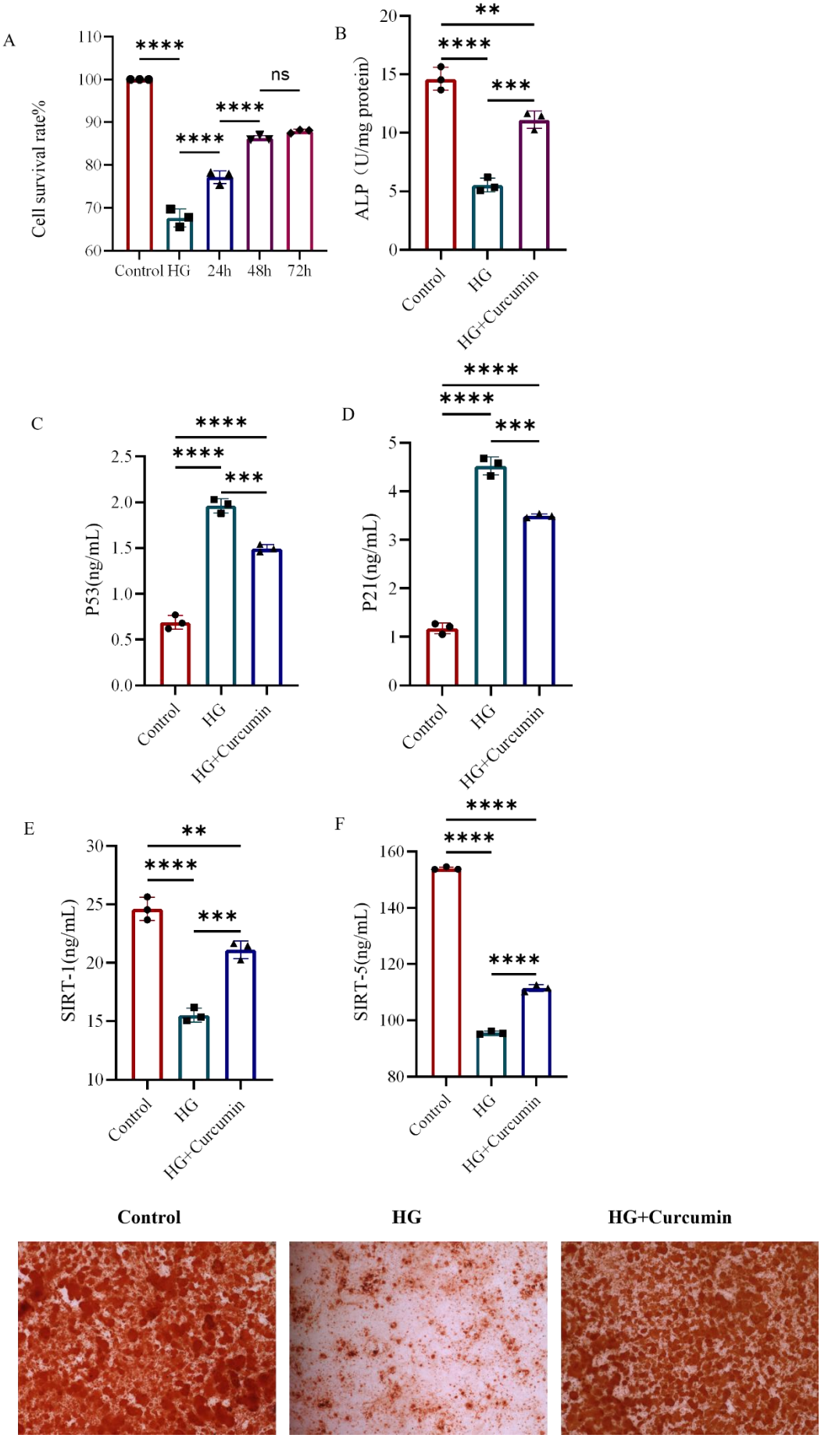

A
Cell survival rate%
110
100
90
80
70
60
****
****
****
ns
Control
HG
24h
48h
72h
B
ALP（U/mg protein）
20
15
10
5
0
**
****
***
Control
HG
HG+Curcumin
C
P53(ng/mL)
2.5
2.0
1.5
1.0
0.5
0.0
****
****
***
Control
HG
HG+Curcumin
D
P21(ng/mL)
5
4
3
2
1
0
****
****
***
Control
HG
HG+Curcumin
E
SIRT-1(ng/mL)
30
25
20
15
10
**
****
***
Control
HG
HG+Curcumin
F
SIRT-5(ng/mL)
160
140
120
100
80
****
****
****
Control
HG
HG+Curcumin
G
Control
HG
HG+Curcumin

