Otero M. et al - Supporting Information

Methods

Electrophoretic Mobility Shift Assay (EMSA) and Supershift analysis

EMSAs were performed using 2 ml of *in vitro*-translated ELF3, prepared as described (27) using the TNT Quick Coupled Transcription/Translation System (Promega), and radiolabeled oligonucleotides encompassing the ETS binding sites in the proximal *MMP13* promoter or mutant sequences. Double-stranded DNA oligonucleotides were end-labeled using T4 polynucleotide kinase and a-32P dATP. Binding reactions were carried out for 30 min at room temperature using 0.8 pmol (~10,000 cpm) of labeled probe in a final volume of 20 ml containing 10 mM Tris (pH 7.5), 50 mM NaCl, 1 mM EDTA, 10 mM KCl, 1mM dithiothreitol (DTT), 10% glycerol, 0.1 mg bovine serum albumin (BSA), and 5 ng poly(dI-dC). Unlabeled wild-type oligonucleotides were added as competitors at 50- or 100-fold excess. For supershift, 1 ml of rabbit polyclonal anti-ELF3 antibody raised against a glutathione S-transferase fusion protein of the N terminus of human ELF3 (East Acres Biologicals), as reported previously (27), was incubated with the binding reaction mixture for 30 min at room temperature before electrophoresis. The protein-DNA complexes were separated on 4.2% polyacrylamide gels using 0.5x Tris borate-EDTA buffer (TBE) buffer (45 mM Tris borate, pH 8.3, and 1 mM EDTA) and visualized by autoradiography.

