Vincent TL, Driscoll C, et al. Transcriptional analysis of micro-dissected articular cartilage in post-traumatic murine osteoarthritis. *Osteoarthritis Cartilage*. 2015;23(4):616-28.
16. Appleton CT, Pitelka V, Henry J, Beier F. Global analyses of gene expression in early experimental osteoarthritis. *Arthritis Rheum*. 2007;56(6):1854-68.
17. Ashwell MS, O'Nat AT, Gonda MG, Mente PL. Gene expression profiling of chondrocytes from a porcine impact injury model. *Osteoarthritis Cartilage*. 2008;16(8):936-46.
18. Loeser RF, Olex AL, McNulty MA, et al. Microarray analysis reveals age-related differences in gene expression during the development of osteoarthritis in mice. *Arthritis Rheum*. 2012;64(3):705-17.
19. Loening AM, James IE, Levenston ME, et al. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. *Arch Biochem Biophys*. 2000;381(2):205-12.
20. Houtman E, van Hoolwerff M, Lakenberg N, et al. Human Osteochondral Explants: Reliable Biomimetic Models to Investigate Disease Mechanisms and Develop Personalized Treatments for Osteoarthritis. *Rheumatol Ther*. 2021.
21. Ramos YF, den Hollander W, Bovee JV, et al. Genes involved in the osteoarthritis process identified through genome wide expression analysis in articular cartilage; the RAAK study. *PLoS One*. 2014;9(7):e103056.
22. Sanchez-Adams J, Leddy HA, McNulty AL, O'Connor CJ, Guilak F. The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. *Curr Rheumatol Rep*. 2014;16(10):451.

23. Mankin HJ, Dorfman H, Lippiello L, Zarins A. Biochemical and metabolic abnormalities in articular cartilage from osteo-arthritic human hips. II. Correlation of morphology with biochemical and metabolic data. *J Bone Joint Surg Am.* 1971;53(3):523-37.
24. Farndale RW, Buttle DJ, Barrett AJ. Improved quantitation and discrimination of sulphated glycosaminoglycans by use of dimethyl-methylene blue. *Biochim Biophys Acta.* 1986;883(2):173-7.
25. Wu TD, Watanabe CK. GMAP: a genomic mapping and alignment program for mRNA and EST sequences. *Bioinformatics.* 2005;21(9):1859-75.
26. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics.* 2015;31(2):166-9.
27. Ewels P, Magnusson M, Lundin S, Kaller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics.* 2016;32(19):3047-8.
28. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* 2014;15(12):550.
29. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* 2016;44(W1):W90-7.
30. Szklarczyk D, Gable AL, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Res.* 2019;47(D1):D607-D13.
31. Al-Sabah A, Stadnik P, Gilbert SJ, Duance VC, Blain EJ. Importance of reference gene selection for articular cartilage mechanobiology studies. *Osteoarthritis Cartilage.* 2016;24(4):719-30.
32. McCulloch RS, Ashwell MS, O'Nan AT, Mente PL. Identification of stable normalization genes for quantitative real-time PCR in porcine articular cartilage. *J Anim Sci Biotechnol.* 2012;3(1):36.
33. Diggle P, Liang K-Y, Zeger SL. Analysis of longitudinal data. Oxford New York: Clarendon Press ; Oxford University Press; 1994. xi, 253 p. p.
34. Reynard LN, Barter MJ. Osteoarthritis year in review 2019: genetics, genomics and epigenetics. *Osteoarthritis Cartilage.* 2020;28(3):275-84.
35. Styrkarsdottir U, Lund SH, Thorleifsson G, et al. Meta-analysis of Icelandic and UK data sets identifies missense variants in SMO, IL11, COL11A1 and 13 more new loci associated with osteoarthritis. *Nat Genet.* 2018;50(12):1681-7.
36. Tachmazidou I, Hatzikotoulas K, Southam L, et al. Identification of new therapeutic targets for osteoarthritis through genome-wide analyses of UK Biobank data. *Nat Genet.* 2019;51(2):230-6.
37. Dunn SL, Soul J, Anand S, Schwartz JM, Boot-Handford RP, Hardingham TE. Gene expression changes in damaged osteoarthritic cartilage identify a signature of non-chondrogenic and mechanical responses. *Osteoarthritis Cartilage.* 2016;24(8):1431-40.
38. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. *Endocr Rev.* 2002;23(6):824-54.
39. Duan C, Xu Q. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. *Gen Comp Endocrinol.* 2005;142(1-2):44-52.
40. Baxter RC. IGF binding proteins in cancer: mechanistic and clinical insights. *Nat Rev Cancer.* 2014;14(5):329-41.
41. Clemmons DR. Role of IGF Binding Proteins in Regulating Metabolism. *Trends Endocrinol Metab.* 2016;27(6):375-91.
42. Kato MV. A secreted tumor-suppressor, mac25, with activin-binding activity. *Mol Med.* 2000;6(2):126-35.
43. Esterberg R, Delalande JM, Fritz A. Tailbud-derived Bmp4 drives proliferation and inhibits maturation of zebrafish chordamesoderm. *Development.* 2008;135(23):3891-901.
44. Laursen LS, Overgaard MT, Soe R, et al. Pregnancy-associated plasma protein-A (PAPP-A) cleaves insulin-like growth factor binding protein (IGFBP)-5 independent of IGF: implications for the mechanism of IGFBP-4 proteolysis by PAPP-A. *FEBS Lett.* 2001;504(1-2):36-40.
45. Hou J, Clemmons DR, Smeekens S. Expression and characterization of a serine protease that preferentially cleaves insulin-like growth factor binding protein-5. *J Cell Biochem.* 2005;94(3):470-84.
46. Loechel F, Fox JW, Murphy G, Albrechtsen R, Wewer UM. ADAM 12-S cleaves IGFBP-3 and IGFBP-5 and is inhibited by TIMP-3. *Biochem Biophys Res Commun.* 2000;278(3):511-5.
47. Jepsen MR, Kloverpris S, Mikkelsen JH, et al. Stanniocalcin-2 inhibits mammalian growth by proteolytic inhibition of the insulin-like growth factor axis. *J Biol Chem.* 2015;290(6):3430-9.

