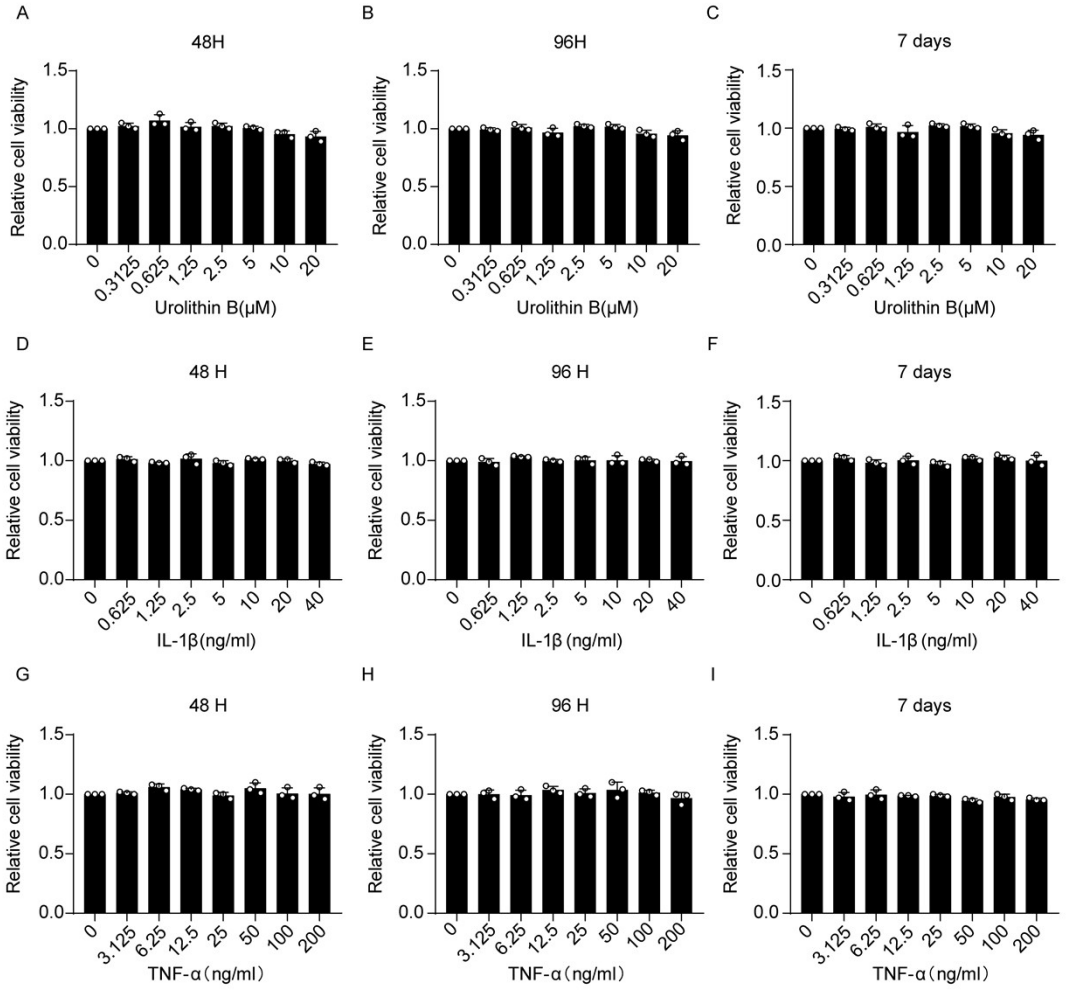
Electronic Supplementary Material (ESI) for Food & Function.

