SUPLEMENTARY DATA

Table 1: Antibodies used for WB, IP and ChIP analysis

Antibody	Source	Reactivity	Company	Catalogue	Dilution
Anti-Mouse-AP conjugated	Goat	Mouse	Sigma	A3562	2500
Anti- Goat- AP conjugated	Rabbit	Goat	Sigma	A2168	2500
Anti-Rabbit-AP conjugated	Goat	Rabbit	Sigma	A3687	2500
Anti-FLAG	Mouse	Artificial peptide	Sigma	F3165	1000
Anti-PBEF1 (NAMPT)	Rabbit	Human	Abcam	Ab24149	700
Anti-GAPDH	Mouse	Human, Mouse Rat Rabbit	Abcam	Ab9484	700
Anti NMNAT1	Goat	Human, Mouse, Rat	Santa Cruz	Sc-30841	500
Anti RNA polII	Mouse	Human, Mouse, Rat	Upstate	05-623	500
Anti-SrT1	Rabbit	Mouse, Human	Upstate	07-131	1000
Anti-act-H3	Rabbit	Mouse, Human	Upstate	06-599	1000
Anti-actK9K14,H3	Rabbit	Human, Mouse, Rat	Cell signaling	9671	1000
Anti-act lysine	Rabbit	All species	Cell signaling	9441	1000
Anti-actK5,H4	Rabbit	Human	Upstate	07-327	500
Anti-alpha Tubulin	Mouse	Human, Mouse, Rat	Santa Cruz	Sc5286	1000
Anti-PCAF	Rabbit	Human, Mouse, Dog	Abcam	Ab12188	500
Anti-Sox9	Rabbit	Human, mouse cow	Abcam	Ab3697	500
Anti-dimethyl-K9,H3	Rabbit	Human	Upstate	07521	1000
Anti-PGC1α	Rabbit	mouse, rat and human	Santa Cruz	Sc-13067	500
Anit-IgG	Rabbit	NA	Santa Cruz	Sc-2027	500
Anti-GCN5	Rabbit	mouse, rat and human	Santa Cruz	Sc-20698	500
Anti- EF1α	Mouse	mouse, rat and human	Upstate	05-235	1000

