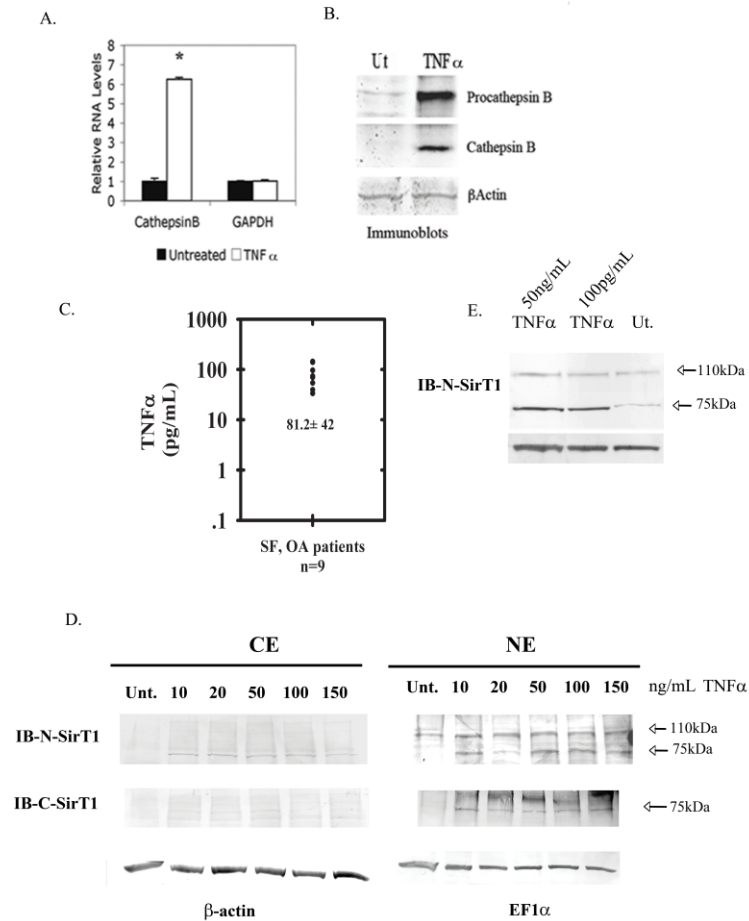


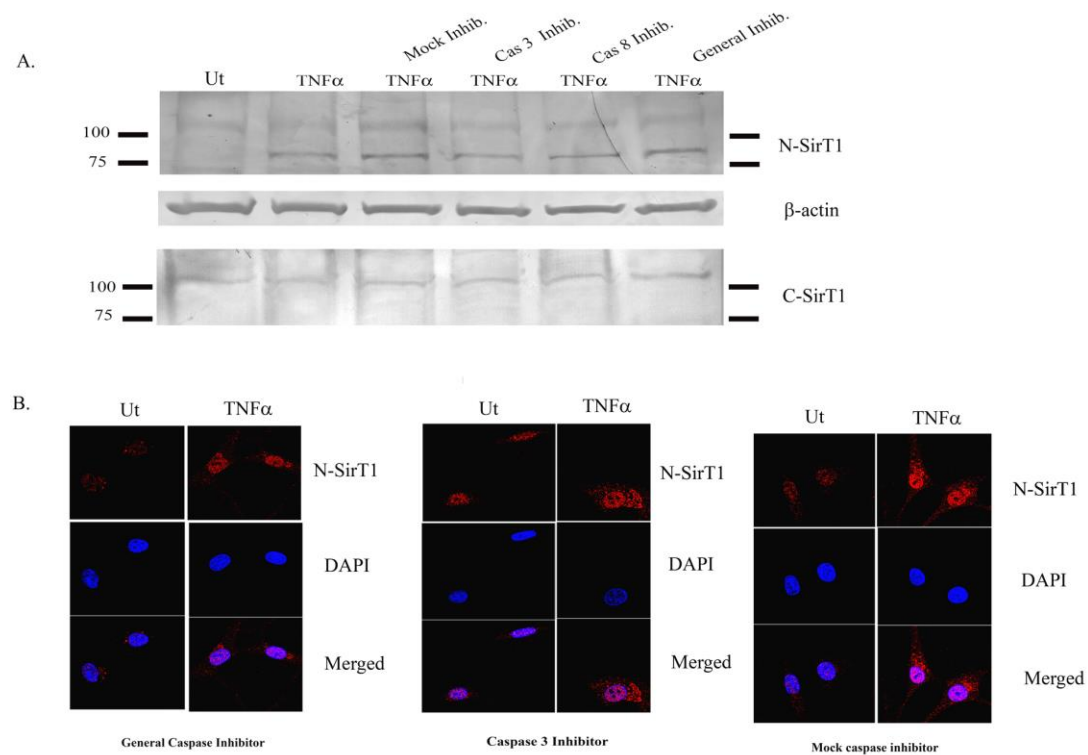
SD-1



SD-1-Cathepsin B levels in TNF α treated hCh. **A.** RNA and **B.** protein extracts from TNF α treated and untreated human chondrocytes (hCh) were analyzed via qPCR (left) and immunoblot analyses (right) for pro- and active Cathepsin B. **C.** ELISA assay for levels of human TNF α in OA-derived (n=9) samples, reveal an average of 81.2 \pm 42pg/mL TNF α in synovial fluid. **D.** Cytoplasmic extracts (CE) and nuclear extracts (NE), obtained from human chondrocytes (p1) exposed to TNF α (10-150ng/mL) reveal that the 75SirT1 fragment is generated in lower concentrations than 50ng/mL. **E.** Protein extracts from chondrocytes exposed to physiological concentration (i.e. approx. 