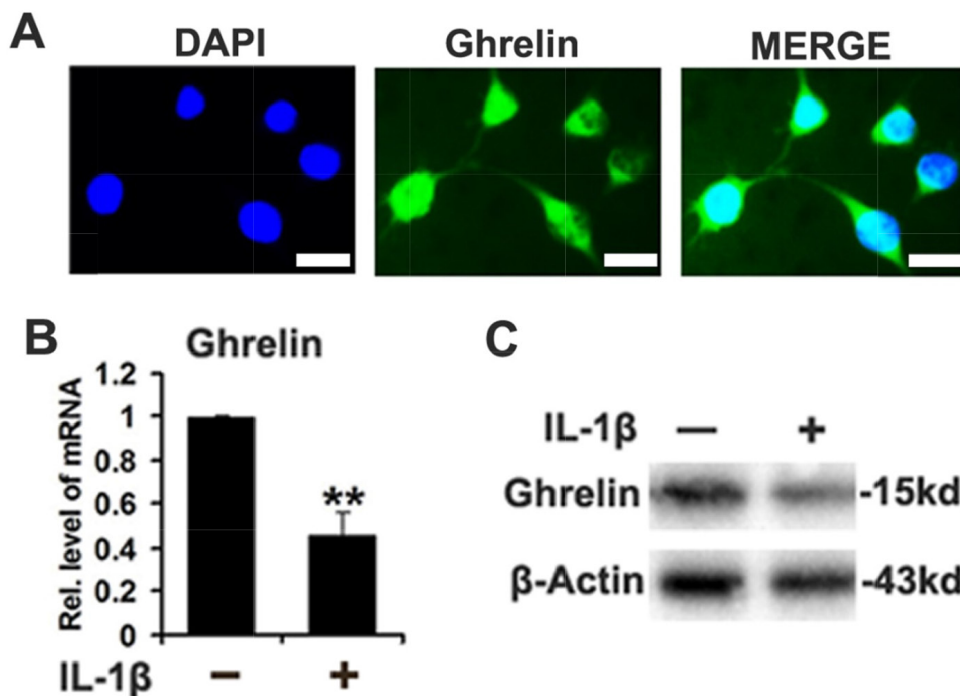
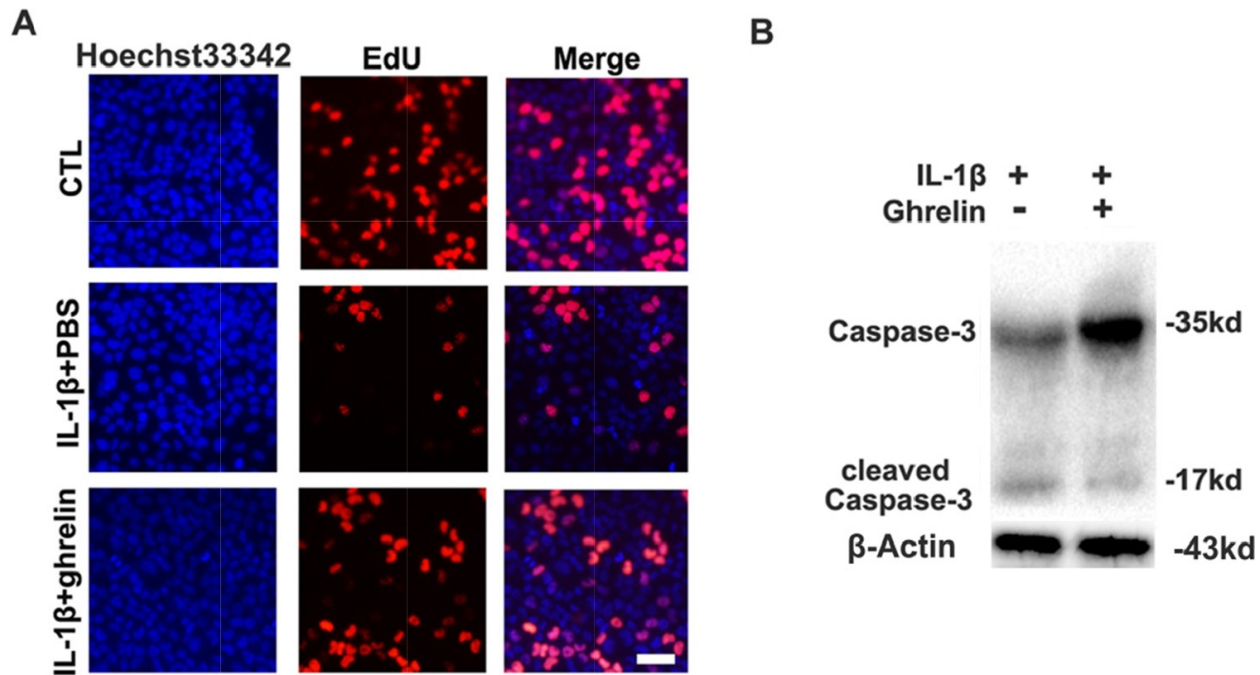


