Sample/Template	details
Source	Cultured human chondrocytes; SW1353 cells; THP-1 cells
Method of preservation	No preservation; Total RNA was extracted directly from PBS washed cell layers
Storage time (if appropriate)	RNA was stored at -80°C and reverse transcribed into cDNA within one month. cDNA was stored at -80°C and analyzed within 6 months.
Handling	
Extraction method	Column based extraction method (NucleoSpin RNA II Kit (Macherey-Nagel, D)).
RNA: DNA-free	All primer pairs used (IL-1ß, TNF-a, iNOS, COX2, GAPDH, ACTB, HPRT1, SDHA, B2M) are intron-spanning primers. The RNA extraction protocol included on-column DNA digestion. Using microfluidics (Bioanalyzer), the quality of each RNA preparation was verified. 'Minus RT controls' were prepared from random RNA samples.
Concentration	All RNA preparations were quantified using microfluidics (Bioanalyzer).
RNA: integrity	Integrity of RNA each preparation was verified using microfluidics (Bioanalyzer).

Inhibition-free	Not all samples were tested for the absence of inhibitors. However, a representative set of 10 samples was used to control for the absence of inhibitors using dilution series of target genes.
Assay optimisation/validation	
Accession numbers	IL-1ß (NM_000576); TNF-a (NM_000594); iNOS (NM_000625); COX2 (NM_000963)
Amplicon details	IL-1ß (132bp); TNF-a (126bp); iNOS (127bp); COX2 (136bp)
Primer sequence	
IL-1ß fw:	CCTATTACAGTGGCAATGAGGATG
IL-1ß rv:	AGTGGTGGTCGGAGATTCG
TNF-a fw:	GCGGTGCTTGTTCCTCAG
TNF-a rv:	GCTACAGGCTTGTCACTCG
iNOS fw:	GTTCTCACGGCACAGGTCTC
iNOS rv:	GCAGGTCACTTATGTCACTTATC
COX2 fw:	CCGAGGTGTATGTATGAGTGTG
COX2 rv:	TGTGTTTGGAGTGGGTTTCAG
GAPDH fw:	GGAGTCCACTGGCGTCTTCAC
GAPDH rv:	GAGGCATTGCTGATGATCTTGAGG
ACTB fw:	CTGGAACGGTGAAGGTGACA
ACTB rv:	AAGGGACTTCCTGTAACAATGCA
B2M fw:	TGCTGTCTCCATGTTTGATGTATCT
B2M rv:	TCTCTGCTCCCCACCTCTAAGT
HPRT1 fw:	TGACACTGGCAAAACAATGCA

HPRT1 rv:	GGTCCTTTTCACCAGCAAGCT
SDHA fw:	TGGGAACAAGAGGGCATCTG
SDHA rv:	CCACCACTGCATCAAATTCATG
*Probe sequence**	no probes used
In silico	All primers were subjected to BLAST analysis
empirical	Primers for IL-1ß, TNF-a, iNOS and COX2 were used at 100 nM
Priming conditions	For cDNA synthesis, random RT primers were used as provided by the manufacturer (High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Austria)).
PCR efficiency	qPCR efficiencies were determined using dilution series of cDNA prepared from chondrocyte mRNA. IL-1ß (E=95.1%; slope= -3.444; R^2=0.996); TNF-a (E=94.6%; slope= -3.458; R^2= 0.999); iNOS (E=99.4%; slope= -3.337; R^2=1); COX2 (E=96.5%; slope= -3.408; R^2=0.999)
Linear dynamic range	The dynamic range of the primers was spanning unknown targets as determined by dilution curves.
Limits of detection	LODs were not determined in this study.
Intra-assay variation	The intra-assay variation was below one Cq.
RT/PCR	

Protocols	**RT:** equal RNA quantities were reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Austria; LOT#: 4368814). **qPCR:** reactions were performed in 25 µl reaction mixtures containing 1 µl cDNA, 12.5 µl SensiMix SYBR Green Master Mix (GenXpress, Austria; LOT#: SMP-110E), 100 nM primers (Metabion, Martinsried, D), and nuclease-free water to 25 µl, and run in duplicate on a Mx3000P QPCR system (Stratagene).
Reagents	see above.
Duplicate RT	Replicates of the RT step were not performed in this study.
NTC	NTCs were included in each run. The absence of both amplification and melting curve peaks were mandatory for the acceptance of the run.
NAC	Minus RT controls were prepared from randomly selected RNA samples.
Positive control	Inter-run calibrators were not used.
Data analysis	

Specialist software	Data were analyzed using the MxPro real-time QPCR software (Stratagene), considering both amplification efficiencies and normalization to GAPDH as reference gene.
Statistical justification	Primary chondrocytes (PCs) were isolated from 3 donors and cultured and analyzed seperately. SW1353 and THP-1 cells were seeded in duplicate. All qPCR reactions were performed in duplicate. Data were exported to the GraphPad Prism statistics software package (GraphPad Prism Software, USA). The Gaussian distribution of the data was verified using the Kolmogorov-Smirnov test. Statistics were performed using one-way analysis of variance (ANOVA) with post hoc Tukey tests, cross-comparing all study groups (95% confidence interval). p-values < 0.05 were considered significant.
Transparent, validated normalisation	The expression stability of 5 candidate reference genes (GAPDH, ACTB, HPRT1, SDHA, B2M) was determined across the samples using geNorm. GAPDH was selected as stable reference gene under the experimental conditions of this study.