Figure S1

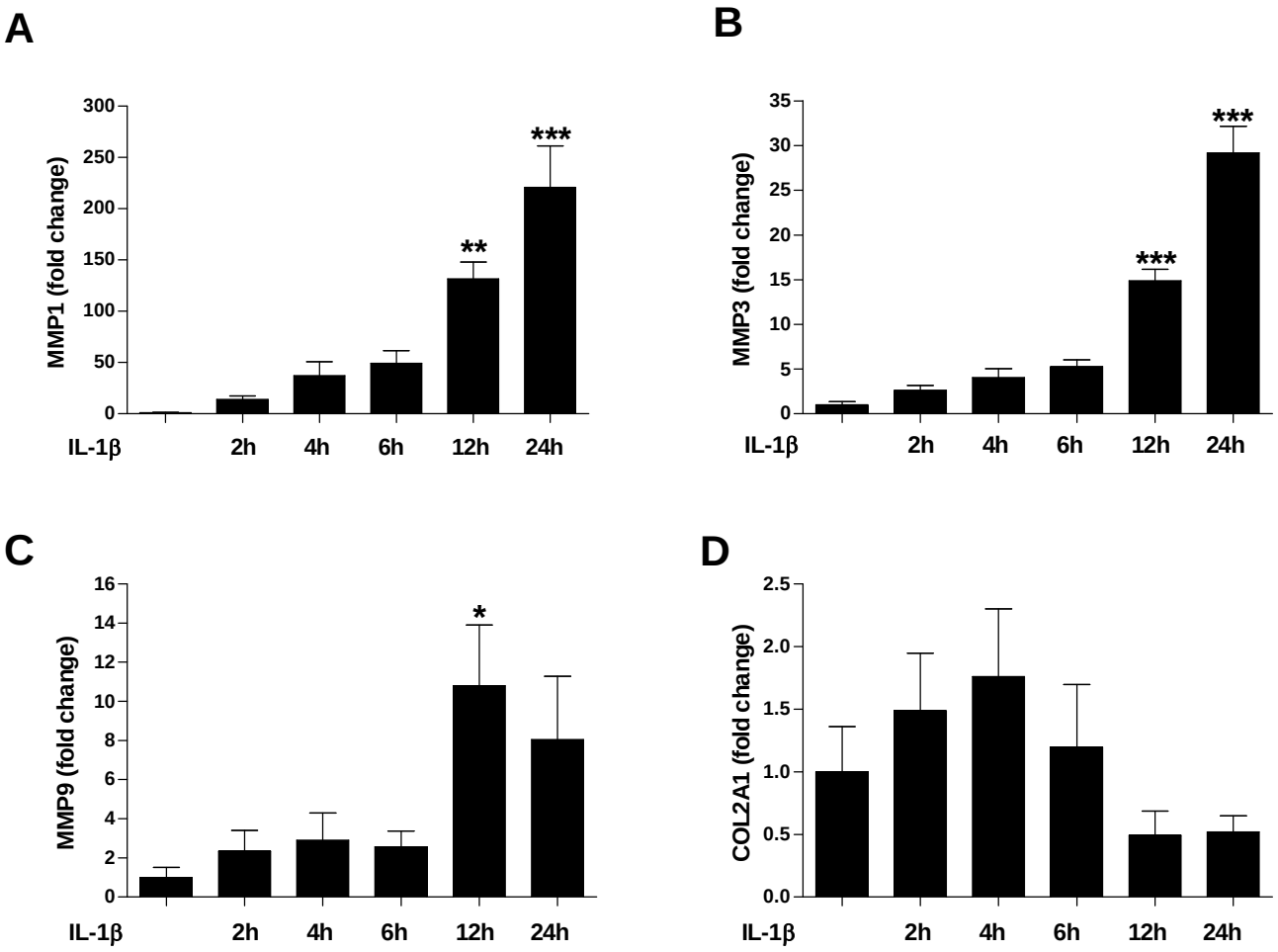

Figure S1. IL-1β-induced ELF3 expression in human primary articular chondrocytes is accompanied by the upregulation of MMPs and repression of COL2A1 expression. Human primary chondrocytes isolated from knee joints obtained from 3 OA patients undergoing total knee replacement were stimulated with 1ng/ml of IL-1β for the indicated times. Total RNAs were isolated and subjected to RT-qPCR analysis for **(A)** MMP-1, **(B)** MMP-3, **(C)** MMP-9 and **(D)** COL2A1 expression. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M. * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$