48. Cobb LJ, Salih DA, Gonzalez I, et al. Partitioning of IGFBP-5 actions in myogenesis: IGF-independent anti-apoptotic function. *J Cell Sci.* 2004;117(Pt 9):1737-46.
49. Tripathi G, Salih DA, Drozd AC, Cosgrove RA, Cobb LJ, Pell JM. IGF-independent effects of insulin-like growth factor binding protein-5 (Igfbp5) in vivo. *FASEB J.* 2009;23(8):2616-26.
50. Clemmons DR, Busby WH, Jr., Garmonj A, et al. Inhibition of insulin-like growth factor binding protein 5 proteolysis in articular cartilage and joint fluid results in enhanced concentrations of insulin-like growth factor 1 and is associated with improved osteoarthritis. *Arthritis Rheum.* 2002;46(3):694-703.
51. Riegger J, Joos H, Palm HG, et al. Striking a new path in reducing cartilage breakdown: combination of antioxidative therapy and chondroanabolic stimulation after blunt cartilage trauma. *J Cell Mol Med.* 2018;22(1):77-88.
52. Matsuzaki T, Alvarez-Garcia O, Mokuda S, et al. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis. *Sci Transl Med.* 2018;10(428).
53. Wu YH, Liu W, Zhang L, et al. Effects of microRNA-24 targeting C-myc on apoptosis, proliferation, and cytokine expressions in chondrocytes of rats with osteoarthritis via MAPK signaling pathway. *J Cell Biochem.* 2018;119(10):7944-58.
54. Tan P, Guan H, Xie L, et al. FOXO1 inhibits osteoclastogenesis partially by antagonizing MYC. *Sci Rep.* 2015;5:16835.
55. Peck B, Ferber EC, Schulze A. Antagonism between FOXO and MYC Regulates Cellular Powerhouse. *Front Oncol.* 2013;3:96.
56. Basisty N, Kale A, Jeon OH, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol.* 2020;18(1):e3000599.
57. McCulloch K, Litherland GJ, Rai TS. Cellular senescence in osteoarthritis pathology. *Aging Cell.* 2017;16(2):210-8.
58. den Hollander W, Pulyakhina I, Boer C, et al. Annotating Transcriptional Effects of Genetic Variants in Disease-Relevant Tissue: Transcriptome-Wide Allelic Imbalance in Osteoarthritic Cartilage. *Arthritis Rheumatol.* 2019;71(4):561-70.
59. Matsui Y, Hasegawa M, Iino T, Imanaka-Yoshida K, Yoshida T, Sudo A. Tenascin-C Prevents Articular Cartilage Degeneration in Murine Osteoarthritis Models. *Cartilage.* 2018;9(1):80-8.
60. Unno H, Hasegawa M, Suzuki Y, et al. Tenascin-C promotes the repair of cartilage defects in mice. *J Orthop Sci.* 2020;25(2):324-30.

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**Supplementary Figure S1.** Venn diagram of coinciding genes between differentially expressed genes in mechanically stressed versus control cartilage from osteochondral explants (DE<sub>MS</sub>) and previously identified differentially expressed genes in preserved versus lesioned OA cartilage (DE<sub>OA</sub>).

**Supplementary Figure S2.** Protein-protein interaction network in STRING of proteins encoded by differentially expressed genes (N=92 genes) not coinciding with OA pathophysiology (DE<sub>ExclusiveMS</sub>).

**Supplementary Figure S3.** Heat-map of proteins present in SASP.

## Supplementary Tables

**Supplementary Table S1** | [A] Donor characteristics of samples for which RNA was sequenced. [B] Donor characteristics of independent samples used for replication of RNA-sequencing findings.

**A**

	<b>Control (N=8 donors)</b>	<b>65%MS (N=7 donors)</b>	<b>All samples (N=9 donors)</b>
<b>Age (Average ± stdev)</b>	61.88 ± 6.06	64.29 ± 9.05	63.78 ± 8.04
<b>Age (Range)</b>	53-70	53-79	53-79
<b>Sex (M/F)</b>	3/5	3/4	3/6
<b>% Female</b>	63%	57%	67%
<b>BMI (Average ± stdev)</b>	30.36 ± 4.90	28.42 ± 3.68	29.88 ± 4.80
<b>BMI (range)</b>	24.9-39.2	24.9-35.11	24.9-39.2

**B**

	<b>All samples (N=10 donors)</b>
<b>Age (Average ± stdev)</b>	66.90 ± 12.11
<b>Age (Range)</b>	52-85
<b>Sex (M/F)</b>	4/6
<b>% Female</b>	60%
<b>BMI (Average ± stdev)</b>	29.09 ± 4.47
<b>BMI (range)</b>	24.78-38.06

**Supplementary Table S2 | Primer sequences used for replication and validation by RT-qPCR.**

<b>Gene name</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
<i>SDHA</i>	TGGAGCTGCAGAACCTGATG	TGTAGTCTTCCCTGGCATGC
<i>YWHAZ</i>	CTGAGGTTGCAGCTGGTGATGACA	AGCAGGCTTTCTCAGGGGAGTTCA
<i>TNC</i>	TGTCATCTCCTACACAGGCG	TCGAGGTCGGTCAGAGCATA
<i>IGFBP4</i>	ATCGAGGCCATCCAGGAAAG	CTGAAGCTGTTGTTGGGGTG
<i>IGFBP5</i>	GTGCTGTGTACCTGCCAAT	CGTCAACGTACTIONCATGCCT
<i>IGFBP6</i>	GTCTACCGAGGGCTCAAAC	GACTTGCCCATCCGATCCAC
<i>CNTFR</i>	AAGGGCTTCTACTGCAGCTG	CATGTAGCGAATGTGGCAGC
<i>WISP2</i>	ATGAGAGGCACACCGAAGAC	TGGGTACGCACCTTTGAGAG
<i>FRZB</i>	ATTGACTTCCAGCACGAGCC	CGAGTGGCGTACTTGTATGAG
<i>COL9A3</i>	AAGTATCTGCCCGCCAGGTC	TCCCTTGAACCCCTGGCATTTC
<i>GADD45A</i>	GCGAGAACGACATCAACATCC	AATGTGGATTTCGTCACCAGCA
<i>PTGES</i>	GGAAGAAGGCCTTTGCCAAC	AGACGAAGCCAGGAAAAGG

**Supplementary Table S3 | Genes differentially expressed in 65%MS (DE<sub>MS</sub>) cartilage compared to control cartilage of human osteochondral explants.**

