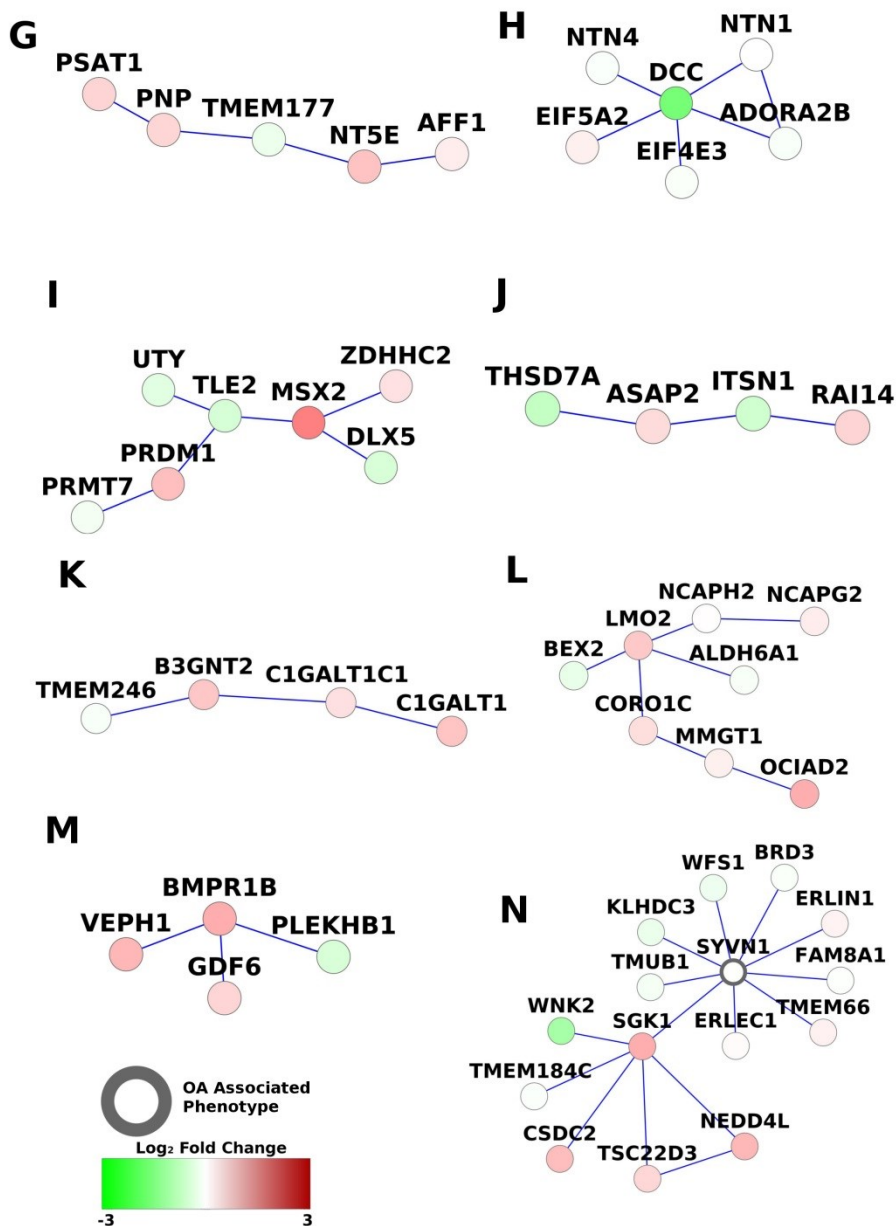
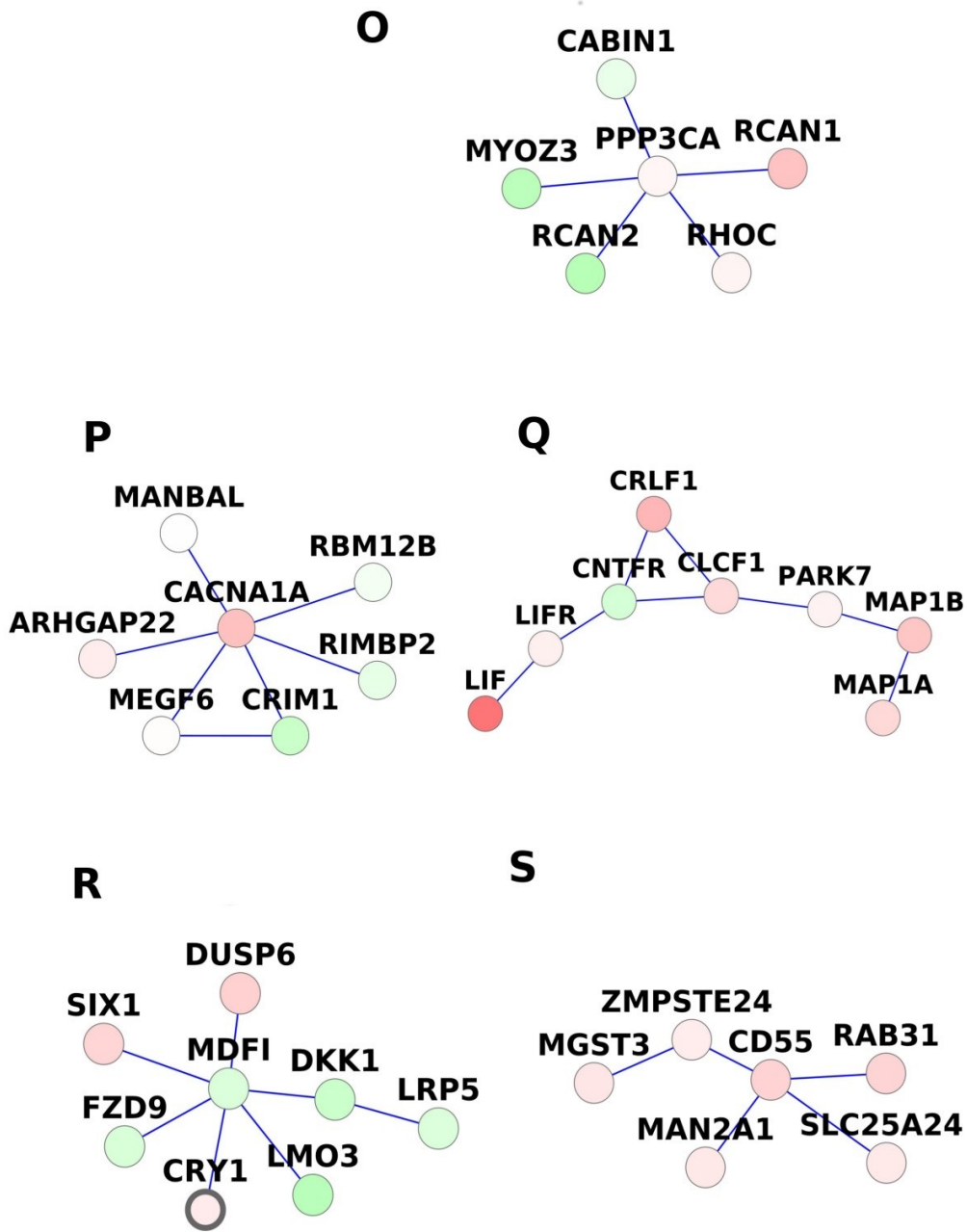