Figure S2

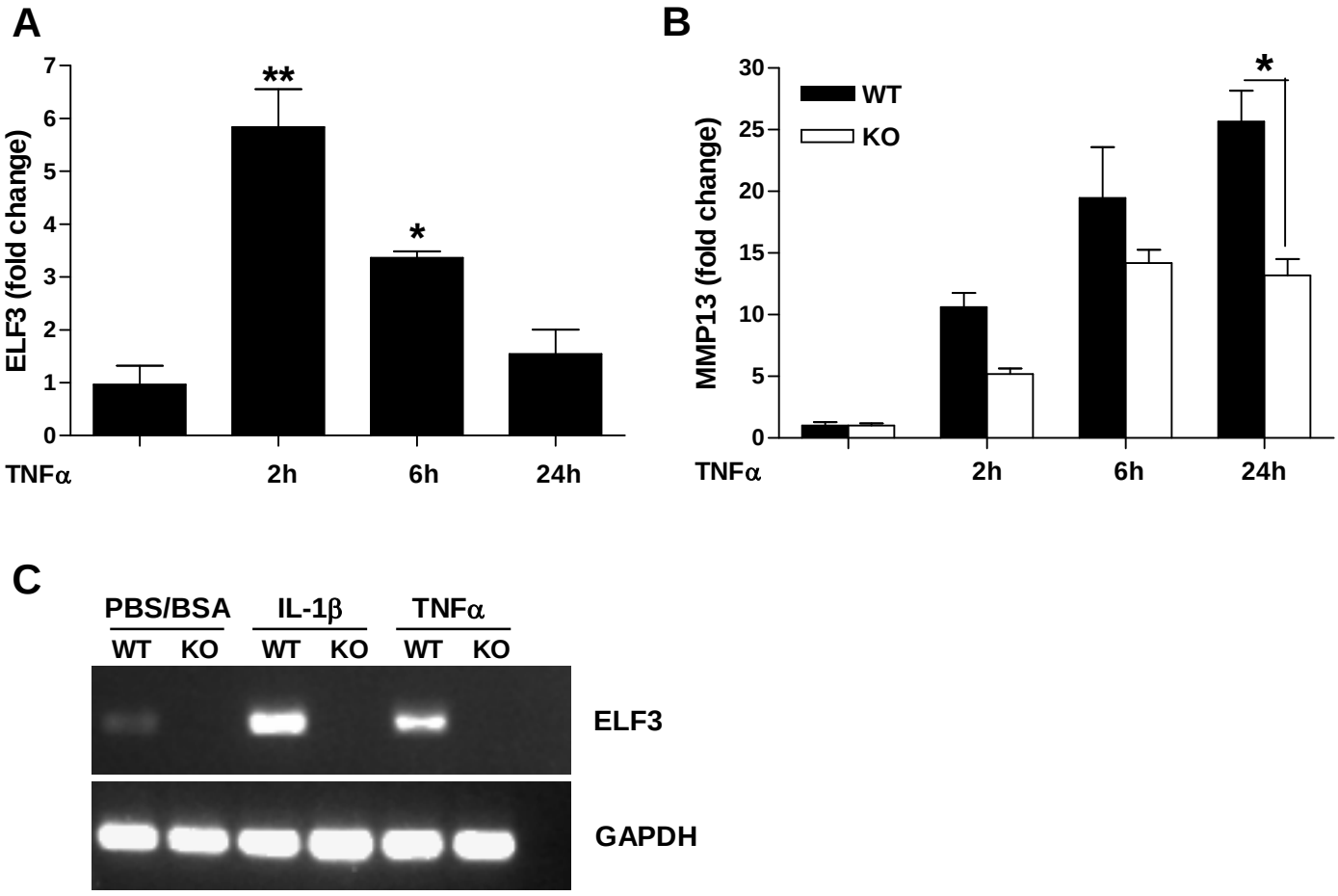

Figure S2. Effects of ELF3 knockout on the TNFα-induced MMP13 expression. **(A)** Mouse primary chondrocytes were isolated from articular cartilage obtained from wild type C57/B6 5- to-6-days old mice and incubated with 10 ng/ml of TNFα for the indicated times. Total RNAs were isolated and ELF3 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M. **(B)** Mouse primary chondrocytes isolated from wild type (WT) and *Elf3* knockout (KO) 5- to 6-days old mice were incubated with 10 ng/ml of TNFα for the indicated times. Total RNAs were isolated and MMP-13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M. **(C)** Expression of ELF3 mRNA in mouse primary chondrocytes isolated from wild type (WT) and *Elf3* knockout (KO) 5- to-6-days old mice. Mouse articular chondrocytes were incubated with vehicle (PBS/BSA), 1 ng/ml of IL-1β or 10 ng/ml of TNFα for 2 h. Total RNAs were isolated and ELF3 mRNA was analyzed by RT-PCR, with the PCR products resolved on a 2% agarose gel. * indicates $p<0.05$, ** indicates $p<0.01$