Supplementary Table. 1 The RNA purity data

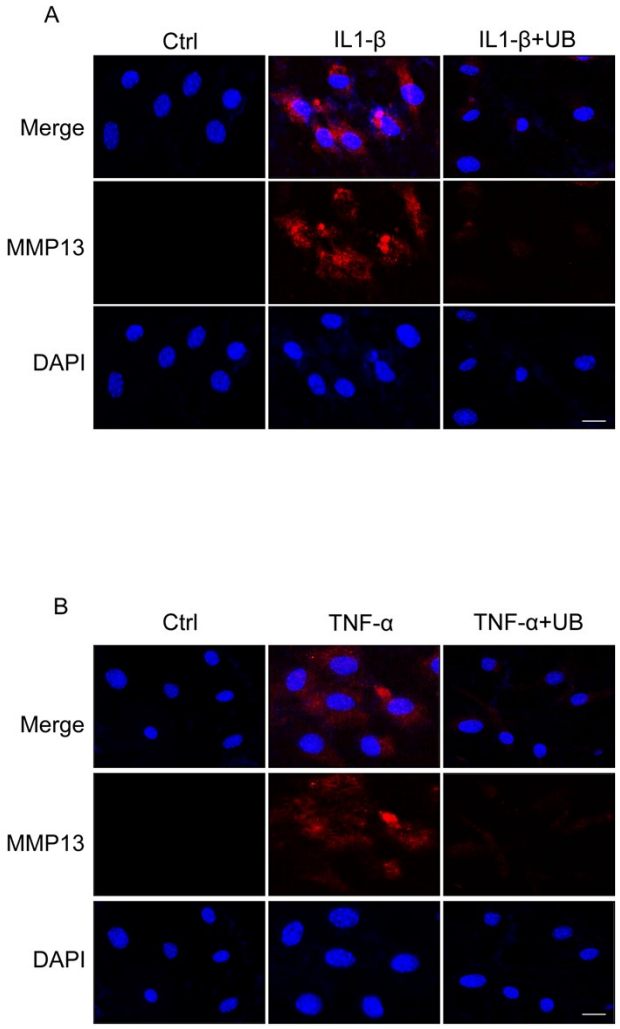
Samples (n=3)	OD (260/280 nm) (n=3)	OD (260/230 nm) (n=3)
Ctrl	1.91/1.85/2.03	2.11/1.89/1.95
IL1-β	1.88/1.94/1.96	2.05/2.06/1.90
IL1-β+UB (5 uM)	1.82/1.82/1.93	1.88/1.80/1.92
IL1-β+UB (10 uM)	1.96/2.03/2.01	2.02/1.95/1.87
Ctrl	2.01/1.97/1.80	1.97/2.09/1.82
TNF-α	1.86/1.79/1.78	1.90/1.96/1.99
TNF-α+UB (5 uM)	1.99/1.90/1.89	2.01/1.94/1.85
TNF-α+UB (10 uM)	2.04/1.96/1.79	1.99/2.06/2.08

Supplementary Figures



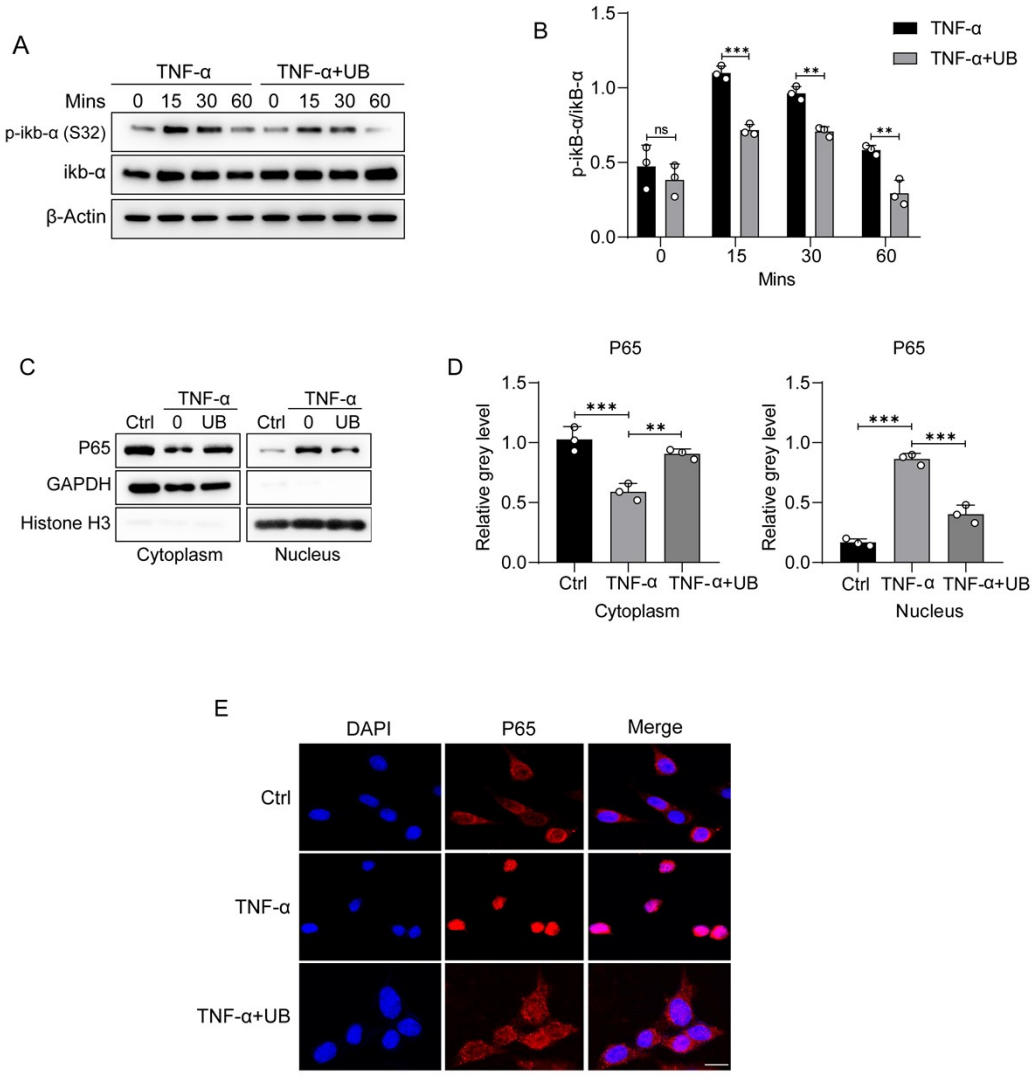
Supplementary Figure. 1 The effects of UB, IL1-β, and TNF-α on chondrocytes viability.