## Ghrelin protects against nucleus pulposus degeneration through inhibition of NF- $\kappa$ B signaling pathway and activation of Akt signaling pathway

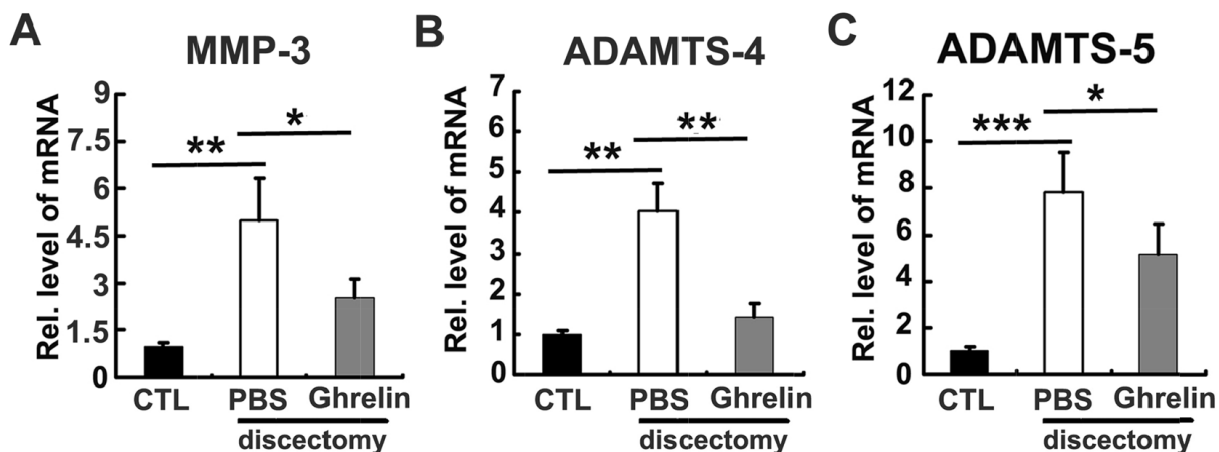
### SUPPLEMENTARY MATERIALS



**Supplementary Figure 1: Expression pattern of ghrelin in NP cells.** (A) Ghrelin is expressed in plasma of primary human NP cells. NP cells were isolated, and cell immunostaining was performed for ghrelin. (B) RNA level of ghrelin is diminished by IL-1 $\beta$ , as assessed by real time PCR. NP cells were cultured with 10 ng/ml IL-1 $\beta$  for 12h, and real time PCR was performed for ghrelin. (C) Protein level of ghrelin in NP cell is reduced through IL-1 $\beta$  treatment, as assayed by Western blot. The values are the mean $\pm$ SD. \* $p$ <0.05 and \*\* $p$ <0.01 vs. Control group. Scale bar=25 $\mu$ m. Each experiment was repeated for three times. Scale bar=25 $\mu$ m.



**Supplementary Figure 2: Ghrelin promotes proliferation and inhibits apoptosis in IL-1β treated NP cells.** (A) The cellular proliferation was detected by EdU proliferation assay. IL-1β treatment diminished the ratio of proliferative cells, while ghrelin reversed cell proliferation in NP cells. Blue dots, all cells; red dots, proliferative cells. (B) Western blot analysis of Caspase-3 and Caspase-3 cleavage in NP cells. Primary NP cells were isolated and stimulated with 10n/ml IL-1β for 12 hours, in presence or absence of 50 nM ghrelin, and then Western blot was performed.



**Supplementary Figure 3: Ghrelin protects against catabolic biomarkers of NP tissue in a rabbit IVD degeneration model.** (A-C) Ghrelin treatment attenuated the induction of MMP-3, ADAMTS-4 as well as ADAMTS-5 in NP tissue of each treatment group, as assayed by real time PCR. NP tissue was collected and real time PCR was performed. The values are the mean±SD. \*p<0.05, \*\* p<0.01 and \*\*\* p<0.005 vs. Control group. Each experiment was repeated for 3 times.