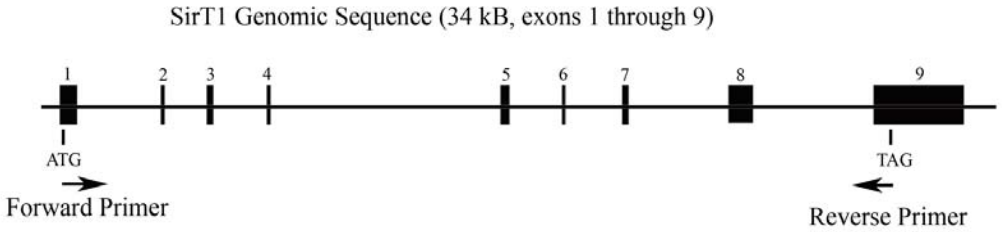
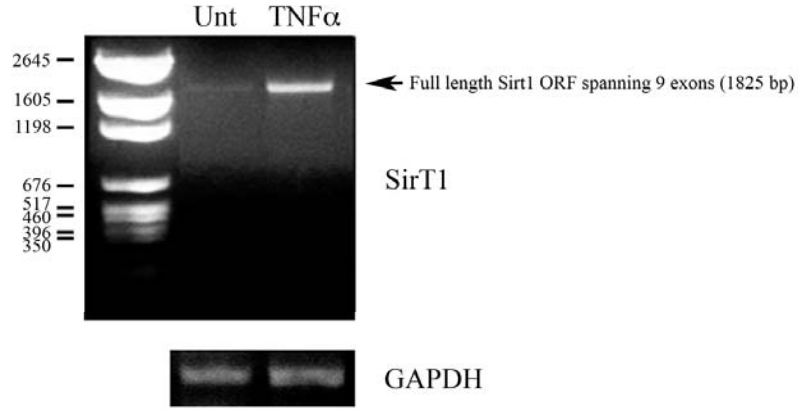
TNFα-Mediated Cleavage and Inactivation of SirT1 in Human Osteoarthritic Chondrocytes
Dvir-Ginzberg *et al*., 2011, Arthritis and Rheumatism

Supplementary Data

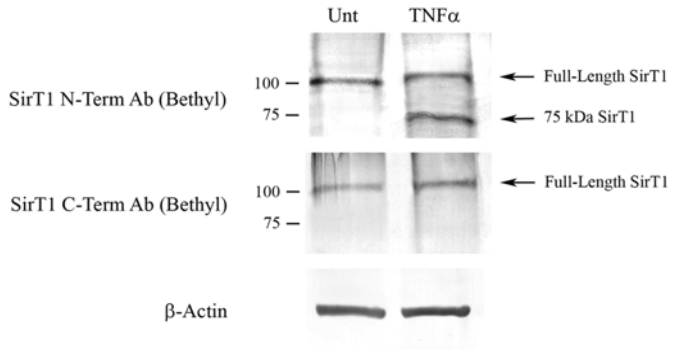




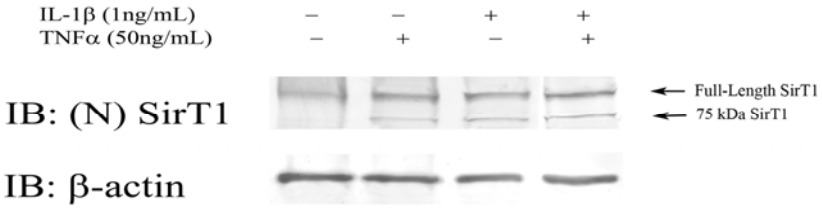
SD1: Total RNA from chondrocytes treated with or without TNFα (50 ng/ml) for 24 hours was processed for RT/PCR with primers that flank the start and stop codons of human SirT1. The full-length open reading frame of SirT1 is 1825 bp as shown.

Supplementary Data

A.

