Figure S1. Curcumenol inhibits TNF-α and IL-1β-induced activation of the MAPK pathway and MMP family proteins in ATDC5 cells. (A and B) Western blot analysis of p-SAPK/JNK, SAPK/JNK, p-ERK, ERK, p-p38 and p38 in ATDC5 chondrocytes stimulated with TNF-α (10 ng/ml) for 10 min. Cells were pretreated with 50 μM curcumenol. Grey scale analysis between p-JNK/JNK, p-ERK/ERK and p-p38/p38. (C and D) Western blot analysis of p-SAPK/JNK, SAPK/JNK, p-ERK, ERK, p-P38 and P38 in ATDC5 chondrocytes stimulated with IL-1β (10 ng/ml) for 10 min. Cells were pretreated with 50 μM curcumenol. Grey scale analysis between p-JNK/JNK, p-ERK/ERK and p-p38/p38. (E and F) Western blot analysis of Col2a1 and MMP3 expression in ATDC5 chondrocytes treated with TNF-α (10 ng/ml) or/and 50 μM curcumenol for 24 h. Grey scale analysis of Col2a1 and MMP3 expression. (G and H) Western blot analysis of Col2a1 and MMP3 expression in ATDC5 chondrocytes treated IL-1β (10 ng/ml) or/and 50 μM curcumenol