Ensembl ID	Gene Name	log2FC	FC	pvalue	FDR
ENSG00000167779	IGFBP6	-2.37	0.19	4.72E-08	3.07E-04
ENSG00000114126	TFDP2	-0.56	0.68	1.07E-07	3.48E-04
ENSG00000100906	NFKBIA	-0.59	0.66	2.51E-07	5.44E-04
ENSG00000064205	WISP2	-1.68	0.31	9.97E-07	1.08E-03
ENSG00000116717	GADD45A	-0.75	0.59	6.73E-07	1.08E-03
ENSG00000171914	TLN2	-1.11	0.46	8.39E-07	1.08E-03
ENSG00000134324	LPIN1	-0.87	0.55	2.79E-06	1.79E-03
ENSG00000140105	WARS	0.53	1.44	2.23E-06	1.79E-03
ENSG00000157514	TSC22D3	-0.77	0.58	2.66E-06	1.79E-03
ENSG00000163453	IGFBP7	-1.24	0.42	2.05E-06	1.79E-03
ENSG00000179051	RCC2	0.90	1.87	3.03E-06	1.79E-03
ENSG00000102760	RGCC	-0.87	0.55	3.57E-06	1.94E-03
ENSG00000196305	IARS	0.43	1.35	4.36E-06	2.18E-03
ENSG00000144908	ALDH1L1	-1.62	0.33	4.78E-06	2.22E-03
ENSG00000068383	INPP5A	-0.77	0.58	5.60E-06	2.43E-03
ENSG00000126803	HSPA2	-0.85	0.55	7.74E-06	2.65E-03
ENSG00000143416	SELENBP1	-1.09	0.47	7.67E-06	2.65E-03
ENSG00000148498	PARD3	-0.78	0.58	6.56E-06	2.65E-03
ENSG00000159461	AMFR	-0.54	0.69	6.98E-06	2.65E-03
ENSG00000204103	MAFB	1.57	2.97	1.43E-05	4.66E-03
ENSG00000119408	NEK6	1.18	2.26	1.91E-05	5.86E-03
ENSG00000142871	CYR61	-0.65	0.64	1.98E-05	5.86E-03
ENSG00000138356	AOX1	-1.06	0.48	2.18E-05	6.10E-03
ENSG00000155324	GRAMD2B	-0.80	0.58	2.25E-05	6.10E-03
ENSG00000107736	CDH23	-1.44	0.37	2.72E-05	6.88E-03
ENSG00000150907	FOXO1	-0.68	0.62	2.86E-05	6.88E-03
ENSG00000163686	ABHD6	-1.05	0.48	2.96E-05	6.88E-03
ENSG00000167191	GPRC5B	-0.87	0.55	2.94E-05	6.88E-03
ENSG00000100612	DHRS7	-0.48	0.71	3.97E-05	7.81E-03
ENSG00000115461	IGFBP5	2.59	6.01	3.54E-05	7.81E-03
ENSG00000115468	EFHD1	-0.80	0.57	3.73E-05	7.81E-03
ENSG00000134107	BHLHE40	1.00	2.00	4.08E-05	7.81E-03
ENSG00000146122	DAAM2	-0.95	0.52	4.08E-05	7.81E-03
ENSG00000173641	HSPB7	-0.89	0.54	3.81E-05	7.81E-03
ENSG00000135069	PSAT1	1.03	2.04	4.52E-05	8.29E-03
ENSG00000148344	PTGES	1.55	2.92	4.58E-05	8.29E-03
ENSG00000166348	USP54	-0.76	0.59	4.79E-05	8.43E-03

ENSG00000139514	SLC7A1	0.57	1.48	4.93E-05	8.44E-03
ENSG00000041982	TNC	1.48	2.80	5.15E-05	8.51E-03
ENSG00000162998	FRZB	-1.63	0.32	5.23E-05	8.51E-03
ENSG00000132970	WASF3	-0.41	0.75	5.36E-05	8.51E-03
ENSG00000103257	SLC7A5	0.89	1.86	6.44E-05	9.96E-03
ENSG00000104324	CPQ	-0.46	0.72	6.58E-05	9.96E-03
ENSG00000166165	CKB	-1.39	0.38	7.19E-05	1.04E-02
ENSG00000168918	INPP5D	-1.25	0.42	7.13E-05	1.04E-02
ENSG00000129757	CDKN1C	-1.23	0.43	7.90E-05	1.09E-02
ENSG00000143878	RHOB	-0.61	0.66	8.05E-05	1.09E-02
ENSG00000183864	TOB2	-0.71	0.61	7.96E-05	1.09E-02
ENSG00000053747	LAMA3	-1.15	0.45	9.40E-05	1.25E-02
ENSG00000145246	ATP10D	-0.49	0.71	9.96E-05	1.30E-02
ENSG00000101825	MXRA5	1.21	2.32	1.11E-04	1.42E-02
ENSG00000122756	CNTFR	-1.88	0.27	1.15E-04	1.44E-02
ENSG00000117020	AKT3	-0.44	0.74	1.26E-04	1.44E-02
ENSG00000134954	ETS1	0.46	1.38	1.26E-04	1.44E-02
ENSG00000165966	PDZRN4	-1.97	0.26	1.26E-04	1.44E-02
ENSG00000169116	PARM1	-1.08	0.47	1.26E-04	1.44E-02
ENSG00000187720	THSD4	-1.53	0.35	1.18E-04	1.44E-02
ENSG00000113739	STC2	-1.07	0.48	1.39E-04	1.53E-02
ENSG00000180354	MTURN	-0.60	0.66	1.37E-04	1.53E-02
ENSG00000185630	PBX1	-0.70	0.62	1.41E-04	1.53E-02
ENSG00000117151	CTBS	-0.44	0.73	1.53E-04	1.63E-02
ENSG00000136997	MYC	0.79	1.73	1.56E-04	1.64E-02
ENSG00000080546	SESN1	-0.84	0.56	1.78E-04	1.84E-02
ENSG00000198755	RPL10A	0.34	1.26	1.88E-04	1.91E-02
ENSG00000080298	RFX3	-0.58	0.67	1.99E-04	1.99E-02
ENSG00000114166	KAT2B	-0.65	0.64	2.09E-04	2.06E-02
ENSG00000127528	KLF2	-1.18	0.44	2.15E-04	2.09E-02
ENSG00000106991	ENG	0.59	1.50	2.31E-04	2.21E-02
ENSG00000100242	SUN2	-0.82	0.57	2.39E-04	2.25E-02
ENSG00000100029	PES1	0.44	1.36	2.49E-04	2.28E-02
ENSG00000185201	IFITM2	0.72	1.65	2.46E-04	2.28E-02
ENSG00000111640	GAPDH	0.58	1.49	2.53E-04	2.29E-02
ENSG00000173848	NET1	-0.90	0.54	2.65E-04	2.36E-02
ENSG00000138336	TET1	-0.47	0.72	2.75E-04	2.42E-02
ENSG00000101782	RIOK3	-0.44	0.74	2.81E-04	2.43E-02
ENSG00000159322	ADPGK	0.35	1.27	2.83E-04	2.43E-02
ENSG00000111885	MAN1A1	-0.99	0.50	2.94E-04	2.48E-02