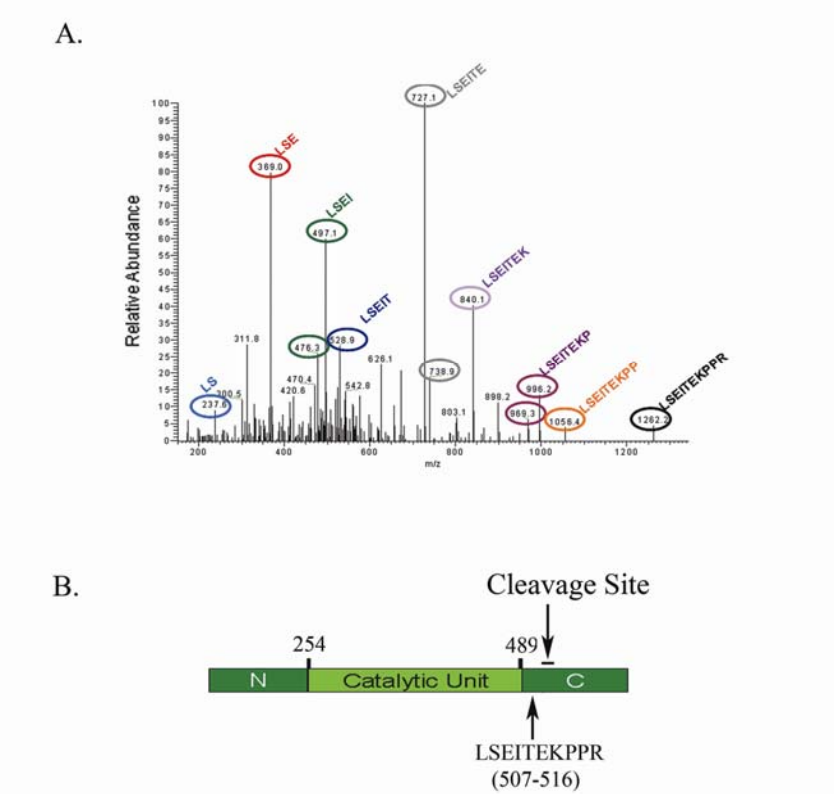


B.



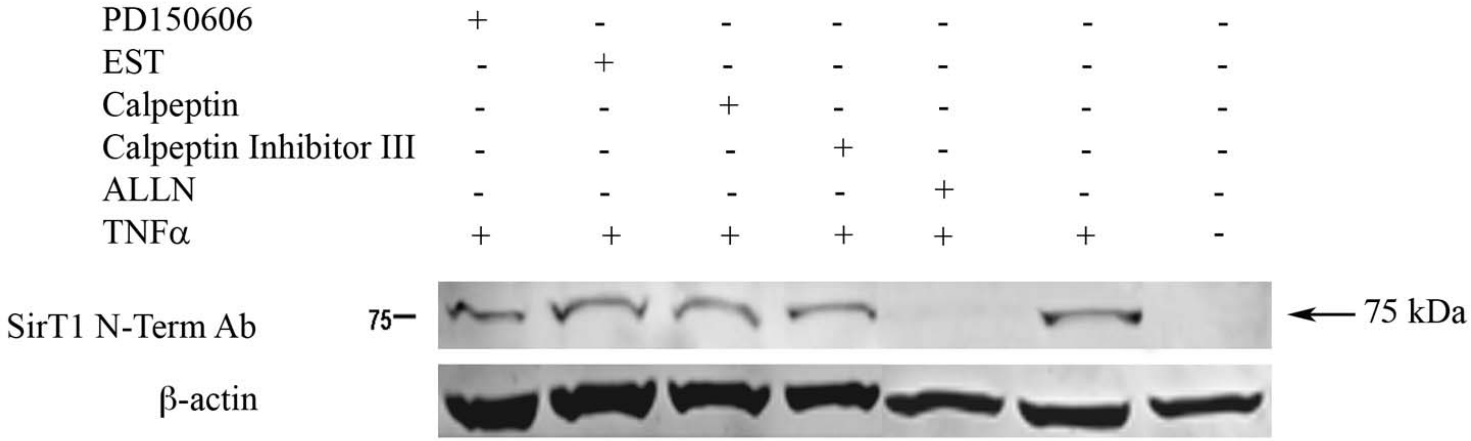
SD2: 75kDa SirT1 is generated under proinflammatory stress. A. Chondrocytes were treated with or without TNFα (50 ng/ml) for 24 hours. Extracts were generated and processed for immunoblotting with the indicated antibodies. **B.** Human chondrocytes were treated with or without TNFα (50 ng/ml) and/or IL-1β (ng/ml) for 24 hours, as indicated above the immunoblot. Extracts were blotted with indicated antibodies. The positions of full-length SirT1 and the 75 kDa SirT1 fragment are shown.