Figure S3

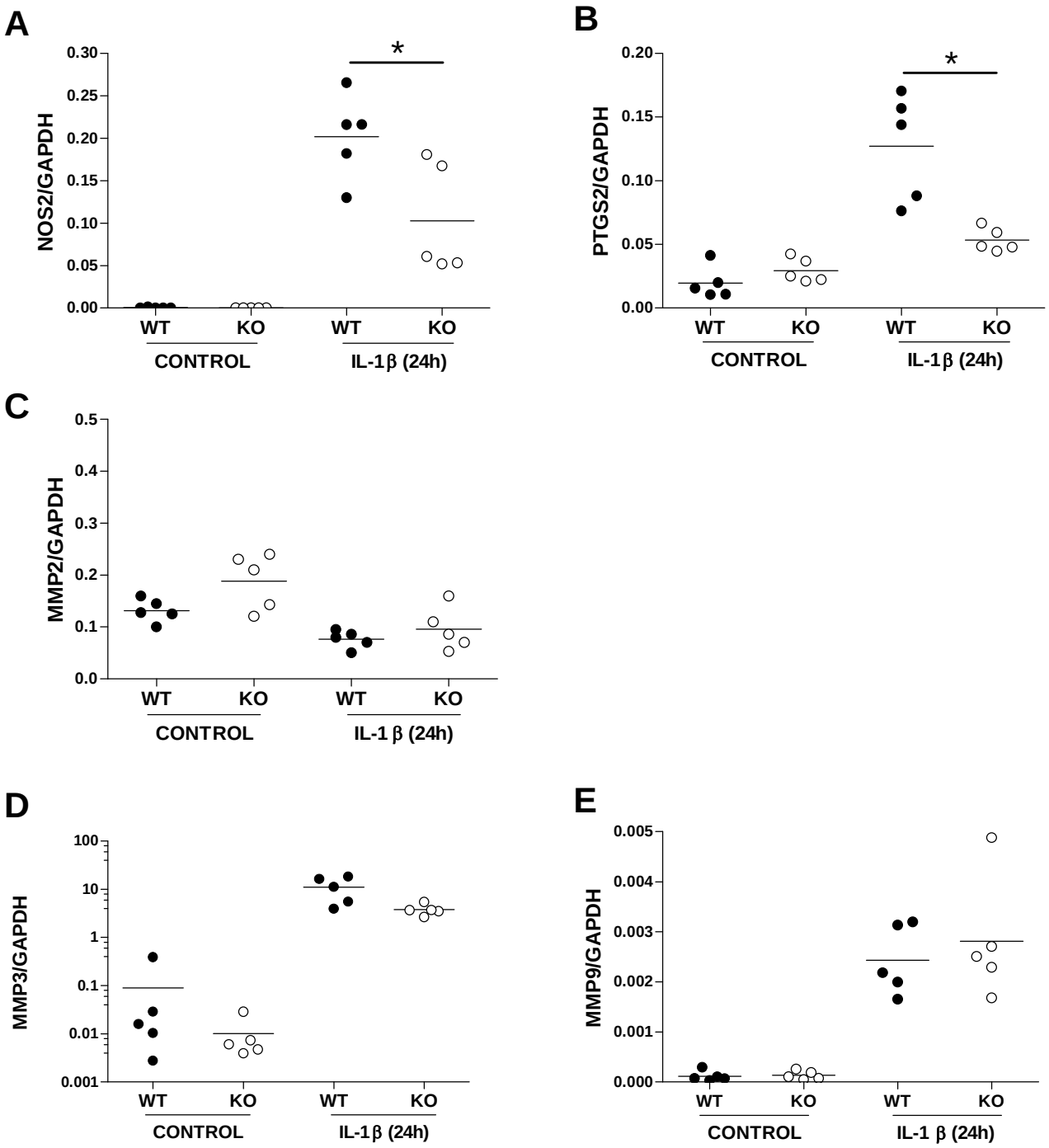

Figure S3. Effects of ELF3 knockout on the IL-1β-induced expression of **(A)** NOS2, **(B)** PTGS2, **(C)** MMP2, **(D)** MMP3 and **(E)** MMP9 in mouse primary chondrocytes. Primary chondrocytes were isolated from articular cartilage obtained from wild type (WT) and *Elf3* knockout (KO) 5- to 6 days old mice, and stimulated with 1ng/ml of IL-1β for 24 h. Total RNAs were isolated and subjected to RT-qPCR analysis. Each value was normalized to GAPDH in the same sample and shown as relative expression to GAPDH. * indicates $p<0.05$