Table 2: Human primers for RT/PCR, siRNA and ChIP Analyses

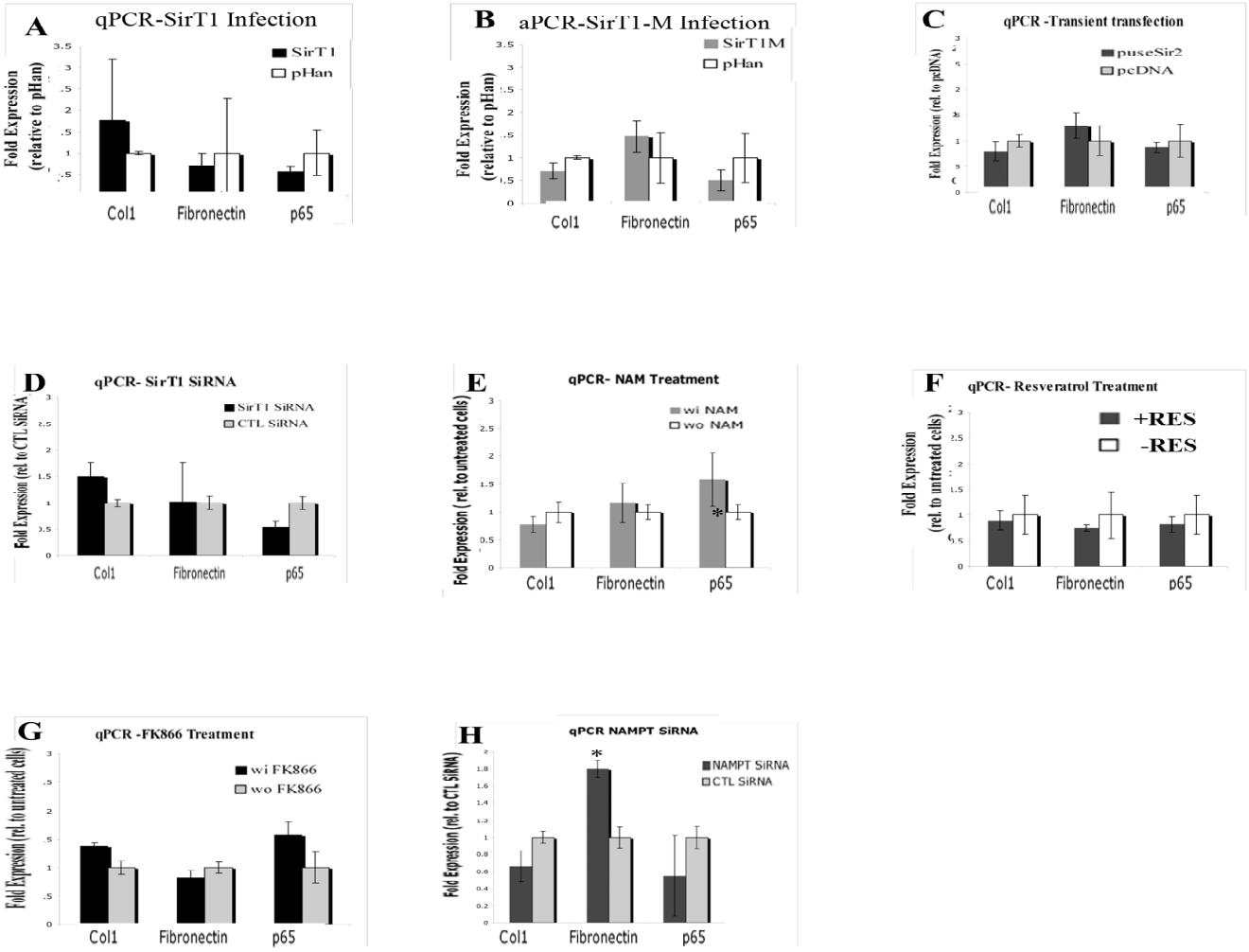
Gene	Primers	Amplicon size (bp)	Annealing Temp (ºC)	Cycle #	Application
GAPDH	F-5'-CCA GGA CAT CAT CAT CCC TGC CTC TAC-3' R5'- GGT CTC TCT CTT CCT CTT GTG C-3'	452	54	25	RT/PCR
Col9(α1)	F5'-CTA CAT TGC AGG TGG CTT ACA A-3' R5'- GGA TTA TCT GCA AGT TTT CCC A-3'	516	54	35	RT/PCR
+Collagen 2(α1)	F5'-CCG CGG TGA GCC ATG ATT CG-3' R5'- CAG GCC CAG GAG GTC CTT TGG G-3'	377(a-form) 171(b-form)	57	35	RT/PCR
Aggrecan	F5'-AGG CTG GGG AGA GAA CTG AAA AG-3' R5'- GCT CAC AAT GGG GTA TCT GAC AG-3'	463	57	35	RT/PCR
COMP	F5'-CAA CTG TCC CCA GAA GAG CAA-3' R5'-TGG TAG CCA AAG ATG ATG AAG CCC-3'	588	57	35	RT/PCR
SirT1	F5'-GCT TAT TTG TCA GAG TTC CCA CCC -3' R5'- CAG CAT TTT CTC ACT GTT CCA GCC 3'	308	60	35	RT/PCR
NMNAT	F5'-TCA TTC AAT CCC ATC AAC AA-3' R5'- CAC AAA TTG GGA ACA GCA AA -3'	461	59	35	RT/PCR
NAMPT	F5'-TGA ATG CCG TGA AAA GAA GA-3' R5'- AAT TTG TTG CCA CTG TGA TT -3'	383	57	35	RT/PCR
GAPDH	F5'-CAA GGC TGA GAA CGG GAA GC-3' R5'- AGG GGG CAG AGA TGA TGA CC -3'	194	57	40	RT/PCR
COMP	F5'-TGT CCC CAG AAG AGC AAC CC-3' R5'- ATT GTC GTC GTC GTC GC -3'	527	59	40	RT/PCR
Agrrecan	F5'-TGC GGG TCA ACA GTG CCT ATC-3' R5'- CAC GAT GCC TTT CAC CAC GAC -3'	181	59	40	RT/PCR
*Collagen 2(α1) Enhancer	F5'-ATC CTC CTT TGT GAG GCT TGT T-3' R5'- AGT ACG AGA GAA CCC ACT GGA C -3'	181	62	40	ChIP
**Collagen 2(α1) Promoter	F5'-AGC GTG ACT CCC AGA GAG G-3' R5'- CAG CGC TCT GCG TCT TCT -3'	200	62	40	ChIP
GAPDH	F5'-TAC TAG CGG TTT TAC GGG CG-3' R5'- TCG AAC AGG AGC AGA GAG CGA -3'	166	59	40	ChIP
PBEF SiRNA Invitrogen	S.5'-GGU GGG UUG UGU UGG UUU GAA AUA A-3' AS.5'- CCA CCC AAC ACA AGC AAA GUU UAU U-3'	NA	NA	NA	SiRNA
SirT1 SiRNA Ambion	S.5'-GGC CAC GGA UAG GUC CAU A-3' AS.5'- UAU GGA CCU AUC CGU GGC C-3'	NA	NA	NA	SiRNA
CTL SiRNA Qiagen (cat# 1027281)	S.cat#229233 AS. cat#229234	NA	NA	NA	SiRNA
Collagen 1(α1)	F 5'- G G A T T C C A G T T C G A G T A T G G -3' R 5'- T G C A G T G G T A G G T G A T G T T C -3'	143	55	45	RT/PCR
Fibronectin 1	F 5'- G C C A T G T G T C T T A C C A T T C A -3' R 5'- T G A A C C A A A A C A G T G T G G T C -3'	153	55	45	RT/PCR
p65 (NFkB)	F 5'- A T G G C T T C T A T G A G G C T G A G -3' R 5'- C A C A G C A T T C A G G T C G T A G T -3'	182	55	45	PT/PCR
MMP1 human	F 5'- C T G G A G G A A A T C T T G C T C A T -3' R 5'- C C C C G A T A T C A G T A G A A T G G -3'	171	55	45	RT/PCR