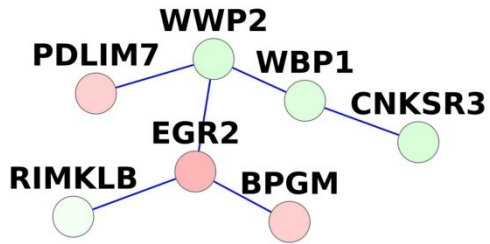
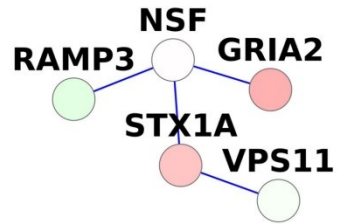
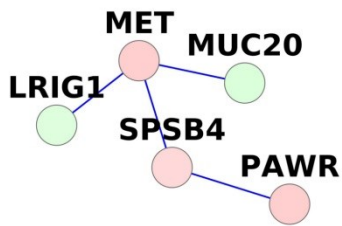
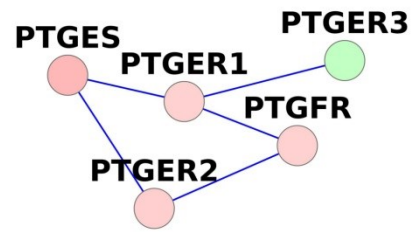
Supplementary Figure 1. Log2 RNA-seq reads of intact vs damaged cartilage. Of the 17160 genes included in the analysis, 4.8% were significantly up-regulated (red) while 4.3% were significantly down-regulated (green). (fold change ≥ 1.5 , adjusted p-value ≤ 0.1).



