## Supplementary Fig. 1



Supplementary Fig. 2



## **Supplementary Figure Legends**

**SFig. 1.** Regulation of MMP-3 expression by TNF- $\alpha$  and IL-1 $\beta$  by nucleus pulposus cells. A) Real-time RT-PCR analysis of cells treated with cytokines show increase in expression of MMP-3. B) Western blot analysis shows increased level of secreted MMP-3 in cells treated with cytokines for 24 h. Values shown are mean <u>+</u> SE from three independent experiments, \* p < 0.05. C) Western blot of MMP-3 in SDC4 silenced NP cells treated with TNF- $\alpha$  and IL-1 $\beta$ . A significant decrease in MMP-3 expression following cytokine treatment is seen in SDC4 silenced cells. D, E) Western blot analysis to measure aggrecan neoepitope (ARGSVIL) formation in conditioned medium of D) rat NP and *E*) human NP cells treated with TNF- $\alpha$  and IL-1 $\beta$  with or without MMP-3 inhibitor NNGH for 24 h. Note, there is no change in ADAMTS dependent neoepitope generation when MMP-3 activity is suppressed in either rat or human NP cells. Representative of two independent experiments.

**SFig. 2.** TNF- $\alpha$  and IL-1 $\beta$  mRNA expression in human degenerate NP and AF tissues. A) Realtime RT-PCR analysis of TNF- $\alpha$  and IL-1 $\beta$  mRNA expression in multiple human NP and AF tissue samples. Sample number for each degenerative grade is indicated. With disc degeneration increasing expression in both the cytokines is seen. Expression in normal control (c) sample was set at 1.0 for both TNF- $\alpha$  and IL-1 $\beta$  and indicated by a horizontal line. C) Human degenerate NP protein extracts used in Western blot analysis, gel electrophoresed and stained with coomasie blue. The distinct bands in each sample verifies the quality of the protein samples and equivalent protein loading.