ENSG00000089220	PEBP1	-0.62	0.65	3.04E-04	2.54E-02
ENSG00000005022	SLC25A5	0.48	1.40	3.16E-04	2.56E-02
ENSG00000137699	TRIM29	-1.13	0.46	3.18E-04	2.56E-02
ENSG00000166033	HTRA1	0.95	1.93	3.17E-04	2.56E-02
ENSG00000184205	TSPYL2	-0.75	0.60	3.42E-04	2.71E-02
ENSG00000143382	ADAMTSL4	-0.66	0.63	3.58E-04	2.81E-02
ENSG00000062716	VMP1	0.60	1.52	3.89E-04	3.01E-02
ENSG00000067141	NEO1	-0.80	0.58	4.24E-04	3.10E-02
ENSG00000078596	ITM2A	-1.00	0.50	4.18E-04	3.10E-02
ENSG00000096060	FKBP5	-0.64	0.64	4.07E-04	3.10E-02
ENSG00000134109	EDEM1	0.33	1.26	4.20E-04	3.10E-02
ENSG00000148180	GSN	-0.60	0.66	4.16E-04	3.10E-02
ENSG00000157600	TMEM164	-0.73	0.60	4.28E-04	3.10E-02
ENSG00000143164	DCAF6	-0.22	0.86	4.34E-04	3.10E-02
ENSG00000071205	ARHGAP10	-0.47	0.72	4.57E-04	3.14E-02
ENSG00000079691	CARMIL1	-0.55	0.68	4.51E-04	3.14E-02
ENSG00000131844	MCCC2	-0.89	0.54	4.58E-04	3.14E-02
ENSG00000168874	ATOH8	-0.91	0.53	4.53E-04	3.14E-02
ENSG00000106624	AEBP1	0.79	1.73	4.98E-04	3.31E-02
ENSG00000154380	ENAH	0.53	1.44	4.97E-04	3.31E-02
ENSG00000253293	HOXA10	-0.36	0.78	4.97E-04	3.31E-02
ENSG00000006016	CRLF1	0.76	1.69	5.15E-04	3.38E-02
ENSG00000106366	SERPINE1	1.02	2.03	5.33E-04	3.47E-02
ENSG00000141753	IGFBP4	1.38	2.59	5.44E-04	3.50E-02
ENSG00000179532	DNHD1	-0.51	0.70	5.53E-04	3.53E-02
ENSG00000106258	CYP3A5	-1.40	0.38	5.74E-04	3.62E-02
ENSG00000082196	C1QTNF3	-1.73	0.30	5.86E-04	3.64E-02
ENSG00000164106	SCRG1	-0.89	0.54	5.87E-04	3.64E-02
ENSG00000151332	MBIP	-0.40	0.76	6.10E-04	3.71E-02
ENSG00000187840	EIF4EBP1	1.06	2.09	6.04E-04	3.71E-02
ENSG00000132824	SERINC3	-0.32	0.80	6.54E-04	3.91E-02
ENSG00000135506	OS9	-0.33	0.80	6.48E-04	3.91E-02
ENSG00000148848	ADAM12	0.62	1.54	6.63E-04	3.92E-02
ENSG00000013016	EHD3	0.42	1.34	6.75E-04	3.93E-02
ENSG00000101439	CST3	-0.74	0.60	6.77E-04	3.93E-02
ENSG00000179941	BBS10	-0.57	0.67	6.83E-04	3.93E-02
ENSG00000104714	ERICH1	-0.46	0.73	7.07E-04	3.93E-02
ENSG00000106351	AGFG2	-0.68	0.63	6.97E-04	3.93E-02
ENSG00000158825	CDA	-1.07	0.48	7.07E-04	3.93E-02
ENSG00000184557	SOCS3	0.78	1.71	7.07E-04	3.93E-02

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ENSG00000162627	SNX7	-0.56	0.68	7.13E-04	3.93E-02
ENSG00000136295	TTYH3	1.05	2.08	7.27E-04	3.97E-02
ENSG00000092445	TYRO3	0.77	1.71	7.45E-04	4.04E-02
ENSG00000010278	CD9	-0.80	0.57	7.82E-04	4.11E-02
ENSG00000049860	HEXB	-0.46	0.73	7.79E-04	4.11E-02
ENSG00000165795	NDRG2	-0.72	0.61	7.65E-04	4.11E-02
ENSG00000170891	CYTL1	-1.65	0.32	7.72E-04	4.11E-02
ENSG00000187514	PTMA	0.49	1.40	8.00E-04	4.17E-02
ENSG00000112559	MDFI	0.76	1.69	8.45E-04	4.36E-02
ENSG00000159307	SCUBE1	-0.93	0.53	8.50E-04	4.36E-02
ENSG00000144857	BOC	-1.08	0.47	8.75E-04	4.45E-02
ENSG00000131067	GGT7	-0.54	0.69	8.90E-04	4.45E-02
ENSG00000171150	SOCS5	-0.40	0.76	8.85E-04	4.45E-02
ENSG00000112306	RPS12	0.26	1.19	9.15E-04	4.54E-02
ENSG00000119938	PPP1R3C	-0.71	0.61	9.54E-04	4.68E-02
ENSG00000164237	CMBL	-0.57	0.67	9.56E-04	4.68E-02
ENSG00000090530	P3H2	-1.17	0.44	1.02E-03	4.71E-02
ENSG00000106617	PRKAG2	-0.51	0.70	1.01E-03	4.71E-02
ENSG00000112118	MCM3	0.39	1.31	1.01E-03	4.71E-02
ENSG00000117868	ESYT2	-0.18	0.88	9.79E-04	4.71E-02
ENSG00000141258	SGSM2	-0.87	0.55	1.01E-03	4.71E-02
ENSG00000143127	ITGA10	-0.47	0.72	9.98E-04	4.71E-02
ENSG00000149485	FADS1	-0.65	0.64	1.04E-03	4.71E-02
ENSG00000162804	SNED1	0.87	1.83	1.04E-03	4.71E-02
ENSG00000169184	MN1	-0.80	0.58	1.04E-03	4.71E-02
ENSG00000169902	TPST1	0.36	1.28	1.04E-03	4.71E-02
ENSG00000185634	SHC4	1.05	2.07	9.91E-04	4.71E-02
ENSG00000130635	COL5A1	0.96	1.95	1.06E-03	4.78E-02
ENSG00000004799	PDK4	-0.88	0.54	1.13E-03	4.84E-02
ENSG00000105974	CAV1	-0.51	0.70	1.12E-03	4.84E-02
ENSG00000131389	SLC6A6	0.98	1.97	1.12E-03	4.84E-02
ENSG00000137745	MMP13	2.38	5.19	1.13E-03	4.84E-02
ENSG00000172348	RCAN2	-0.88	0.54	1.10E-03	4.84E-02
ENSG00000172493	AFF1	-0.39	0.76	1.09E-03	4.84E-02
ENSG00000187151	ANGPTL5	-1.18	0.44	1.11E-03	4.84E-02
ENSG00000100814	CCNB1IP1	-0.33	0.80	1.14E-03	4.84E-02
ENSG00000092758	COL9A3	-1.62	0.32	1.16E-03	4.89E-02
ENSG00000160111	CPAMD8	-1.37	0.39	1.19E-03	4.98E-02
ENSG00000170776	AKAP13	-0.41	0.75	1.19E-03	4.98E-02

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Legend: Log<sub>2</sub>FC, log<sub>2</sub> fold change; FC, fold change; FDR, False discovery rate

**Supplementary Table S4 | Gene enrichment found in Enrichr.** Enrichment for [A] 156 DE<sub>MS</sub> genes in and [B] 92 DE<sub>ExclusiveMS</sub> for the gene ontology terms: biological process, molecular function and cellular component 2018, and pathways: KEGG 2019 human and reactome.