Supplementary Figure 2. PhenomeExpress Analysis. Network analysis incorporating cross-species gene-phenotype associations, identified 23 differentially expressed networks based on direct protein-protein interactions in the damaged cartilage. Sub-networks included; pyridine-containing compound biosynthetic (**G**), process anterior/posterior axon guidance (**H**), organ morphogenesis (**I**), regulation of Ras GTPase activity (**J**), O-glycan processing (**K**), chromosome condensation (**L**), ovarian cumulus expansion (**M**), ER-associated ubiquitin-dependent protein catabolic process (**N**). The fold change of the proteins is shown by the node colour and OA associated phenotype annotated proteins used to generate the sub-networks are shown with a black border.

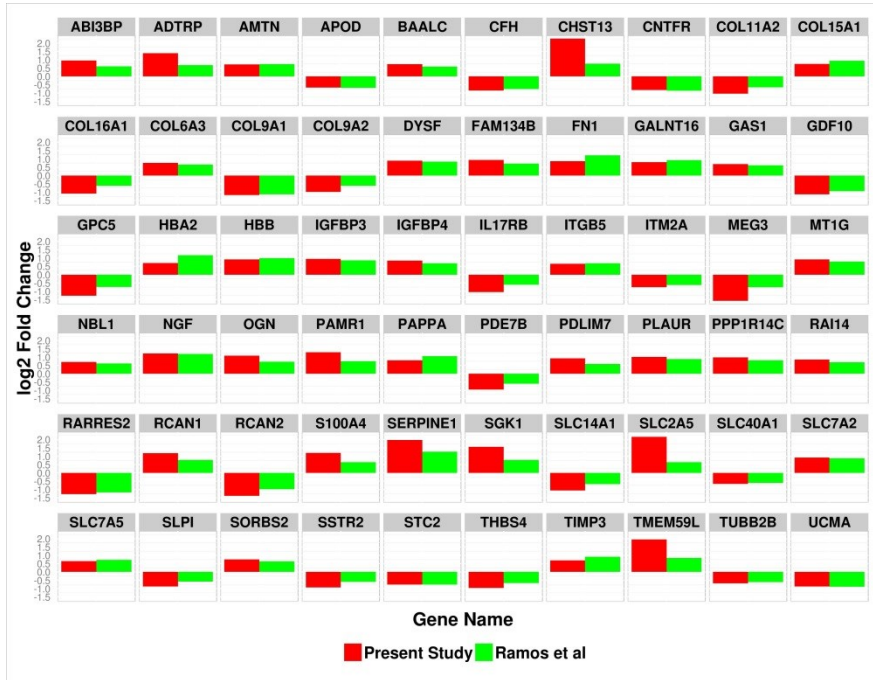


Supplementary Figure 2 Cont. PhenomeExpress Analysis. calcineurin-NFAT signaling cascade (**O**), acetylcholine secretion (**P**), neurotransmission (**Q**), positive regulation of tyrosine phosphorylation of Stat3 protein (**R**), regionalization, CAAX-box protein processing (**S**).