100pg/mL, see panel C) of TNF α present enhanced levels of 75SirT1, similar to those treated with 50ng/mL TNF α . TNF α concentrations are indicated above blots D and E. Arrows point towards the full-length SirT1 (i.e. FLSirT1, 110kDa) and cleaved SirT1 (i.e. 75SirT1, 75kDa).

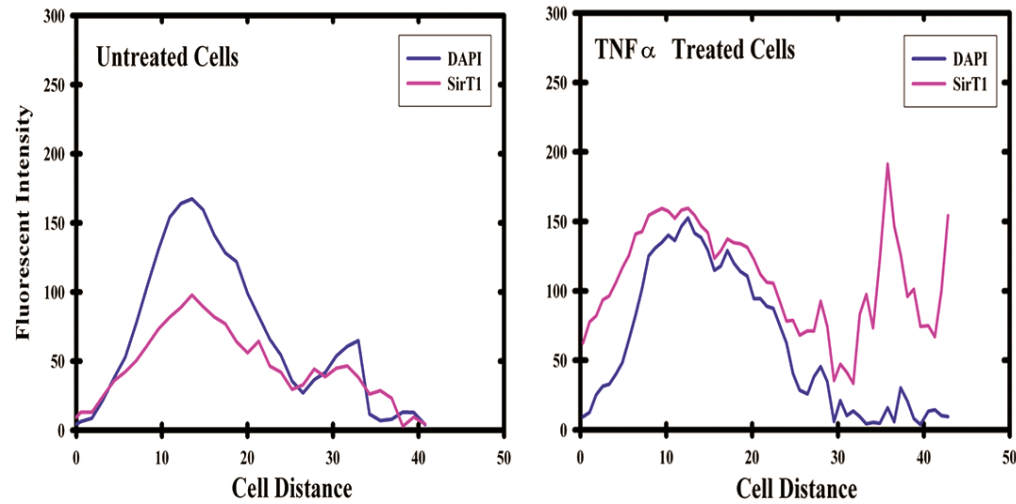
SUPPLEMENTAL DATA

SD-2



SD-2: 75SirT1 is not generated through caspase-mediated cleavage following TNF α stimulation. **A.** Human OA chondrocytes plated (p0) and treated with TNF α and various caspase inhibitors (indicated above the blot), were immunoblotted with N-terminally reactive SirT1 (N-SirT1) antibody. Blots display no change in 75SirT1 formation when treated with TNF α and caspase inhibitors. **B.** Confocal images of OA hCh treated with general caspase inhibitor (left panel); caspase 3 inhibitor (middle panel) and mock caspase inhibitor (right panel). The data overall indicate that 75SirT1 cytoplasmic export is not dependent on the caspase pathway.