for 24 h. Grey scale analysis of Col2a1 and MMP3 expression. *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. p-, phosphorylated; Col2a1, collagen type II α 1 chain.

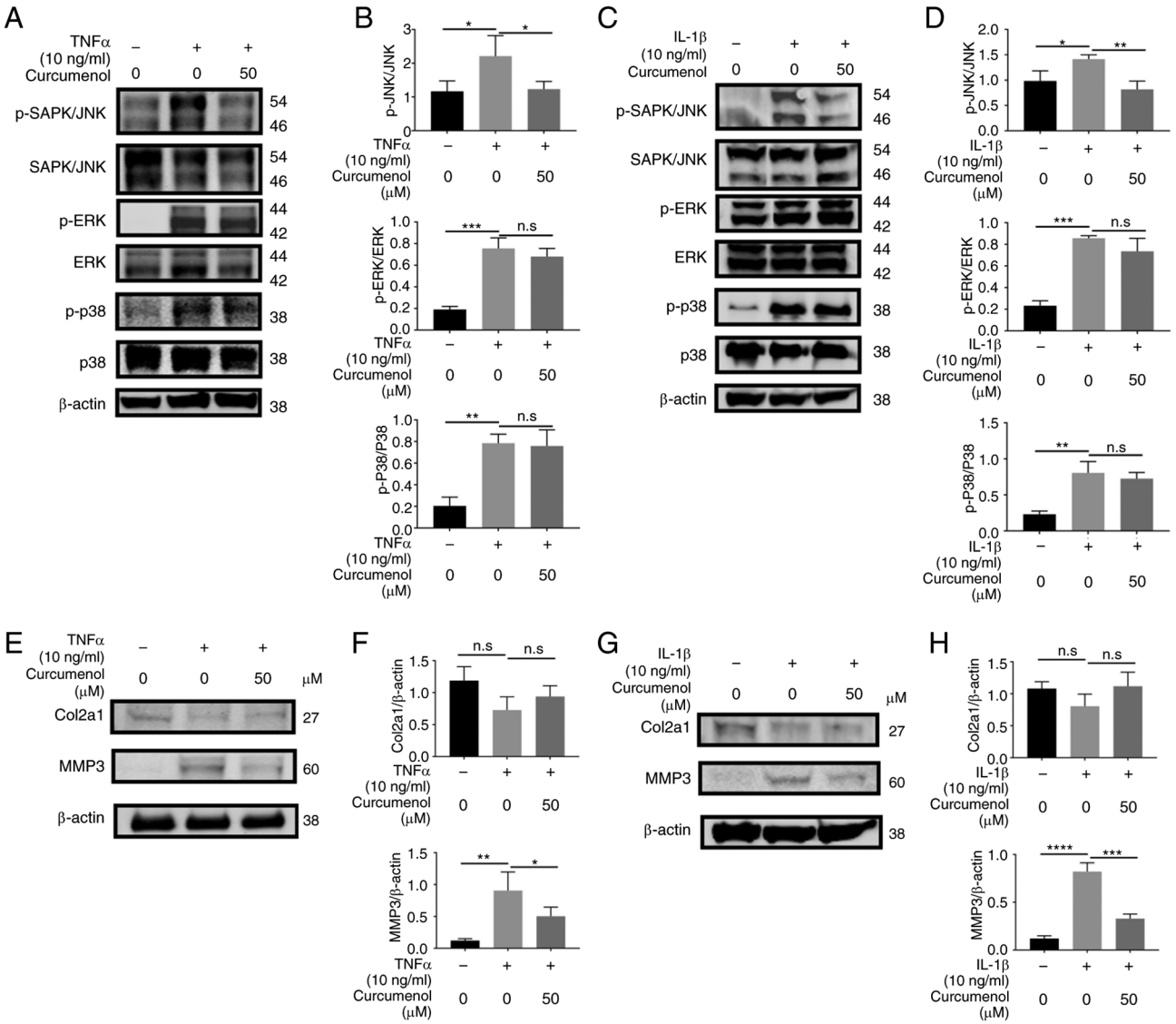

Figure S2. Curcumenol modifies catabolism status induced by TNF-α and IL-1β in pellet culture. (A) Safranin O-Fast Green staining of ATDC5 chondrocytes using pellet culture, at a density of 15 million cells per ml, stimulated with TNF-α (10 ng/ml) or/and curcumenol (50 μM) for 21 days. (B) Quantification of the ratio between SO/FG of pellets was analyzed using Image Pro Plus 6.0. (C) Safranin O-Fast Green staining of ATDC5 chondrocytes using pellet culture, at a density of 15 million cells per ml, stimulated with IL-1β (10 ng/ml) or/and curcumenol (50 μM) for 21 days. (D) Quantification of the ratio between Safranin O/Fast Green of pellets was analyzed. ***P<0.001 and ****P<0.0001. SO, Safranin O; FG, Fast Green.

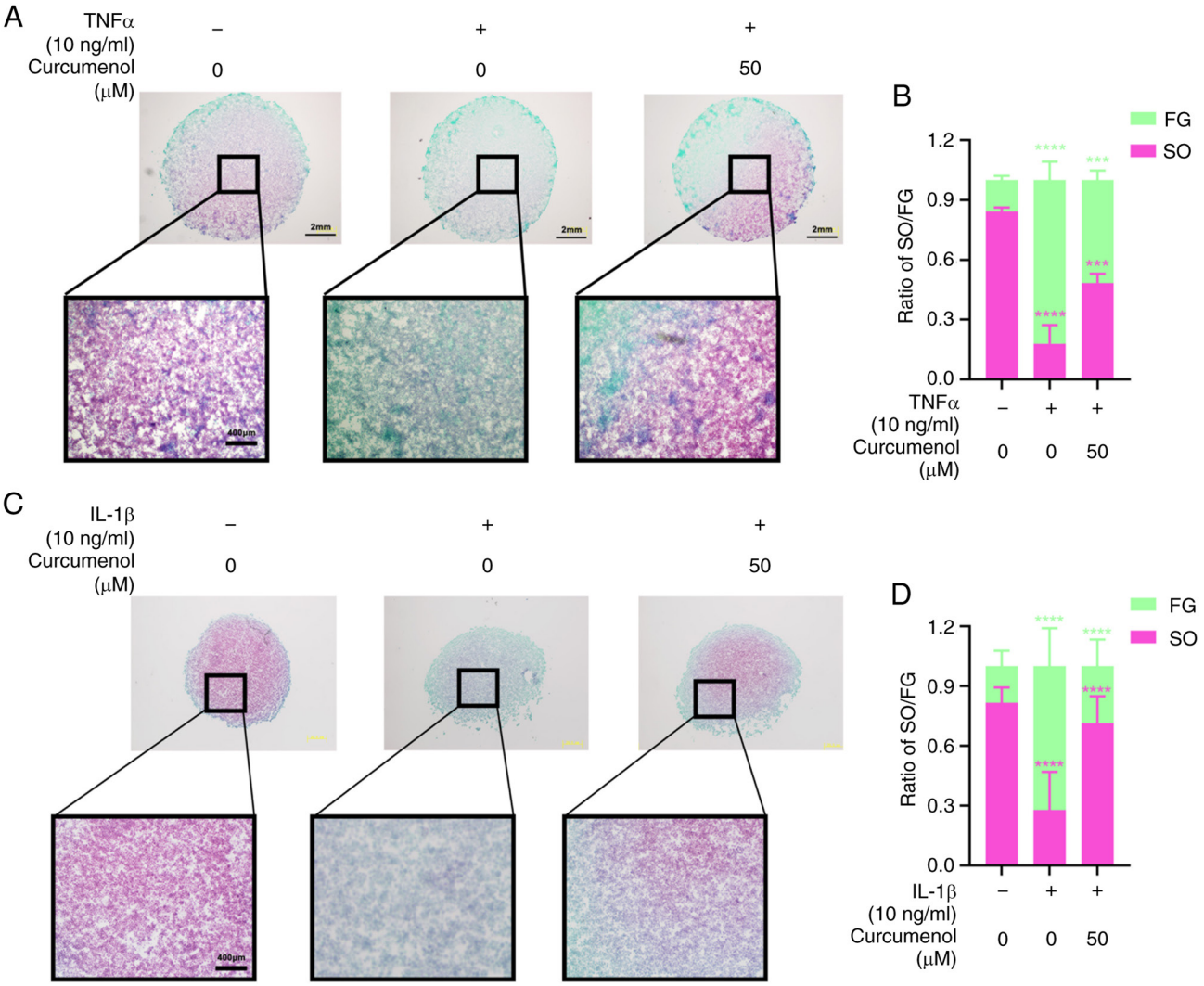

Figure S3. Curcumenol exerts an anti-inflammatory effect, and rescues the senescence of primary chondrocytes by inhibiting the NF-κB and MAPK pathways *in vitro*. (A and B) Senescence β-galactosidase staining of primary chondrocytes stimulated with TNF-α (10 ng/ml) or/and curcumenol (50 μM) for 1 day, and the ratio of positive cells analyzed using Image Pro Plus 6.0. (C and D) Senescence β-galactosidase staining of primary chondrocytes stimulated with IL-1β (10 ng/ml) or/and curcumenol (50 μM) for 1 day, and the ratio of positive cells. (E) Western blot analysis of p-Akt, Akt, p-IKKα, IKKα, p-P65, P65, p-IκBα, IκBα, p-SAPK/JNK, SAPK/JNK, p-ERK, ERK, p-P38 and P38 expression in primary chondrocytes stimulated with TNF-α (10 ng/ml) for 10 min. Cells were pretreated with 50 μM curcumenol. (F) Grey scale analysis between p-P65/P65, p-JNK/JNK and p-IκBα/IκBα shown in panel (E). *P<0.05, **P<0.01, ***P<0.001 and ****P<0.0001. p-, phosphorylated.