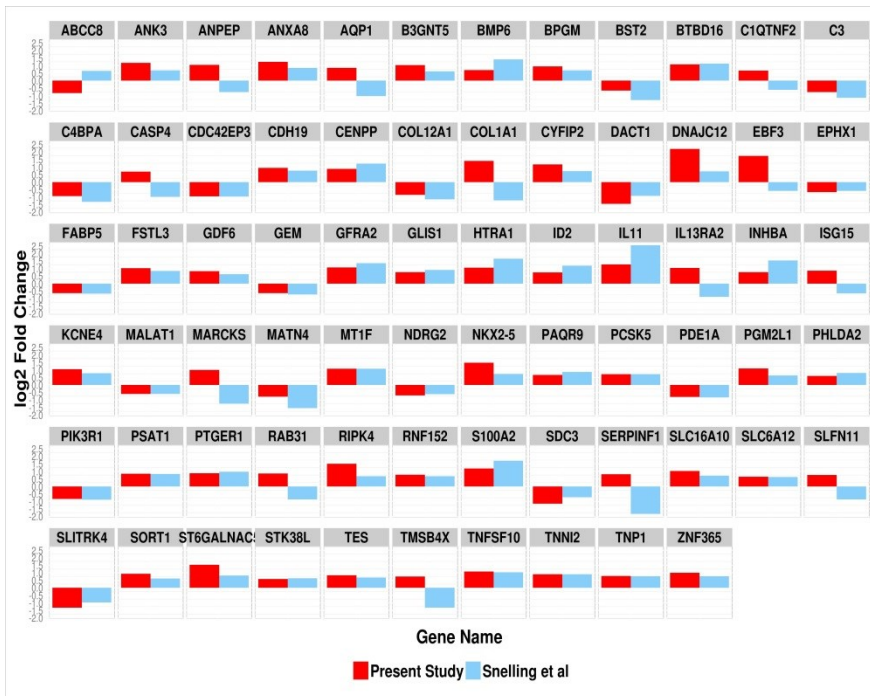
T**U****V****W**

Supplementary Figure 2 Cont. PhenomeExpress Analysis. rhombomere formation (**T**), positive regulation of receptor recycling (**U**), hepatocyte growth factor receptor signaling pathway (**V**), response to lipopolysaccharide (**W**).

A



B



Supplementary Figure 3. Comparison of intact vs damaged OA cartilage transcriptome studies. The log₂ fold change of the differentially expressed genes in the three comparable datasets, the present study compared to Ramos *et al* (A) and Snelling *et al* (B).

Supplementary Table 1. Primer sequences used in real-time PCR for RNA-seq validation.

Gene	Forward Primer	Reverse Primer
GAPDH	5'-AGGGCTGCTTTTAACTCTGGT-3'	5'-CCCCACTTGATTTTGGAGGGA-3'
ACTB	5'-CCACCATGTACCCAGGCATT-3'	5'-CACATCTGCTGGAAGGTGGA-3'
COL1A2	5'-GGCTACCCAACCTGCCTTCA-3'	5'GCAGTGGTAGGTGATGTTCTGAGA3'
COL2A1	5'GGCAATAGCAGGTTACGTACA-3'	5'-CGATAACAGTCTTGCCCCACTT-3'
ACAN	5'-TCGAGGACAGCGAGGCC-3'	5'-TCGAGGGGTAGCGGTAGAGA-3'
SOX9	5'-GACTTCCGCGACGTGGAC-3'	5'-GTTGGGCGGCAGGTACTG-3'
TNFAIP6	5'-CTCCATATGGCTTGAACGAGCAGC-3'	5'-GCCCTTAGCCATCCATCCAGCA-3'
TNFRSF11B	5'-TCCTGGATTTGGAGTGGTGC-3'	5'-CTCCTCACACAGGGTAACATCTAT-3'
HAPLN1	5'-ACTTGAGAGCATCCGAACTCC-3'	5'-GGGGGCCATTTTCTGCTTG-3'

Supplementary Table 3. The top 50 up-regulated genes in damaged cartilage relative to intact cartilage