**A**

Term	Overlap	P-value	Adj P-value	Odds Ratio	Combined Score	Genes
endoplasmic reticulum lumen (GO:0005788)	12/270	1.44E-06	6.42E-04	5.70	76.64	CST3;IGFBP5;COL5A1;OS9;IGFBP4;ADAMTSL4;STC2;TNC;P3H2;IGFBP7;COL9A3;CYR61
negative regulation of cellular process (GO:0048523)	17/534	9.82E-07	5.01E-03	4.08	56.46	TSPYL2;CDA;WARS;-CAV1;P3H2;ETS1;-CYR61;WISP2;RHOB;KAT2B;RGCC;FRZB;MYC;BHLHE40;CD9;IGFBP7;IGFBP6
Cellular senescence	8/160	3.75E-05	1.15E-02	6.41	65.33	GADD45A;MYC;SERPINE1;AKT3;EIF4EBP1;SLC25A5;ETS1;-FOXO1
protein kinase regulator activity (GO:0019887)	7/107	2.08E-05	1.20E-02	8.39	90.40	CDKN1C;SOCS3;GPRC5B;RGCC;M-BIP;PRKAG2;SOCS5
Insulin resistance	6/108	2.03E-04	1.25E-02	7.12	60.55	NFKBIA;SOCS3;PPP1R3C;AKT3;PRKAG2;FOXO1
Focal adhesion	8/199	1.72E-04	1.33E-02	5.15	44.67	SHC4;CAV1;ITGA10;AKT3;LAMA3;TNC;COL9A3;TLN2
Longevity regulating pathway	6/102	1.49E-04	1.53E-02	7.54	66.47	SESN1;AKT3;EIF4EBP1;PRKAG2;HSPA2;FOXO1
Insulin signaling pathway	7/137	1.01E-04	1.56E-02	6.55	60.26	SHC4;SOCS3;PPP1R3C;AKT3;EIF4EBP1;PRKAG2;FOXO1
Chronic myeloid leukemia	5/76	3.20E-04	1.64E-02	8.43	67.87	SHC4;NFKBIA;GADD45A;MYC;AKT3
insulin-like growth factor II binding (GO:0031995)	3/7	1.59E-05	1.83E-02	54.95	607.02	IGFBP5;IGFBP4;IGFBP6
focal adhesion (GO:0005925)	11/356	1.14E-04	2.54E-02	3.96	35.97	ENAH;EHD3;GSN;CAV1;TNC;CD9;TLN2;RPL10A;DCAF6;RHOB;ENG
insulin-like growth factor I binding (GO:0031994)	3/13	1.26E-04	2.89E-02	29.59	265.73	IGFBP5;IGFBP4;IGFBP6
insulin-like growth factor binding (GO:0005520)	3/14	1.59E-04	3.05E-02	27.47	240.28	IGFBP5;IGFBP4;IGFBP6
kinase binding (GO:0019900)	12/418	1.11E-04	3.20E-02	3.68	33.51	SHC4;KAT2B;GPRC5B;RGCC;WARS;GADD45A;CAV1;RC-C2;NEK6;PEBP1;PRKAG2;RHOB
extracellular matrix organization (GO:0030198)	10/229	1.30E-05	3.31E-02	5.60	62.99	SCUBE1;MMP13;COL5A1;ITGA10;-SERPINE1;ADAM12;LAMA3;TNC;HTRA1;COL9A3
Vitamin B6 metabolism	2/6	8.88E-04	3.42E-02	42.74	300.26	PSAT1;AOX1

Small cell lung cancer	5/93	8.10E-04	3.56E-02	6.89	49.06	NFKBIA;GADD45A;MYC;AKT3;LAMA3
kinase inhibitor activity (GO:0019210)	5/59	9.65E-05	3.70E-02	10.86	100.45	CDKN1C;SOCS3;WARS;MBIP;SOCS5
protein alpha-1,2-de-mannosylation (GO:0036508)	4/24	3.35E-05	4.27E-02	21.37	220.16	OS9;EDEM1;AMFR;MAN1A1
negative regulation of cell proliferation (GO:0008285)	12/363	2.88E-05	4.90E-02	4.24	44.31	CDKN1C;KAT2B;RGCC;WARS;IGFBP5;FRZB;MYC;BHLHE40;P3H2;IGFBP7;IGFBP6;ETS1

**B**

<b>Term</b>	<b>Overlap</b>	<b>P-value</b>	<b>Adj P-value</b>	<b>Odds Ratio</b>	<b>Combined Score</b>	<b>Genes</b>
kinase inhibitor activity (GO:0019210)	5/59	7.59E-06	8.74E-03	18.42	217.17	CDKN1C; SOCS3;WARS;MBIP ;SOCS5
focal adhesion (GO:0005925)	9/356	3.84E-05	1.71E-02	5.50	55.88	ENAH;EHD3;GSN;C-D9;TLN2;RPL10A;DCAF6;RHOB;ENG

**Supplementary Table S5 | DE<sub>MS</sub> genes coinciding with previously reported DE genes in OA pathophysiology (DE<sub>OA</sub>). [A] DE<sub>MS</sub> genes with same direction of effect as DE<sub>OA</sub> genes. [B] DE<sub>MS</sub> genes with opposite direction of effect as DE<sub>OA</sub> genes.**

A	65%MS versus control cartilage (n=14/ group)				Lesioned versus Preserved OA cartilage (n=35/ group)			
	Gene Name	log- 2FC	FC	pvalue	FDR	log- 2FC	FC	pvalue
<i>TFDP2</i>	-0.56	0.68	1.07E-07	3.48E-04	-0.23	0.85	2.02E-03	2.27E-02
<i>WISP2</i>	-1.68	0.31	9.97E-07	1.08E-03	-1.20	0.44	1.48E-04	3.06E-03
<i>ALDH1L1</i>	-1.62	0.33	4.78E-06	2.22E-03	-1.02	0.49	5.56E-09	7.24E-07
<i>INPP5A</i>	-0.77	0.58	5.60E-06	2.43E-03	-0.31	0.81	1.69E-03	1.99E-02
<i>GRAMD2B</i>	-0.80	0.58	2.25E-05	6.10E-03	-0.30	0.81	1.18E-04	2.54E-03
<i>GPRC5B</i>	-0.87	0.55	2.94E-05	6.88E-03	-1.02	0.49	2.10E-09	3.32E-07
<i>DAAM2</i>	-0.95	0.52	4.08E-05	7.81E-03	-0.50	0.71	2.40E-04	4.48E-03
<i>EFHD1</i>	-0.80	0.57	3.73E-05	7.81E-03	-0.40	0.76	2.37E-03	2.54E-02
<i>FRZB</i>	-1.63	0.32	5.23E-05	8.51E-03	-1.88	0.27	4.07E-12	1.87E-09
<i>TOB2</i>	-0.71	0.61	7.96E-05	1.09E-02	-0.26	0.84	4.72E-05	1.22E-03
<i>CNTFR</i>	-1.88	0.27	1.15E-04	1.44E-02	-1.69	0.31	3.32E-12	1.56E-09
<i>PDZRN4</i>	-1.97	0.26	1.26E-04	1.44E-02	-1.37	0.39	2.70E-05	7.76E-04
<i>PARM1</i>	-1.08	0.47	1.26E-04	1.44E-02	-0.65	0.64	2.63E-05	7.61E-04
<i>STC2</i>	-1.07	0.48	1.39E-04	1.53E-02	-1.03	0.49	1.05E-08	1.24E-06
<i>MTURN</i>	-0.60	0.66	1.37E-04	1.53E-02	-0.42	0.75	3.76E-06	1.58E-04
<i>KAT2B</i>	-0.65	0.64	2.09E-04	2.06E-02	-0.33	0.80	1.33E-03	1.66E-02
<i>ADAMTSL4</i>	-0.66	0.63	3.58E-04	2.81E-02	-0.42	0.75	1.26E-03	1.61E-02
<i>ITM2A</i>	-1.00	0.50	4.18E-04	3.10E-02	-0.60	0.66	8.86E-05	2.02E-03
<i>NEO1</i>	-0.80	0.58	4.24E-04	3.10E-02	-0.32	0.80	1.38E-03	1.71E-02
<i>FKBP5</i>	-0.64	0.64	4.07E-04	3.10E-02	-0.20	0.87	5.39E-03	4.59E-02
<i>ATOX1</i>	-0.91	0.53	4.53E-04	3.14E-02	-0.48	0.72	1.67E-03	1.97E-02
<i>HOXA10</i>	-0.36	0.78	4.97E-04	3.31E-02	-0.36	0.78	2.32E-04	4.37E-03
<i>AGFG2</i>	-0.68	0.63	6.97E-04	3.93E-02	-0.30	0.81	5.47E-03	4.64E-02
<i>NDRG2</i>	-0.72	0.61	7.65E-04	4.11E-02	-0.70	0.62	1.67E-08	1.83E-06
<i>CYTL1</i>	-1.65	0.32	7.72E-04	4.11E-02	-0.46	0.73	4.94E-03	4.31E-02
<i>SCUBE1</i>	-0.93	0.53	8.50E-04	4.36E-02	-1.26	0.42	2.03E-08	2.15E-06
<i>BOC</i>	-1.08	0.47	8.75E-04	4.45E-02	-0.70	0.62	3.96E-07	2.54E-05
<i>CMBL</i>	-0.57	0.67	9.56E-04	4.68E-02	-0.41	0.75	1.34E-07	1.02E-05
<i>RCAN2</i>	-0.88	0.54	1.10E-03	4.84E-02	-2.03	0.25	8.20E-15	9.19E-12
<i>CPAMD8</i>	-1.37	0.39	1.19E-03	4.98E-02	-1.19	0.44	2.90E-09	4.23E-07
<i>IARS</i>	0.43	1.35	4.36E-06	2.18E-03	0.40	1.32	1.35E-04	2.85E-03
<i>BHLHE40</i>	1.00	2.00	4.08E-05	7.81E-03	0.30	1.23	4.89E-04	7.78E-03
<i>PSAT1</i>	1.03	2.04	4.52E-05	8.29E-03	0.77	1.70	1.14E-03	1.49E-02