+This set of primers will differentiate between the two splice variants of collagen 2(α1); the a-form is 377 bp while the b-form is 171 bp.

*These primers were used in the ChIP assay for the collagen 2(α1) enhancer.

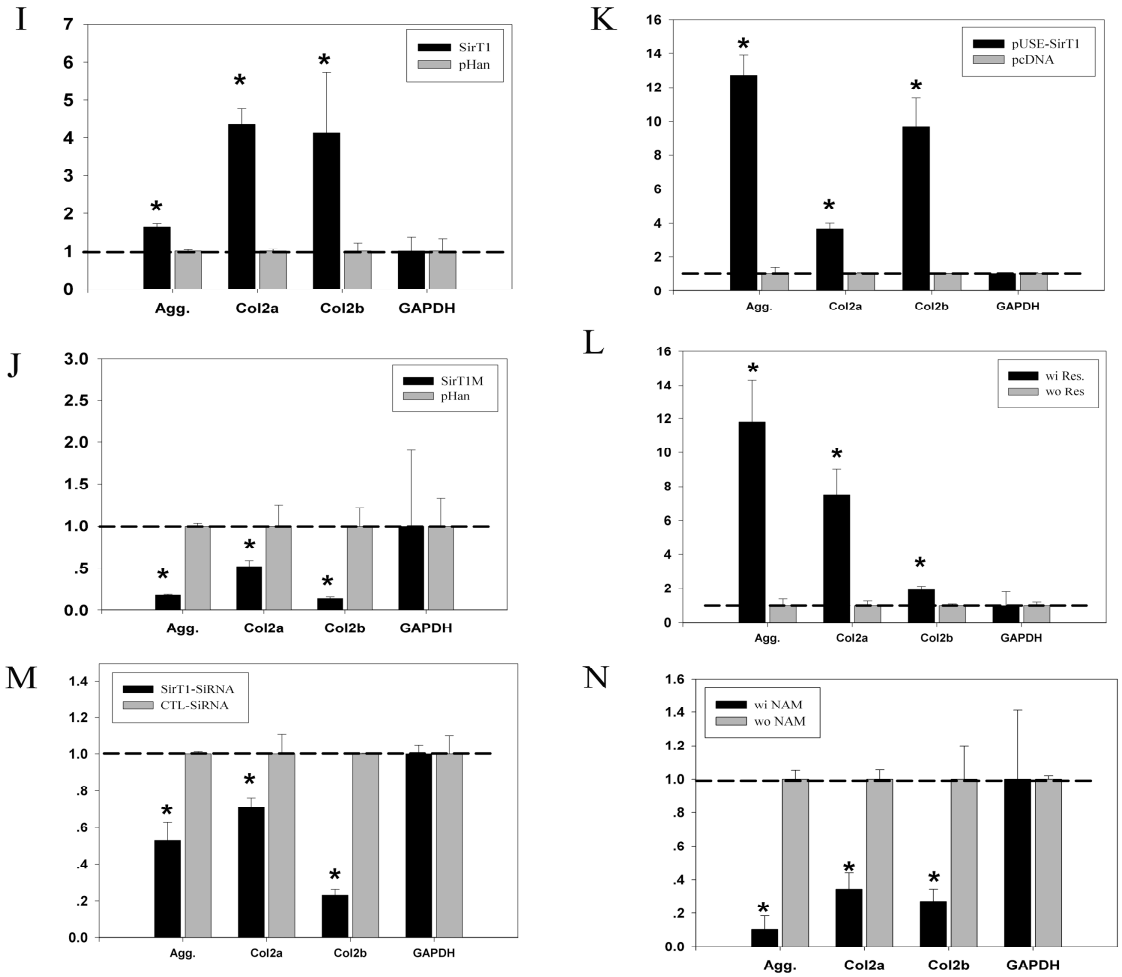
**These primers were used in the ChIP assays for the collagen 2(α1) promoter