Gene Name	Gene Symbol	FC	Adjusted p-value
Family with sequence similarity 167, member A	FAM167A	7.49	1.29x10 ⁻³⁴
Leukemia inhibitory factor	LIF	6.51	1.24x10 ⁻²¹
Msh homeobox 2	MSX2	5.62	2.11x10 ⁻¹⁹
Carbohydrate (chondroitin 4) sulfotransferase 13	CHST13	4.83	1.67x10 ⁻¹²
Tumor necrosis factor, alpha-induced protein 6	TNFAIP6	4.67	1.85x10 ⁻¹⁹
DnaJ (Hsp40) homolog, subfamily C, member 12	DNAJC12	4.63	2.95x10 ⁻¹³
RP11-267A15.1	RP11-267A15.1	4.50	5.17x10 ⁻¹²
Solute carrier family 2 (facilitated glucose/fructose transporter), member 5	SLC2A5	4.49	1.27x10 ⁻³⁵
RP11-536I6.2	RP11-536I6.2	4.16	3.81x10 ⁻¹⁰
Forkhead box F1	FOXF1	3.98	2.41x10 ⁻¹¹
Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	SERPINE1	3.94	1.81x10 ⁻²⁴
Transmembrane protein 59-like	TMEM59L	3.89	1.41x10 ⁻¹¹
Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2	PREX2	3.87	1.85x10 ⁻¹⁹
Teneurin transmembrane protein 3	TENM3	3.78	6.89x10 ⁻¹⁶
Neurotrophin 3	NTF3	3.77	3.54x10 ⁻¹²
ADAM metallopeptidase with thrombospondin type 1 motif, 14	ADAMTS14	3.74	1.56x10 ⁻¹⁴
Anillin, actin binding protein	ANLN	3.72	3.32x10 ⁻¹⁷
Urocanate hydratase 1	UROC1	3.67	6.29x10 ⁻¹⁴
Protocadherin 10	PCDH10	3.66	9.41x10 ⁻¹²
Chloride intracellular channel 3	CLIC3	3.62	5.27x10 ⁻¹³
Collagen, type VII, alpha 1	COL7A1	3.59	1.51x10 ⁻¹⁴
High mobility group AT-hook 2	HMGA2	3.58	6.00x10 ⁻⁰⁹
Creatine kinase, brain	CKB	3.57	5.55x10 ⁻¹⁹
AC093850.2	AC093850.2	3.52	2.34x10 ⁻⁰⁹
Target of myb1 (chicken)-like 1	TOM1L1	3.50	1.93x10 ⁻¹¹
Hes-related family bHLH transcription factor with YRPW motif 2	HEY2	3.49	3.86x10 ⁻¹³
Dual specificity phosphatase 4	DUSP4	3.48	1.38x10 ⁻¹³
Versican	VCAN	3.46	6.97x10 ⁻¹⁸
Eva-1 homolog A (C. elegans)	EVA1A	3.38	7.24x10 ⁻⁰⁸
Early B-cell factor 3	EBF3	3.33	1.58x10 ⁻⁰⁷
Leucine rich repeat containing 8 family, member E	LRRC8E	3.32	8.84x10 ⁻¹¹
WNT1 inducible signaling pathway protein 1	WISP1	3.30	1.85x10 ⁻¹⁹
FAT atypical cadherin 3	FAT3	3.28	4.32x10 ⁻¹⁰
Laminin, beta 3	LAMB3	3.19	4.93x10 ⁻¹³
Centrosomal protein 55kDa	CEP55	3.18	2.20x10 ⁻⁰⁷
Solute carrier family 38, member 5	SLC38A5	3.11	1.16x10 ⁻²²
Hormonally up-regulated Neu-associated kinase	HUNK	3.11	4.78x10 ⁻¹¹
RP11-160A10.2	RP11-160A10.2	3.11	8.74x10 ⁻⁰⁸
Family with sequence similarity 132, member B	FAM132B	3.05	3.04x10 ⁻⁰⁶
Carbonic anhydrase XII	CA12	3.03	6.11x10 ⁻⁰⁸
SLIT and NTRK-like family, member 6	SLITRK6	3.03	3.96x10 ⁻⁰⁶
T-box 3	TBX3	3.03	7.84x10 ⁻⁰⁷
Bone morphogenetic protein receptor, type IB	BMPR1B	3.01	2.16x10 ⁻⁰⁶
Potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	KCNS3	2.99	4.56x10 ⁻⁰⁹
Periostin, osteoblast specific factor	POSTN	2.99	2.38x10 ⁻⁰⁶
Serum/glucocorticoid regulated kinase 1	SGK1	2.97	6.71x10 ⁻²⁵
OCIA domain containing 2	OCIAD2	2.97	3.16x10 ⁻¹⁵
Fibroblast growth factor 9	FGF9	2.94	1.62x10 ⁻⁰⁶
Non-SMC condensin I complex, subunit G	NCAPG	2.94	3.36x10 ⁻⁰⁶
Cyclin-dependent kinase 17	CDK1	2.93	5.66x10 ⁻⁰⁷