Figure S4

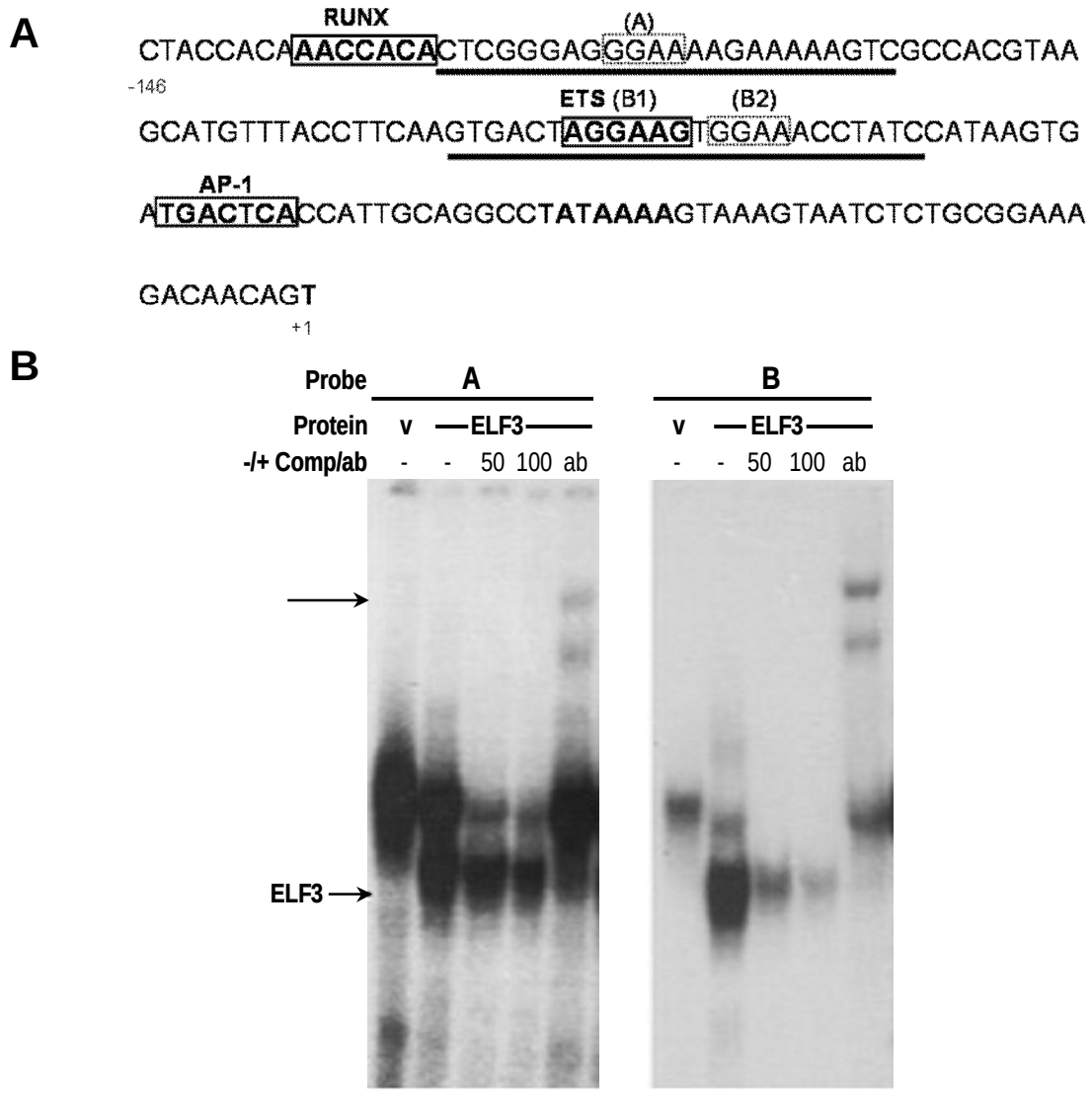

Figure S4. ELF3 binds to the EBS sequences contained in the proximal *MMP13* promoter. **(A)** Schematic representation of the proximal human MMP13 promoter sequence, containing the TATA box (bold), the evolutionarily conserved RUNX2, ETS/PEA3 and AP-1 binding sites (bold, closed boxes), along with less conserved putative ETS binding sites (A and B2, dotted boxes). The sequence of the oligonucleotides utilized for the EMSA analysis, comprising the ETS binding sites A (probe A) and B1+B2 (probe B), is underlined. **(B)** End-labeled double-stranded oligonucleotides containing the ETS sites A and B (containing tandem EBS) of the *MMP13* promoter were incubated with *in vitro* translated protein made with empty vector (v) or pCI-ELF3. Self-competitor oligonucleotides (at 50x and 100x) and ELF3 antibody (ab) were added as indicated. Note that the EMSA for probe A is over-exposed compared to that for probe B for better visualization of the supershifted bands.