Supplementary Data. Dvir-Ginzberg et al., 2008



Figures A-H. qPCR for non-lineage specific genes. To verify that SirT1 on gene expression was specific for cartilage genes we also assessed expression of non-lineage specific genes such as collagen 1, fibronectin and p65(NFkB). In nearly all the treatment conditions shown these genes showed no signficiant changes in expression levels (LSD, $p<0.05$). The exceptions were that p65 was lower in sirT1 siRNA-treated cells and higher in NAMPT siRNA-treated cells.

Supplementary Data. Dvir-Ginzberg et al., 2008



Figures I-N. qPCR experiments for chondrcyte-specific gene expression. All experiments were repeated via qPCR to confirm the results of the RT-PCR. The genes inspected were Aggrecan, Collagen2 and GAPDH as an internal control. In the stable and transient transfections with SirT1 (I, K, respectively) Expression of Aggrecan, Collagen 2a and Collagen 2b wasfound to be significantly higher than pHan vector control. The same genes were found to be suppressed in the SirT1M stable cells compared to the control (J). Resveratrol treated cells displayed elevated levels of Aggrecan and Collagen 2a as compared to untreated cells(L). NAM-treated cells showed a significant reduction in Aggrecan, Collagen 2a and Collagen 2b. Overall, the qPCR results are in agreement with a the RT-PCR results. The results are averages of triplicates of 2 different experiments (n=6).

Supplemenary data

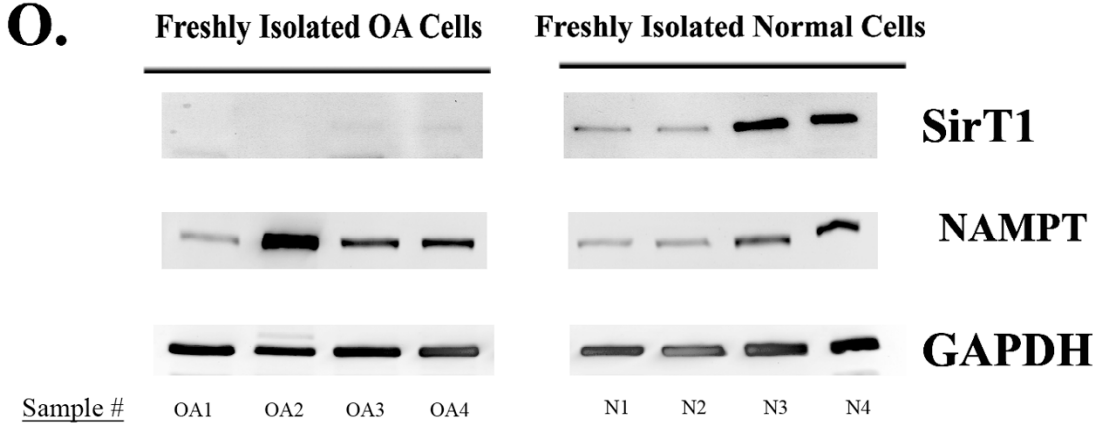


Figure O. Protein extracts were generated from chondrocytes freshly isolated (not passed in culture) from either cadavers (Normal) with no sign of arthritis (ave. age 73) or from osteoarthritic (OA) patients (ave age 62). The protein samples were immunoblotted and probed for SirT1, NAMPT and GAPDH. Results indicate higher levels of SirT1 expression in Normal versus. OA chondrocytes while NAMPT expression levels appeared to be higher in the OA samples. Note that the cadaver chondrocytes were viable since they can proliferate in culture. Also, assessment of MMP13 expression shows that all the Normal samples did not express this gene while all the OA samples expressed high levels (data not shown).