<i>PTGES</i>	1.55	2.92	4.58E-05	8.29E-03	1.61	3.06	2.33E-15	3.61E-12
<i>SLC7A1</i>	0.57	1.48	4.93E-05	8.44E-03	0.53	1.44	1.43E-06	7.10E-05
<i>TNC</i>	1.48	2.80	5.15E-05	8.51E-03	0.50	1.41	7.53E-04	1.09E-02
<i>SLC7A5</i>	0.89	1.86	6.44E-05	9.96E-03	0.99	1.99	2.91E-10	6.25E-08
<i>MXRA5</i>	1.21	2.32	1.11E-04	1.42E-02	0.53	1.44	3.42E-04	5.94E-03
<i>HTRA1</i>	0.95	1.93	3.17E-04	2.56E-02	1.26	2.39	1.80E-14	1.65E-11
<i>EDEM1</i>	0.33	1.26	4.20E-04	3.10E-02	0.23	1.17	2.38E-03	2.55E-02
<i>CRLF1</i>	0.76	1.69	5.15E-04	3.38E-02	1.60	3.04	4.59E-13	2.96E-10
<i>SERPINE1</i>	1.02	2.03	5.33E-04	3.47E-02	1.57	2.97	3.22E-13	2.24E-10
<i>IGFBP4</i>	1.38	2.59	5.44E-04	3.50E-02	0.67	1.60	2.27E-03	2.47E-02
<i>ADAM12</i>	0.62	1.54	6.63E-04	3.92E-02	0.99	1.98	4.58E-06	1.85E-04
<i>TYRO3</i>	0.77	1.71	7.45E-04	4.04E-02	0.94	1.92	1.19E-11	5.00E-09
<i>SHC4</i>	1.05	2.07	9.91E-04	4.71E-02	1.07	2.10	1.43E-11	5.87E-09
<i>COL5A1</i>	0.96	1.95	1.06E-03	4.78E-02	0.46	1.38	4.94E-03	4.31E-02
<i>SLC6A6</i>	0.98	1.97	1.12E-03	4.84E-02	0.60	1.51	1.13E-04	2.45E-03

Legend: Log2FC, log2 fold change; FC, fold change; FDR, False discovery rate

<b>B</b>	<b>Control versus 65% MS cartilage (n=14/group)</b>				<b>Lesioned versus Preserved OA cartilage (n=35/ group)</b>			
	<b>Gene Name</b>	<b>log2FC</b>	<b>FC</b>	<b>pvalue</b>	<b>FDR</b>	<b>log2FC</b>	<b>FC</b>	<b>pvalue</b>
<i>TMEM164</i>	-0.73	0.60	4.28E-04	3.10E-02	0.24	1.18	5.09E-03	4.41E-02
<i>FOXO1</i>	-0.68	0.62	2.86E-05	6.88E-03	0.29	1.22	6.52E-04	9.72E-03
<i>CAV1</i>	-0.51	0.70	1.12E-03	4.84E-02	0.33	1.25	4.58E-03	4.07E-02
<i>AKAP13</i>	-0.41	0.75	1.19E-03	4.98E-02	0.33	1.26	1.11E-04	2.42E-03
<i>CDA</i>	-1.07	0.48	7.07E-04	3.93E-02	0.39	1.31	2.31E-03	2.50E-02
<i>IGFBP7</i>	-1.24	0.42	2.05E-06	1.79E-03	0.47	1.38	3.42E-03	3.30E-02
<i>RGCC</i>	-0.87	0.55	3.57E-06	1.94E-03	0.47	1.39	1.59E-05	5.13E-04
<i>AKT3</i>	-0.44	0.74	1.26E-04	1.44E-02	0.52	1.43	1.01E-04	2.25E-03
<i>SNX7</i>	-0.56	0.68	7.13E-04	3.93E-02	0.53	1.45	3.24E-04	5.67E-03
<i>C1QTNF3</i>	-1.73	0.30	5.86E-04	3.64E-02	0.60	1.52	9.93E-04	1.35E-02
<i>CKB</i>	-1.39	0.38	7.19E-05	1.04E-02	0.63	1.55	1.37E-03	1.70E-02
<i>TSC22D3</i>	-0.77	0.58	2.66E-06	1.79E-03	0.67	1.59	2.81E-09	4.16E-07
<i>TRIM29</i>	-1.13	0.46	3.18E-04	2.56E-02	0.78	1.72	1.48E-05	4.85E-04
<i>P3H2</i>	-1.17	0.44	1.02E-03	4.71E-02	1.69	3.23	0.00E+00	0.00E+00
<i>MAFB</i>	1.57	2.97	1.43E-05	4.66E-03	-0.69	0.62	1.32E-03	1.66E-02
<i>MDFI</i>	0.76	1.69	8.45E-04	4.36E-02	-0.42	0.75	5.76E-03	4.82E-02