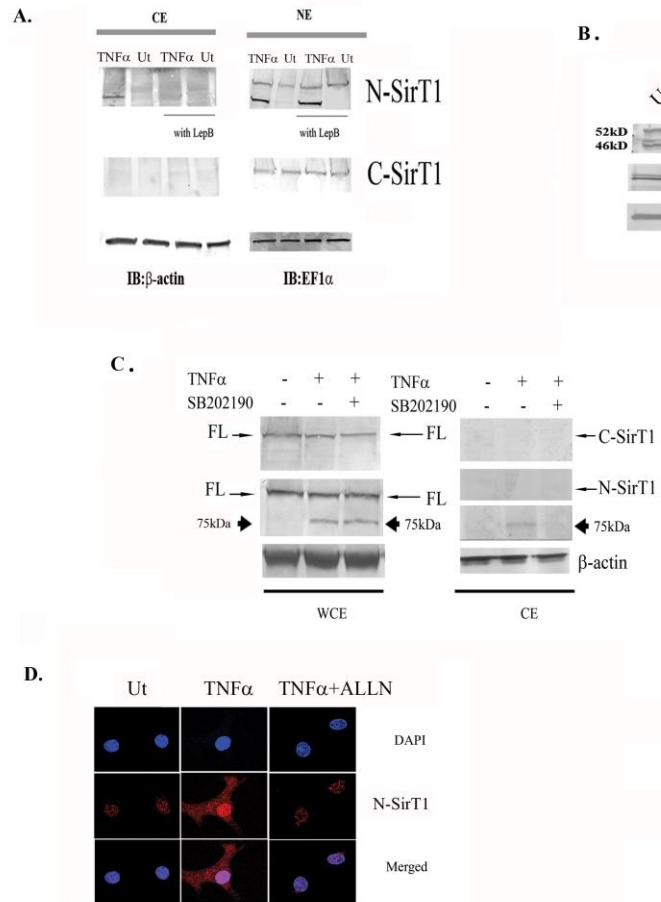
SD-3



SD-3: 75SirT1 is exported to the cytoplasm following TNF α treatment. Human OA chondrocytes were treated with and without TNF α and stained with N-SirT1 antibody (denoted in the pink line) and DAPI (denoted in the blue line). Fluorescent intensity of N-SirT1 and DAPI as a function of distance along the cell axis was quantified in n=50 OA hCh of each treatment (derived from 4 different cell sources). The results show approx. 50% of N-terminally intact SirT1 (pink line) is export outside the nucleus (blue line).