Figure S5

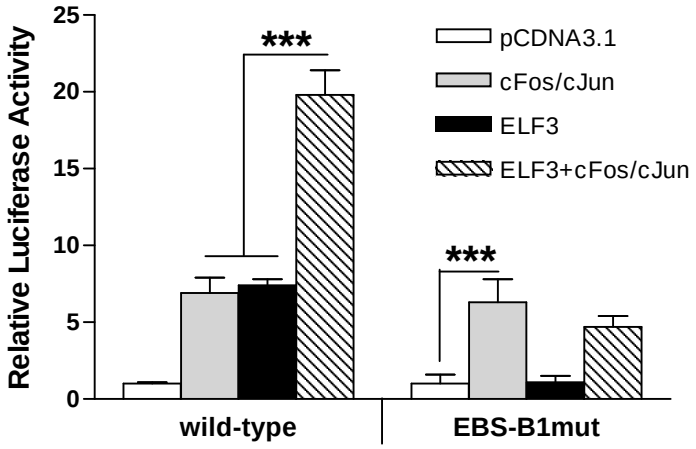

Figure S5. Mutation of the evolutionarily conserved B1(-78) ETS binding site disrupts both ELF3-driven MMP13 transactivation and ELF3-enhancement of the AP-1-driven MMP13 activation. T/C-28a2 cells were co-transfected with 325 ng of the wild-type -267/+27 bp-*MMP13* promoter sequence or the EBS-B1 mutant construct, and 25 ng of expression vectors encoding ELF3, cFos and cJun. Luciferase activities were normalized to the protein input. *** indicates $p<0.001$

Figure S6

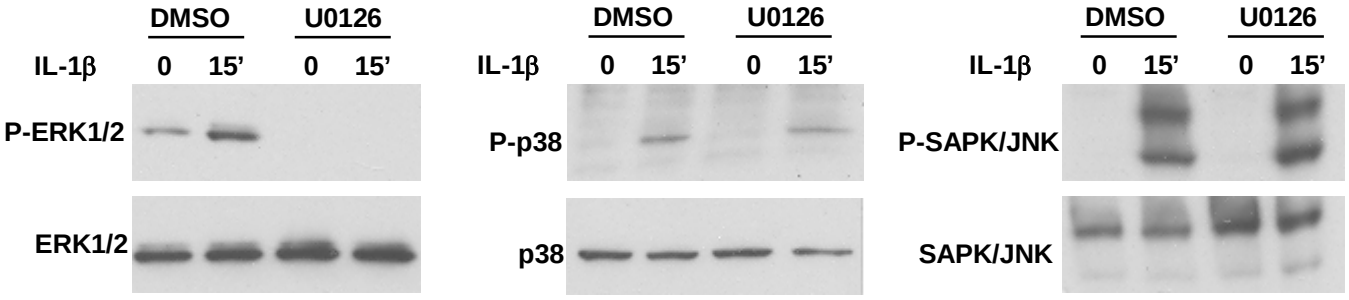

Figure S6. Pretreatment with 2.5μM of the MEK1/2 inhibitor, U0126, specifically blocks ERK1/2 phosphorylation without affecting the IL-1β-induced p38 or SAPK/JNK phosphorylation in T/C-28a2 immortalized chondrocytes.