Supplementary Table 4. The top 50 down-regulated genes in damaged cartilage relative to intact cartilage

Gene Name	Gene Symbol	FC	Adjusted p-value
Deleted in colorectal carcinoma	DCC	6.37	1.60x10 ⁻²⁵
Transmembrane protein 176A	TMEM176A	3.72	1.96x10 ⁻¹²
Tachykinin, precursor 1	TAC1	3.35	4.26x10 ⁻¹²
CD52 molecule	CD52	3.33	1.25x10 ⁻⁰⁸
WNK lysine deficient protein kinase 2	WNK2	3.30	5.55x10 ⁻¹⁹
Angiotensin II receptor, type 2	AGTR2	3.22	8.01x10 ⁻⁰⁷
Leucine rich repeat transmembrane neuronal 2	LRRTM2	3.09	1.40x10 ⁻¹¹
Tumor necrosis factor receptor superfamily, member 4	TNFRSF4	3.07	1.31x10 ⁻⁰⁸
Even-skipped homeobox 1	EVX1	3.06	3.24x10 ⁻⁰⁶
Chromosome 1 open reading frame 173	C1orf173	3.04	4.90x10 ⁻⁰⁷
Solute carrier family 22	SLC22A11	3.01	6.91x10 ⁻⁰⁸
Maternally expressed 3	MEG3	2.99	8.80x10 ⁻¹⁴
Calcium channel, voltage-dependent, alpha 2/delta subunit 2	CACNA2D2	2.98	3.58x10 ⁻¹⁷
Neurexin 2	NRXN2	2.94	7.82x10 ⁻¹⁰
Calpain 6	CAPN6	2.90	4.15x10 ⁻⁰⁶
Seizure related 6 homolog (mouse)-like 2	SEZ6L	2.90	4.15x10 ⁻⁰⁸
Neurotrophic tyrosine kinase, receptor, type 3	NTRK3	2.89	1.21x10 ⁻⁰⁵
3-hydroxymethyl-3-methylglutaryl-CoA lyase-like 1	HMGCLL1	2.83	1.84x10 ⁻⁰⁷
Insulin-like growth factor 2 mRNA binding protein 2	IGF2	2.77	4.17x10 ⁻¹⁸
NFAT activating protein with ITAM motif 1	NFAM1	2.74	4.49x10 ⁻¹⁰
Transmembrane protein 176B	TMEM176B	2.73	1.89x10 ⁻⁰⁶
Family with sequence similarity 101, member A	FAM101A	2.73	2.37x10 ⁻⁰⁹
Mesothelin	MSLN	2.73	1.24x10 ⁻⁰⁵
Family with sequence similarity 198, member A	FAM198A	2.72	1.78x10 ⁻¹¹
Dishevelled-binding antagonist of beta-catenin 1	DACT1	2.72	2.83x10 ⁻¹⁸
Sarcalumenin	SRL	2.70	8.36x10 ⁻⁰⁶
Zinc finger protein 385C	ZNF385C	2.68	3.58x10 ⁻¹³
Myosin, heavy chain 14, non-muscle	MYH14	2.68	1.04x10 ⁻⁰⁹
Kinesin family member 1A	KIF1A	2.66	2.09x10 ⁻⁰⁷
Cadherin-related family member 1	CDHR1	2.65	6.98x10 ⁻⁰⁸
R-spondin 3	RSPO3	2.65	1.73x10 ⁻⁰⁶
Regulator of calcineurin 2	RCAN2	2.61	1.23x10 ⁻⁰⁸
RP11-588K22.2	RP11-588K22.2	2.59	4.78x10 ⁻¹⁰
ALX homeobox 4	ALX4	2.58	7.28x10 ⁻⁰⁷
Small G protein signaling modulator 1	SGSM1	2.58	3.05x10 ⁻⁰⁸
LIM domain only 3 (rhombotin-like 2)	LMO3	2.57	1.61x10 ⁻⁰⁵
Guanylate cyclase 1, soluble, alpha 3	GUCY1A3	2.56	1.73x10 ⁻¹⁴
PR domain containing 16	PRDM16	2.55	3.18x10 ⁻⁰⁵
Myozenin 3	MYOZ3	2.53	5.39x10 ⁻¹³
Long intergenic non-protein coding RNA 284	LINC00284	2.53	4.26x10 ⁻⁰⁷
Glutamate receptor, ionotropic, N-methyl D-aspartate 2C	GRIN2C	2.52	2.22x10 ⁻⁰⁷
Serine/threonine kinase 32B	STK32B	2.51	6.71x10 ⁻²⁵
SLIT and NTRK-like family, member 4	SLITRK4	2.50	6.55x10 ⁻⁰⁸
Cardiomyopathy associated 5	CMYA5	2.48	3.94x10 ⁻¹⁰
Chordin-like 2	CHRD2	2.47	7.69x10 ⁻¹⁰
Piezo-type mechanosensitive ion channel component 2	PIEZO2	2.45	5.70x10 ⁻²¹
RP11-561C5.3	RP11-561C5.3	2.43	4.01x10 ⁻⁰⁵
Kyphoscoliosis peptidase	KY	2.43	1.04x10 ⁻⁰⁴
Vitrin	VIT	2.43	3.19x10 ⁻⁰⁴
Retinoic acid receptor responder	RARRES2	2.42	1.52x10 ⁻⁰⁶