SUPPLEMENTAL DATA

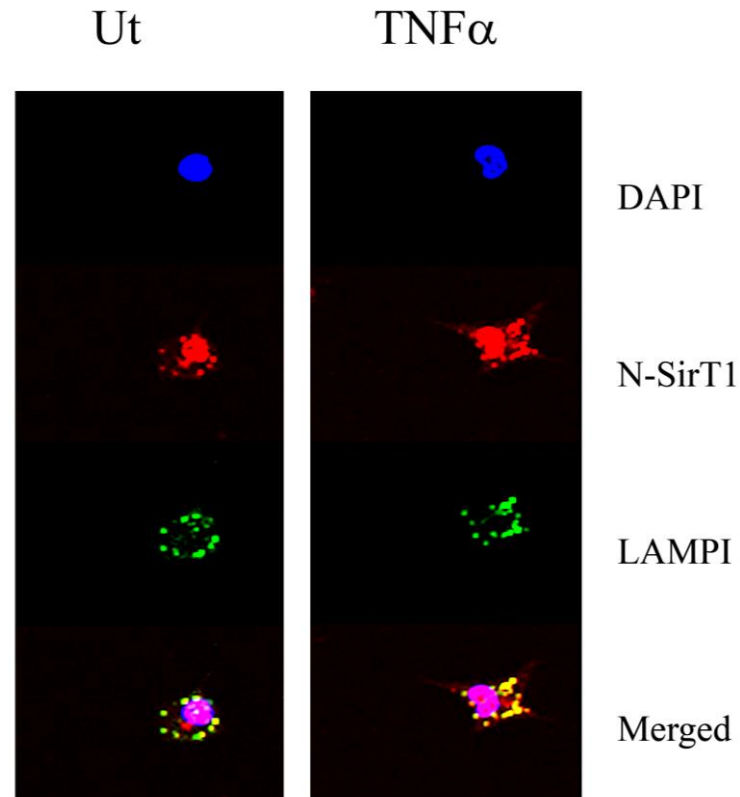
SD-4



SD-4: 75SirT1 is exported to the cytoplasm in a MAPK p38, CRM1-dependent fashion, following TNF α -treatment. **A.** Immunoblot analyses of OA hCh treated with TNF α and LepB, show that inhibiting CRM1-mediated export of SirT1, results in reduced 75SirT1 in the cytoplasmic compartment (CE; left panel, consistent with Figure 2D), but does not affect the level of generated 75SirT1 (NE; right panel). **B.** Immunoblot analyses of crude extracts from OA hCh treated and untreated with TNF α , revealed no difference in JNK phosphorylation, supporting that 75SirT1 is exported via TNF α -induced MAPK p38 pathway (upper panel of Figure 2E). **C.** Whole cell extracts (left panel; WCE) and cytoplasmic extracts (right panel; CE) were obtained from hCh (treated as indicated above the blots) and immunoblotted for N-SirT1, C-SirT1 and β -actin antibodies. SB202190 (1 μ g/mL) inhibits p38 activity and was supplemented as indicated above the blots. Inhibiting MAPK p38 does not affect SirT1 cleavage, however it does prevent export of SirT1 (as seen in Figure 2E). "FL" denotes the full-length SirT1 and "75kDa"- denotes the N-terminally intact cleaved 75SirT1 fragment. **D.** Confocal images of endogenous SirT1 stained with N-SirT1 antibody, in TNF α treated (TNF α) and untreated OA hCh (Ut). ALLN inhibits Cathepsin B-mediated cleavage of SirT1 and was supplemented as indicated above the micrographs. The images show that similar to untreated cells, N-terminally intact SirT1 (red fluorescence) is not located in the cytoplasm in the presence of ALLN and TNF α .

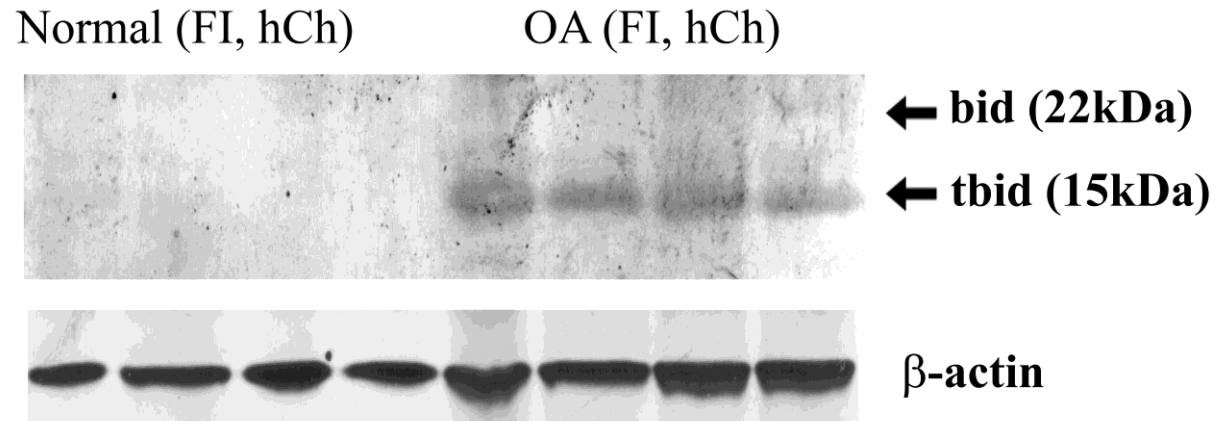
SUPPLEMENTAL DATA

SD-5



SD-5: 75SirT1 does not display enhanced lysosomal colocalization following TNF α stimulation. Human chondrocytes were treated and untreated with 50ng/mL TNF α (denoted as "TNF α " and "Ut", respectively). Confocal images of coimmunostained OA hCh (green fluorescence- Lysosomal-associated membrane protein 1 or LAMP-I and red fluorescence -N-SirT1 antibodies) revealed no significant difference in SirT1 colocalization with lysosomes, amongst the treatments.

SD-6



SD-6: tBid is enhanced in OA vs. normal freshly isolated human chondrocytes. Human chondrocytes were isolated from age-matched normal and OA samples and protein extracts were subjected to western blot analyses. While OA samples showed augmented tBid levels, normal samples showed undetected tBid or Bid levels (n=7).