Legend: Log2FC, log2 fold change; FC, fold change; FDR, False discovery rate

**Supplementary Table S6 | Exclusive mechanical response genes (DE<sub>ExclusiveMS</sub>).**

Gene name	log2FC	FC	pvalue	FDR
IGFBP6	-2.37	0.19	4.72E-08	3.07E-04
NFKBIA	-0.59	0.66	2.51E-07	5.44E-04
TLN2	-1.11	0.46	8.39E-07	1.08E-03
GADD45A	-0.75	0.59	6.73E-07	1.08E-03
RCC2	0.90	1.87	3.03E-06	1.79E-03
WARS	0.53	1.44	2.23E-06	1.79E-03
LPIN1	-0.87	0.55	2.79E-06	1.79E-03
AMFR	-0.54	0.69	6.98E-06	2.65E-03
PARD3	-0.78	0.58	6.56E-06	2.65E-03
SELENBP1	-1.09	0.47	7.67E-06	2.65E-03
HSPA2	-0.85	0.55	7.74E-06	2.65E-03
CYR61	-0.65	0.64	1.98E-05	5.86E-03
NEK6	1.18	2.26	1.91E-05	5.86E-03
AOX1	-1.06	0.48	2.18E-05	6.10E-03
ABHD6	-1.05	0.48	2.96E-05	6.88E-03
CDH23	-1.44	0.37	2.72E-05	6.88E-03
HSPB7	-0.89	0.54	3.81E-05	7.81E-03
IGFBP5	2.59	6.01	3.54E-05	7.81E-03
DHRS7	-0.48	0.71	3.97E-05	7.81E-03
USP54	-0.76	0.59	4.79E-05	8.43E-03
WASF3	-0.41	0.75	5.36E-05	8.51E-03
CPQ	-0.46	0.72	6.58E-05	9.96E-03
INPP5D	-1.25	0.42	7.13E-05	1.04E-02
RHOB	-0.61	0.66	8.05E-05	1.09E-02
CDKN1C	-1.23	0.43	7.90E-05	1.09E-02
LAMA3	-1.15	0.45	9.40E-05	1.25E-02
ATP10D	-0.49	0.71	9.96E-05	1.30E-02
THSD4	-1.53	0.35	1.18E-04	1.44E-02
ETS1	0.46	1.38	1.26E-04	1.44E-02
PBX1	-0.70	0.62	1.41E-04	1.53E-02
CTBS	-0.44	0.73	1.53E-04	1.63E-02
MYC	0.79	1.73	1.56E-04	1.64E-02
SESN1	-0.84	0.56	1.78E-04	1.84E-02
RPL10A	0.34	1.26	1.88E-04	1.91E-02
RFX3	-0.58	0.67	1.99E-04	1.99E-02
KLF2	-1.18	0.44	2.15E-04	2.09E-02
ENG	0.59	1.50	2.31E-04	2.21E-02

SUN2	-0.82	0.57	2.39E-04	2.25E-02
IFITM2	0.72	1.65	2.46E-04	2.28E-02
PES1	0.44	1.36	2.49E-04	2.28E-02
GAPDH	0.58	1.49	2.53E-04	2.29E-02
NET1	-0.90	0.54	2.65E-04	2.36E-02
TET1	-0.47	0.72	2.75E-04	2.42E-02
ADPGK	0.35	1.27	2.83E-04	2.43E-02
RIOK3	-0.44	0.74	2.81E-04	2.43E-02
MAN1A1	-0.99	0.50	2.94E-04	2.48E-02
PEBP1	-0.62	0.65	3.04E-04	2.54E-02
SLC25A5	0.48	1.40	3.16E-04	2.56E-02
TSPYL2	-0.75	0.60	3.42E-04	2.71E-02
VMP1	0.60	1.52	3.89E-04	3.01E-02
GSN	-0.60	0.66	4.16E-04	3.10E-02
DCAF6	-0.22	0.86	4.34E-04	3.10E-02
MCCC2	-0.89	0.54	4.58E-04	3.14E-02
CARMIL1	-0.55	0.68	4.51E-04	3.14E-02
ARHGAP10	-0.47	0.72	4.57E-04	3.14E-02
ENAH	0.53	1.44	4.97E-04	3.31E-02
AEBP1	0.79	1.73	4.98E-04	3.31E-02
DNHD1	-0.51	0.70	5.53E-04	3.53E-02
CYP3A5	-1.40	0.38	5.74E-04	3.62E-02
SCRG1	-0.89	0.54	5.87E-04	3.64E-02
EIF4EBP1	1.06	2.09	6.04E-04	3.71E-02
MBIP	-0.40	0.76	6.10E-04	3.71E-02
OS9	-0.33	0.80	6.48E-04	3.91E-02
SERINC3	-0.32	0.80	6.54E-04	3.91E-02
BBS10	-0.57	0.67	6.83E-04	3.93E-02
CST3	-0.74	0.60	6.77E-04	3.93E-02
EHD3	0.42	1.34	6.75E-04	3.93E-02
SOCS3	0.78	1.71	7.07E-04	3.93E-02
ERICH1	-0.46	0.73	7.07E-04	3.93E-02
TTYH3	1.05	2.08	7.27E-04	3.97E-02
HEXB	-0.46	0.73	7.79E-04	4.11E-02
CD9	-0.80	0.57	7.82E-04	4.11E-02
PTMA	0.49	1.40	8.00E-04	4.17E-02
SOCS5	-0.40	0.76	8.85E-04	4.45E-02
GGT7	-0.54	0.69	8.90E-04	4.45E-02
RPS12	0.26	1.19	9.15E-04	4.54E-02

PPP1R3C	-0.71	0.61	9.54E-04	4.68E-02
TPST1	0.36	1.28	1.04E-03	4.71E-02
MN1	-0.80	0.58	1.04E-03	4.71E-02
SNED1	0.87	1.83	1.04E-03	4.71E-02
FADS1	-0.65	0.64	1.04E-03	4.71E-02
ITGA10	-0.47	0.72	9.98E-04	4.71E-02
SGSM2	-0.87	0.55	1.01E-03	4.71E-02
ESYT2	-0.18	0.88	9.79E-04	4.71E-02
MCM3	0.39	1.31	1.01E-03	4.71E-02
PRKAG2	-0.51	0.70	1.01E-03	4.71E-02
ANGPTL5	-1.18	0.44	1.11E-03	4.84E-02
AFF1	-0.39	0.76	1.09E-03	4.84E-02
MMP13	2.38	5.19	1.13E-03	4.84E-02
PDK4	-0.88	0.54	1.13E-03	4.84E-02
CCNB1IP1	-0.33	0.80	1.14E-03	4.84E-02
COL9A3	-1.62	0.32	1.16E-03	4.89E-02

Legend: Log2FC, log2 fold change; FC, fold change; FDR, False discovery rate

**Supplementary Table S7 | Previously reported OA risk loci present in our DE gene dataset.**

Gene name	Control versus 65%MS cartilage (n=14/group)		Lesioned versus Pre-served OA cartilage (N=35/group)		Risk Loci (GWAS)		
	FC	FDR	FC	FDR	Risk SNPs	OR	Suggested mechanism
<i>TNC</i>	2.80	8.51E-03	1.41	1.09E-02	rs13321, rs2480930 and rs1330349	1.09	Allelic expression imbalance (AEI)
<i>SCUBE1</i>	0.53	4.36E-02	0.42	2.15E-06	rs528981060	1.68	