(A-C) Chondrocytes were treated with different concentrations of UB for 48 h, 96 h, and 7 days, and the CCK-8 assay kit was used to assess the impact of UB on chondrocyte viability (n = 3). (D-F) Chondrocytes were treated with different concentrations of IL1-β for 48 h, 96 h, and 7 days, and the CCK-8 assay kit was used to assess the impact of UB on chondrocyte viability (n = 3). (G-I) Chondrocytes were treated with different concentrations of TNF-α for 48 h, 96 h, and 7 days, and the CCK-8 assay kit was used to assess the impact of UB on chondrocyte viability (n = 3). All data are presented as Mean ± SD. Statistical significance was denoted as *P<0.05, **P<0.05, ***P<0.05.



Supplementary Figure. 2 Urolithin B inhibits the MMP13 expression in chondrocytes.

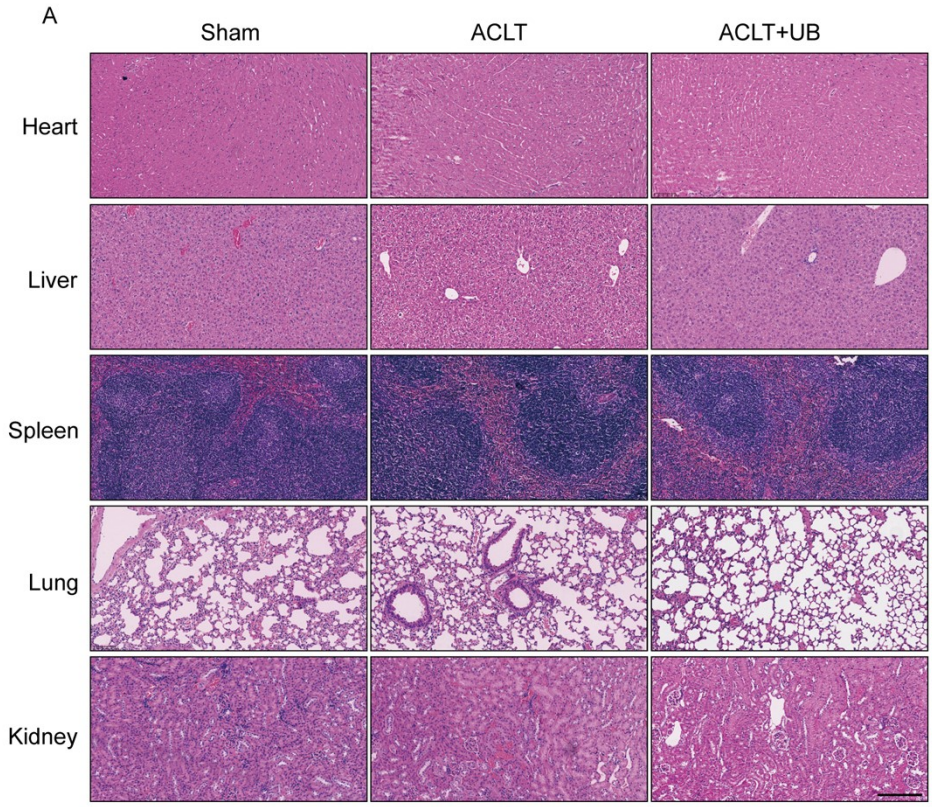
(A-B) Chondrocytes were co-treated with 10 ng/ml IL-1β or 50 ng/ml TNF-α along with 10 μM UB for 4 days. Immunofluorescence staining was performed to visualize the expression of MMP13 (n = 3). All data are presented as Mean ± SD. Statistical significance was denoted as *P<0.05, **P<0.05, ***P<0.05.