Supplementary Data



SD3: Identification of 75 kDa protein fragment as SirT1. SirT1 was overexpressed in human chondrocytes and immunoprecipitated. the IPs were subjected to electrophoresis and the gel stained with MagicBlue. The band corresponding to 75 kDa was excised and trypsin digested. This was followed by protein sequencing using the NanoLC-MS/MS peptide sequencing technology (Protech). The peptide fragments were identificed as shown above. The LSEITEKPPR sequence was a predominant peptide located between positions 507 and 516 of human SirT1.

Supplementary Data



SD4: Chondrocytes were treated with or without TNFα (50 ng/ml) in the presence or absence of the indicted protease inhibitors for 24 hours. Extracts from the treated cells were immunoblotted with the indicated antibodies as shown. The 75 kDa SirT1 fragment of SirT1 is shown.

Supplementary Data

Table 1: Human primers RT/PCR and ChIP analyses.

Gene	Primers	Amplicon size (bp)	Annealing Temp (^{o}C)	Cycle #	Application
Human SirT1	F5'-GCT TAT TTG TCA GAG TTC CCA CCC -3' R5'- CAG CAT TTT CTC ACT GTT CCA GCC 3'	308	60	40	qPCR
Human Col2a1	F5'-GGA AAC TTT GCT GCC CAG ATG-3' R5'- TCA CCA GGT TCA CCA GGA TTG C -3'	167	59	40	q/PCR
Human Col2a1 (a&b forms)	F5'-CCG-CGG-TGA-GCC-ATG-ATT-CG -3' R5'-CAG-GCC-CAG-GAG-GTC-CTT-TGG -3'	377(a) 171(b)	57	35	qPCR
Human Coll11a1	F5'-GCC ACC GTT TCG TTT TCC AC-3' R5'- GCC CTT TGA CTT TTT CCC CC -3'	178	57	40	qPCR
Aggrecan	F5'-TGC GGG TCA ACA GTG CCT ATC-3' R5'- CAC GAT GCC TTT CAC CAC GAC -3'	181	59	40	qPCR
Human GAPDH	F5'-CAA GGC TGA GAA CGG GAA GC-3' R5'- AGG GGG CAG AGA TGA TGA CC -3'	194	57	40	RT/PCR, qPCR
Collagen 2a1 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-qPCR
Collagen 2a1 Promoter	F5'-AGC GTG ACT CCC AGA GAG G-3' R5'- CAG CGC TCT GCG TCT TCT -3'	200	62	40	ChIP-qPCR
ADAM metallopeptidase with thrombospondin type 1, motif, 4 (ADAMTS4)	F5' ACAAAGATCCAGGAAAGGAGGGCT R5 AGGGCTGAGGACCGTTAAAGGAAA	117	59	40	qPCR
ADAM metallopeptidase with thrombospondin type 1, motif, 5 (aggrecanase-2) (ADAMTS5)	F5' TTCAACGTCAAGCCATGGCAACTG R5' TGACGATAGGCAAACTGCACTCCT	93	60	40	qPCR

Supplementary Data

Table 2: Antibodies used for immunoblotting, immunoprecipitation and ChIP analyses.

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	All species	Sigma	F3165	1000
Anti-His	Mouse	All species	Millipore	MAB3118	1000
Anti-β-actin	Mouse	Human, Mouse Rat Rabbit	Abcam	Ab8226	2000
Anti-N-SirT1	Rabbit	Mouse, Human	Millipore	07-131	1000
Anti-N-SirT1	Rabbit	Human	Bethyl	A300-687A	1000
Anti-C-SirT1	Rabbit	Mouse,	Bethyl	A300-688A	1000
Anti Cathepsin B * Recognizes the Active subunit and Procathepsin B	Rabbit	Human	Millipore	AB4064	1000
Anti Cathepsin L * Recognizes the Active subunit	Rabbit	Human	Millipore	AB4097	1000
Anti-Sox9	Rabbit	Human, mouse cow	Abcam	Ab3697	500
Anti-PGC1α	Rabbit	NA	Santa Cruz	Sc-2027	500
Anit-IgG	Rabbit	mouse, rat and human	Santa Cruz	Sc-20698	500