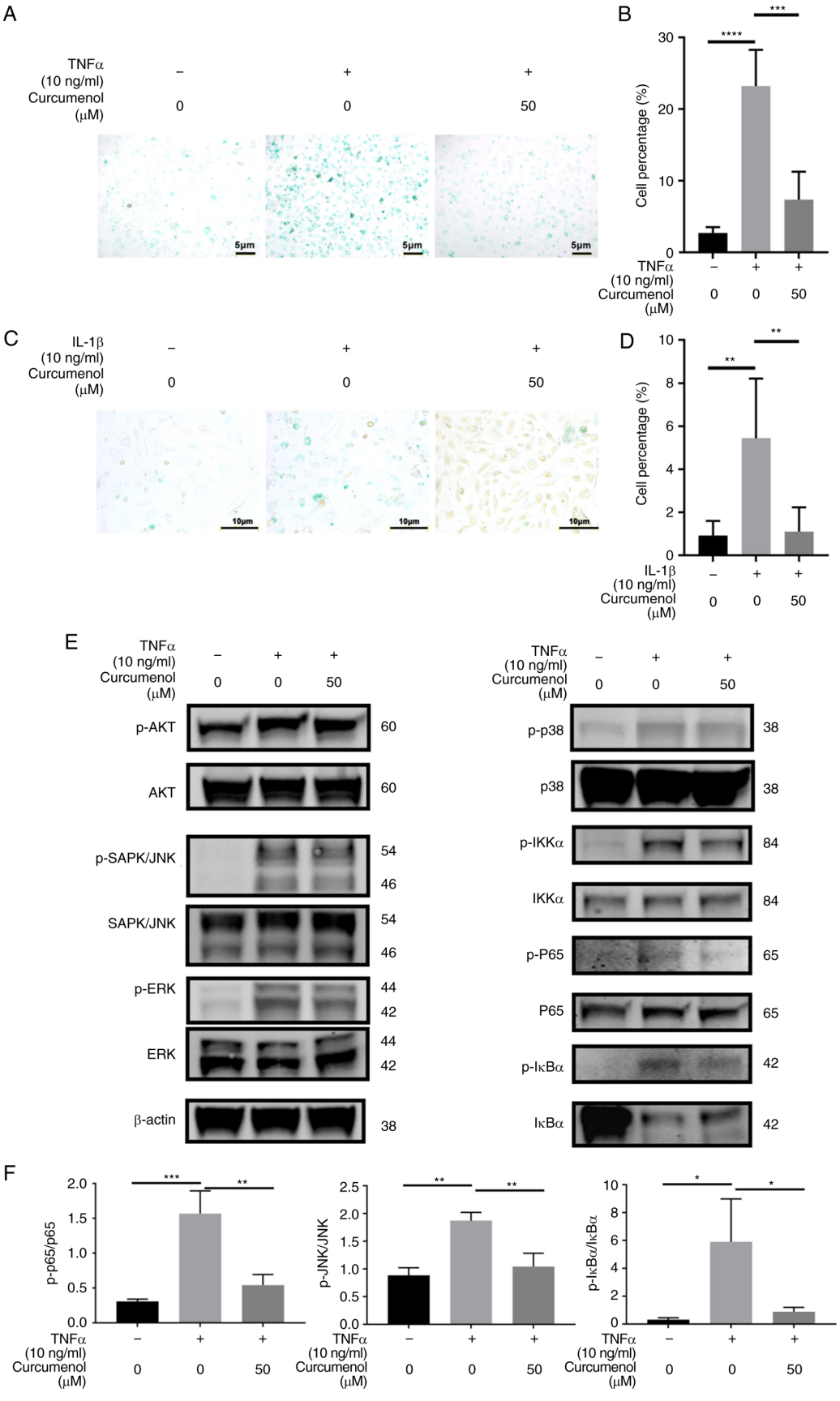

Figure S4. Reactive effect of Curcumenol in inhibiting the NF-κB and MAPK pathways *in vitro*. ATDC5 chondrocytes were pretreated with incomplete media for 2 h at 37˚C, and then stimulated with TNF-α (10 ng/ml) or/and curcumenol (50 μM) for 10 min at 37˚C (A) Western blot analysis of p-SAPK/JNK, SAPK/JNK, p-P65, P65, p-IκBα and IκBα expression in ATDC5 chondrocytes. (B) Grey scale analysis between p-JNK/JNK, p-P65/P65 and p-IκBα/IκBα. **P<0.01, ***P<0.001 and ****P<0.0001. p-, phosphorylated.