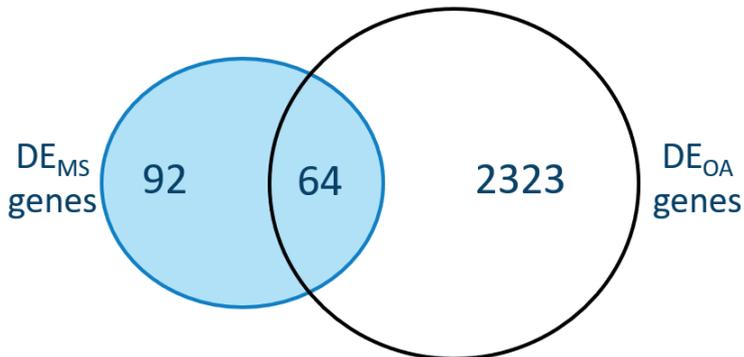
Legend: Log2FC, log2 fold change; FC, fold change; FDR, False discovery rate

**Supplementary Table S8 | All insulin growth factor binding proteins (IGFBPs) and related DE genes identified in our analysis.**

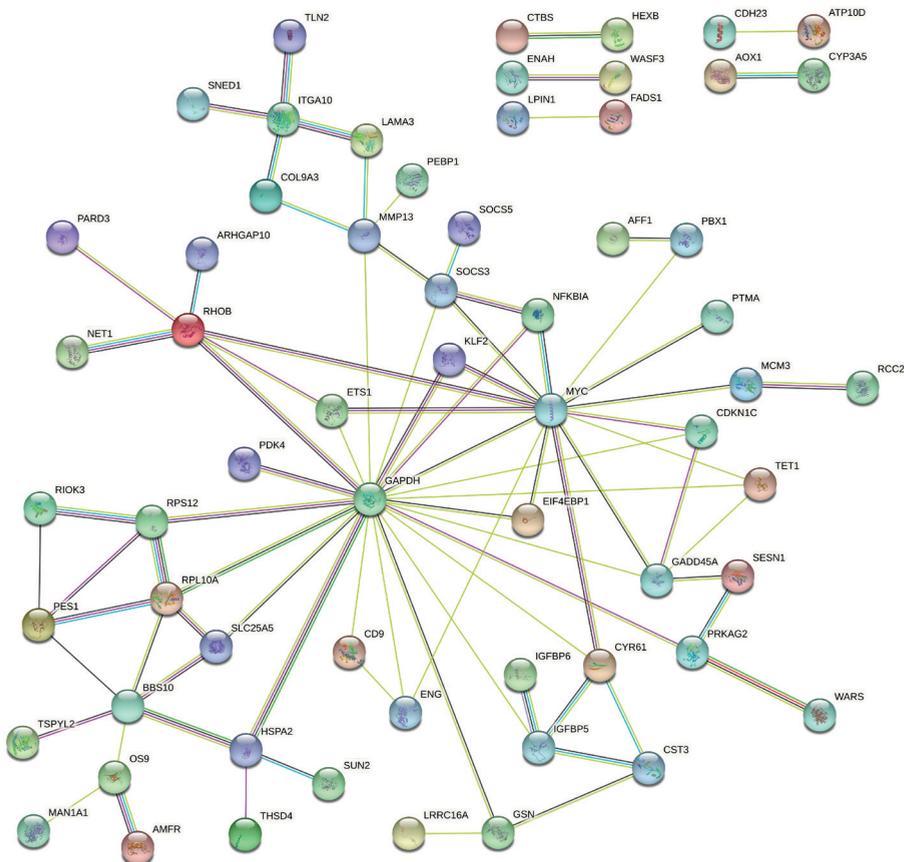
Gene name	log2FC	FC	pvalue	FDR
IGFBP4	1.38	2.59	5.44E-04	3.50E-02
IGFBP5	2.59	6.01	3.54E-05	7.81E-03
IGFBP6	-2.37	0.19	4.72E-08	3.07E-04
IGFBP7	-1.24	0.42	2.05E-06	1.79E-03
HTRA1	0.95	1.93	3.17E-04	2.56E-02
STC2	-1.07	0.48	1.39E-04	1.53E-02
ADAM12	0.62	1.54	6.63E-04	3.92E-02

Legend: Log2FC, log2 fold change; FC, fold change; FDR, False discovery rate

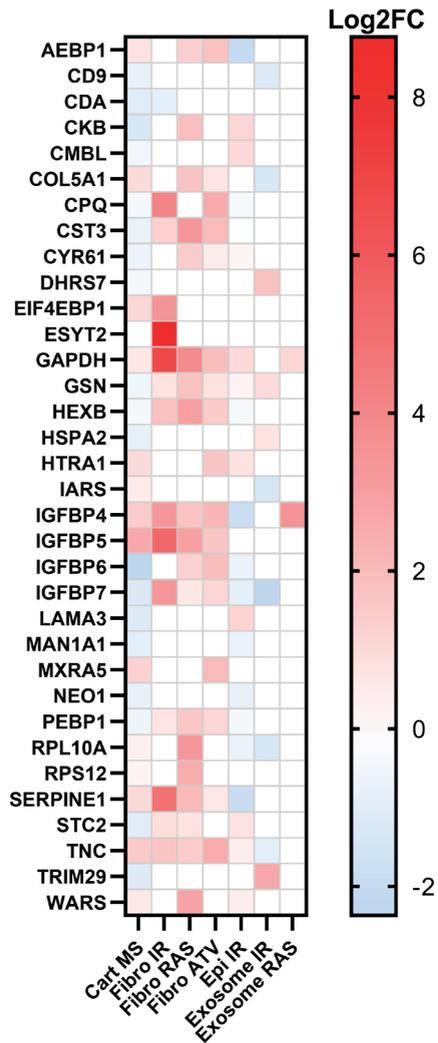
## Supplementary Figures



**Supplementary Figure S1** | Venn diagram of coinciding genes between differentially expressed genes in mechanically stressed versus control cartilage from osteochondral explants (DE<sub>MS</sub>) and previously identified differentially expressed genes in preserved versus lesioned OA cartilage (DE<sub>OA</sub>) [1].



**Supplementary Figure S2** | Protein-protein interaction network in STRING of proteins encoded by differentially expressed genes (N=92 genes) not coinciding with OA pathophysiology (DE<sub>ExclusiveMS</sub>).



**Supplementary Figure S3 | Heat-map of proteins present in SASP.** The heat-map depicts the log<sub>2</sub> fold change (Log<sub>2</sub>FC) of gene expression changes in response to mechanical stress (Cart MS; first column) and proteins changes to several senescence inducing treatments found in the SASPatlas [2]. Abbreviations: cart, cartilage; MS, Mechanical stress; Fibro, fibroblasts; IR, X-irradiation; RAS, oncogenic RAS overexpression; ATV, atazanavir treatment; Epi, epithelial.

## REFERENCES

1. Coutinho de Almeida R, Ramos YFM, Mahfouz A, et al. RNA sequencing data integration reveals an miRNA interactome of osteoarthritis cartilage. *Annals of the rheumatic diseases*. 2019;78(2):270-7.
2. Basisty N, Kale A, Jeon OH, et al. A proteomic atlas of senescence-associated secretomes for aging biomarker development. *PLoS Biol*. 2020;18(1):e3000599.