Supplementary Table 5. Upstream Transcription factor analysis. Motif, Chip-Seq, text-mining and coexpression based methods were used to identify possible upstream transcription factors (TFs) controlling the expression of the up-regulated differentially expressed genes in damaged OA cartilage. For each TF detected by at least 2 methods the z-score or BH-adjusted p-value is shown for each tool as is the fold-change and p-value of the TF. TFs not included in a method are shown by NA while non-significant z-scores and p-values are shown by NS. Significantly differentially expressed TFs are highlighted in bold.

Gene Symbol	FC	padj	Motif z-Score	Chip-Seq z-Score	Textmining padj	Coexpression z-Score
AHR	1.75	0.01	NS	NS	0.02	3.71
BMP2	1.18	0.28	NA	NA	9.48x10 ⁻⁰⁴	4.83
BRCA1	1.32	0.16	NS	NS	4.76x10 ⁻⁰⁴	3.10
CTCF	-1.05	0.71	NS	4.29	8.43x10 ⁻⁰³	NS
DNMT3B	1.28	0.45	NA	NA	0.03	3.34
E2F1	1.78	0.01	NS	4.01	3.39x10 ⁻⁰⁴	3.11
E2F4	-1.06	0.67	NS	3.95	3.39x10 ⁻⁰⁴	NS
EZH2	1.50	0.11	NA	3.12	0.02	3.31
FOS	-1.02	0.96	4.94	NS	0.03	3.74
FOSB	-1.07	0.87	4.94	NA	NS	3.55
FOSL1	1.62	0.04	4.94	NS	NS	3.46
FOXM1	2.35	1.38x10 ⁻⁰⁴	3.14	3.47	1.24x10 ⁻⁰³	3.01
HES1	-1.20	0.24	4.21	NA	1.58x10 ⁻⁰³	NS
HOXA10	-1.26	0.04	3.39	NA	4.76x10 ⁻⁰⁴	NS
ID3	-1.09	0.70	NA	NA	7.27x10 ⁻⁰³	3.40
JUN	1.11	0.80	4.05	NS	2.50x10 ⁻⁰³	NS
JUNB	1.03	0.92	4.94	NS	0.04	3.19
JUND	1.10	0.73	4.94	NS	0.03	NS
SNAI2	-1.36	0.03	NS	NA	0.06	4.82
ZBTB16	-1.09	0.78	NS	NA	2.97x10 ⁻⁰³	3.68