Supplementary Table 1. Real-time PCR primers and Conditions.

GENE (species)	Primer sequences	Size (bp)	Anneal (°C)	GenBank Accesion
ELF3 (human)	Forward: 5'-CAACTATGGGGCCAAAAGAA-3' Reverse: 5'-TTCCGACTCTGGAGAACCTC-3'	176	57	NM_004433
COL2A1 (human)	Forward: 5'-TCACGTACACTGCCCTGAAG-3' Reverse: 5'-TGCAACGGATTGTGTTGTTT-3'	213	57	NM_001844
MMP1 (human)	Forward: 5'-CTGGAATTGGCCACAAAGTT-3' Reverse: 5'-TCCTGCAGTTGAACCAGCTA-3'	144	57	NM_002421
MMP3 (human)	Forward: 5'-GCATCCACACCCTAGGTTTC-3' Reverse: 5'-TGGCTCCATGGAATTTCTCT-3'	137	57	NM_002422
MMP9 (human)	Forward: 5'-CTGGGCAGATTCCAAACCT-3' Reverse: 5'-TACACGCGAGTGAAGGTGAG-3'	170	57	NM_004994
MMP13 (human)	Forward: 5'-TCAGGAAACCAGGTCTGGAG-3' Reverse: 5'-TGACGCGAACAATACGGTTA-3'	196	57	NM_002427
GAPDH (human)	Forward: 5'-CAAAGTTGTCATGGATGACC-3' Reverse: 5'-CCATGGAGAAGGCTGGGG-3'	195	57	NM_002046
HPRT1 (human)	Forward: 5'-AAAGGACCCCACGAAGTGTT-3' Reverse: 5'-TCAAGGGCATATCCTACAACAA-3'	85	57	NM_000194
ELF3 (mouse)	Forward: 5'-GGCCCTCATGGCTGCCACCT-3' Reverse: 5'TTGGGATCTTGTCTGAGGTCCTGGA3'	187	60	NM_001163131.1
MMP2 (mouse)	Forward: 5'-TCGTGGCAGCCCATGAGTTCG-3' Reverse: 5'- CATCGGGGGAGGGCCCATAGAG-3'	156	57	NM_008610.2
MMP3 (mouse)	Forward: 5'-GTCCCTCTATGGAACTCCCACAGCA-3' Reverse: 5'-GGACTTCTCCCCGGAGGGTGC-3'	140	60	NM_010809.1
MMP9 (mouse)	Forward: 5'-TACCCGAGTGGACGCGACCG-3' Reverse: 5'-ATGTGGTCGCACACCAGAGGC-3'	140	60	NM_013599.2
MMP13 (mouse)	Forward: 5'-ATGGTCCAGGCGATGAAGACCCC-3' Reverse: 5'-GTGCAGGCGCCAGAAGAATCTGT-3'	140	60	NM_008607
NOS2 (mouse)	Forward: 5'-TGCAACATGGGAGCCACAGCA-3' Reverse: 5'-AGGGTGGTGCGGCTGGACTT-3'	156	60	NM_010927.3
PTGS2 (mouse)	Forward: 5'-CTGCTGCCCGACACCTTCAACA-3' Reverse: 5'-CATTTCTTCCCCCAGCAACCCGG-3'	151	60	NM_011198.3
GAPDH (mouse)	Forward: 5'-GGGCTCATGACCACAGTCCATGC-3' Reverse: 5'-CCTTGCCCACAGCCTTGGCA-3'	142	60	GU214026
HPRT1 (mouse)	Forward: 5'-TCCCAGCGTCGTGATTAGCGA-3' Reverse: 5'-GGGCCACAATGTGATGGCCTCC-3'	179	60	NM_013556