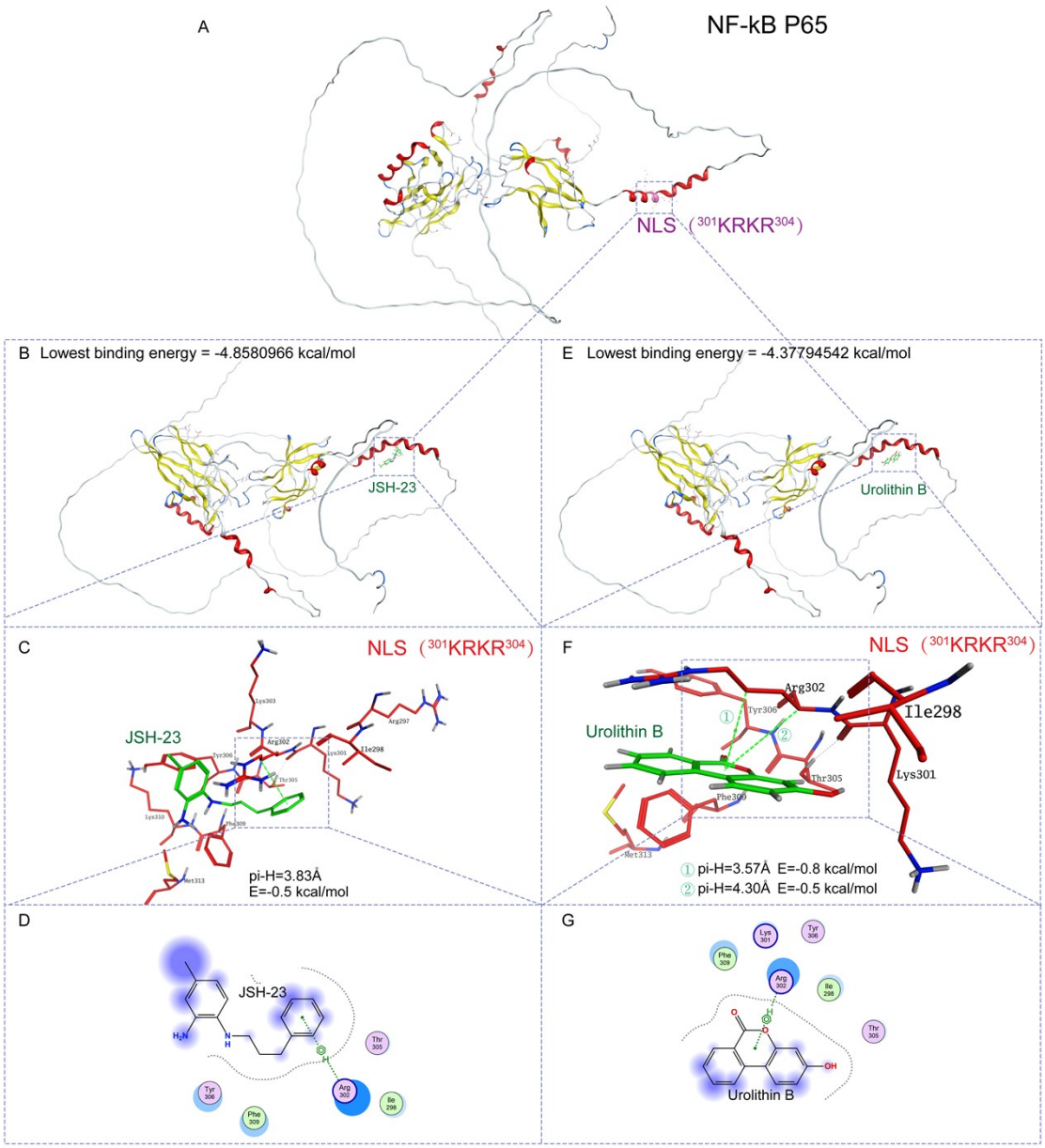
Supplementary Figure. 3 Urolithin B restrains the NF-κB pathway activation induced by TNF-α.

(A) ATDC5 cells were pretreated with UB for 2 hours, followed by treatment with TNF-α (50 ng/ml) with or without UB for 0, 15, 30, and 60 minutes. Total cellular proteins were extracted and subjected to Western blotting to assess the phosphorylation levels of IkB-α (n = 3). (B) Quantitative analysis was performed to evaluate the phosphorylation levels of IkB-α (n = 3). (C) ATDC5 cells were pretreated with UB for 2 hours, followed by treatment with TNF-α (50 ng/ml) with or without UB for 60 minutes. Nuclear and cytoplasmic proteins were separated

and analyzed using Western blotting to determine the distribution of P65 (n = 3). (D) Quantitative analysis was conducted to measure the grayscale values of the P65 protein bands (n = 3). (E) A cellular immunofluorescence assay was performed to examine the nuclear-cytoplasmic distribution of P65 (n = 3). All data are presented as Mean ± SD. Statistical significance was denoted as *P<0.05, **P<0.05, ***P<0.05.



Supplementary Figure. 4 The H&E staining of mice organs.



Supplementary Figure. 5 Urolithin B and JSH-23 interact with the ARG302 residue in the NLS motif of NF-kB p65.