Figure S1. AZ inhibits mouse ATDC5 chondrocyte apoptosis. (A) ATDC5 cells were treated with DMSO and IL-1 β (20 ng/ml) for 24 h or were untreated, and were then treated with AZ (25, 50 and 100 μ M) for another 24 or 48 h, and cell viability was assessed by Cell Counting Kit-8 assay. Chondrocytes were pretreated with or without IL-1 β (20 ng/ml) for 24 h and were then treated with AZ (100 μ M) for another 24 h, and (B) apoptosis was measured by flow cytometry and (C) cells were visualized by toluidine blue staining. (D) Western blotting and ImageJ analysis were used to determine relative protein expression levels of Notch1, SOCS3, caspase 3 and cleaved caspase 3. $^{\#}P$ <0.05 vs. control; $^{*}P$ <0.05 vs. IL-1 β . AZ, acetyl zingerone; SOCS3, suppressor of cytokine signaling 3.

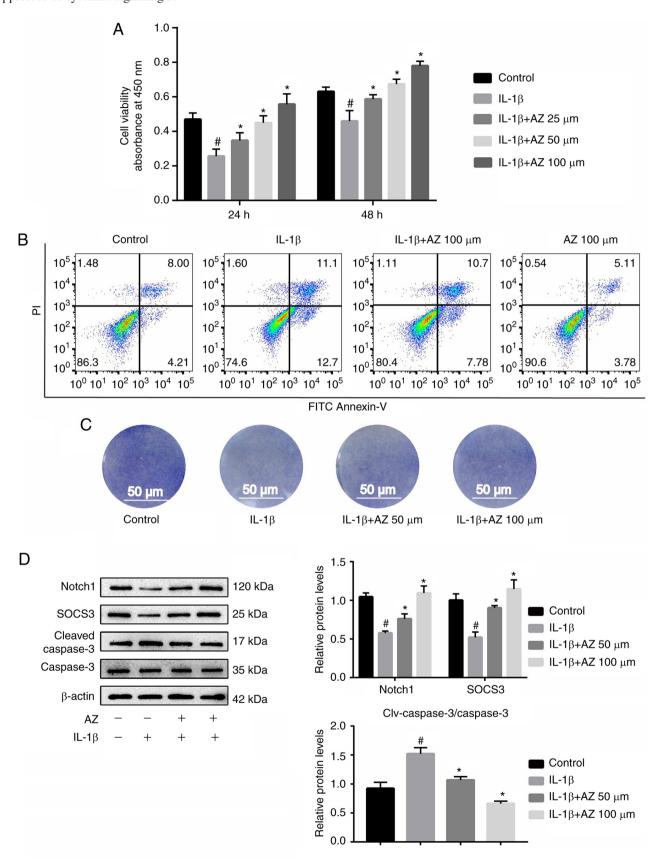


Figure S2. AZ inhibits ferroptosis in mouse ATDC5 chondrocytes. (A) GSH and (B) MDA levels in each group were measured using GSH and MDA assays. ATDC5 cells were pretreated with or without IL-1 β (20 ng/ml) for 24 h, then treated with AZ (100 μ M) or Fer-1 (60 nmol/ml) for another 24 h. (C) ATDC5 cells were pretreated with or without IL-1 β (20 ng/ml) for 24 h, then treated with AZ (100 μ M) and Fer-1 (60 nmol/ml) for another 24 h, and GPX4 expression was determined by western blotting. *P<0.05 vs. control; *P<0.05 vs. IL-1 β . AZ, acetyl zingerone; Fer-1, ferrostatin-1; GPX4, glutathione peroxidase 4; GSH, glutathione; MDA, malondialdehyde.