Supplementary Figure 1： A： Time-dependent analysis of curcumin treatment CCK8 analysis showed no statistical difference between the effects at 48 hours and 72 hours. B: The control group, high-sugar group, and curcumin-treated group were tested for alkaline phosphatase (ALP) content in MC3T3-E1 cells using the p-nitrophenyl phosphate (pNPP) colorimetric method. C: The control group, high-sugar group, and curcumin-treated group were tested for P53 content in MC3T3-E1 cells using ELISA. D: The control group, high-sugar group, and curcumin-treated group were tested for P21 content in MC3T3-E1 cells using ELISA. E: The control group, high-sugar group, and curcumin-treated group were analyzed using ELISA to measure SIRT-1 levels in MC3T3-E1 cells. F: The control group, high-sugar group, and curcumin-treated group were analyzed using ELISA to measure SIRT-5 levels in MC3T3-E1 cells. G: Representative image of cell alizarin red staining. *p <0.05, ***p <0.001, ****p <0.0001

Method

Colorimetric method using p-nitrophenyl phosphate (pNPP)

The alkaline phosphatase (ALP) activity of osteoblasts was quantified using a colorimetric assay based on the hydrolysis of *p*-nitrophenyl phosphate (pNPP) to *p*-nitrophenol (pNP)， the specific method is as follows

Cell Preparation:MC3T3-E1 osteoblasts were seeded in 24-well plates (1×10^4 cells/well) and treated under experimental conditions (e.g., high glucose ± curcumin).After treatment, cells were washed twice with ice-cold PBS and lysed in 200 μL RIPA buffer containing protease inhibitors.

Enzymatic Reaction:50 μL cell lysate was mixed with 50 μL pNPP substrate solution.The mixture was incubated at 37°C for 30 min (optimized to ensure linear product formation).

Reaction Termination & Detection:The reaction was stopped by adding 100 μL of 2 M NaOH.

Absorbance was measured at 405 nm using a microplate reader (BioTek Synergy H1).

Standard Curve & Quantification:A standard curve was generated using pNP solutions (0–100 μM).ALP activity was expressed as μmol pNP produced per min per mg protein.Protein concentration was determined by BCA assay (Pierce™). All of the above kits were purchased from Nantong Aoxiang Biotechnology Co., Ltd.

ELISA experiment

The protein expression levels of P53, P21, SIRT-1, and SIRT-5 were quantified using enzyme-linked immunosorbent assays (ELISA), the specific method is as follows:

Cell Preparation:MC3T3-E1 osteoblasts were seeded in 24-well plates (1×10^4 cells/well) and treated under experimental conditions (e.g., high glucose ± curcumin). After treatment, cells were washed twice with ice-cold PBS and lysed in 200 μL RIPA buffer containing 1× protease and phosphatase inhibitors.

Protein Extraction & Quantification:Total protein concentration was determined using a BCA protein assay kit (Pierce™). Lysates were centrifuged at 12,000 × g for 15 min at 4°C, and supernatants were aliquoted for ELISA.

ELISA Procedure:Coating:96-well plates were coated with 100 μL/well of capture antibody (specific to each target: P53/P21/SIRT-1/SIRT-5) diluted in coating buffer (0.1 M carbonate-bicarbonate, pH 9.6), and incubated overnight at 4°C.

Blocking:Wells were washed 3× with PBS containing 0.05% Tween-20 (PBST), then blocked with 200 μL/well of 5% BSA-PBS for 2 h at 37°C.

Sample & Standard Incubation:Blocking solution was removed; 100 μL of cell lysate (diluted to 2 μg/μL in assay buffer) or serial-diluted recombinant protein standards (0–500 pg/mL) were added per well. Plates were incubated for 2 h at 37°C.

Detection Antibody:Wells were washed 5× with PBST, then incubated with 100 μL/well of biotinylated detection antibody (target-specific) for 1 h at 37°C.

Streptavidin-HRP Conjugation:After washing, 100 μL/well of streptavidin-HRP conjugate (1:5,000 dilution) was added and incubated for 30 min at 37°C in the dark.

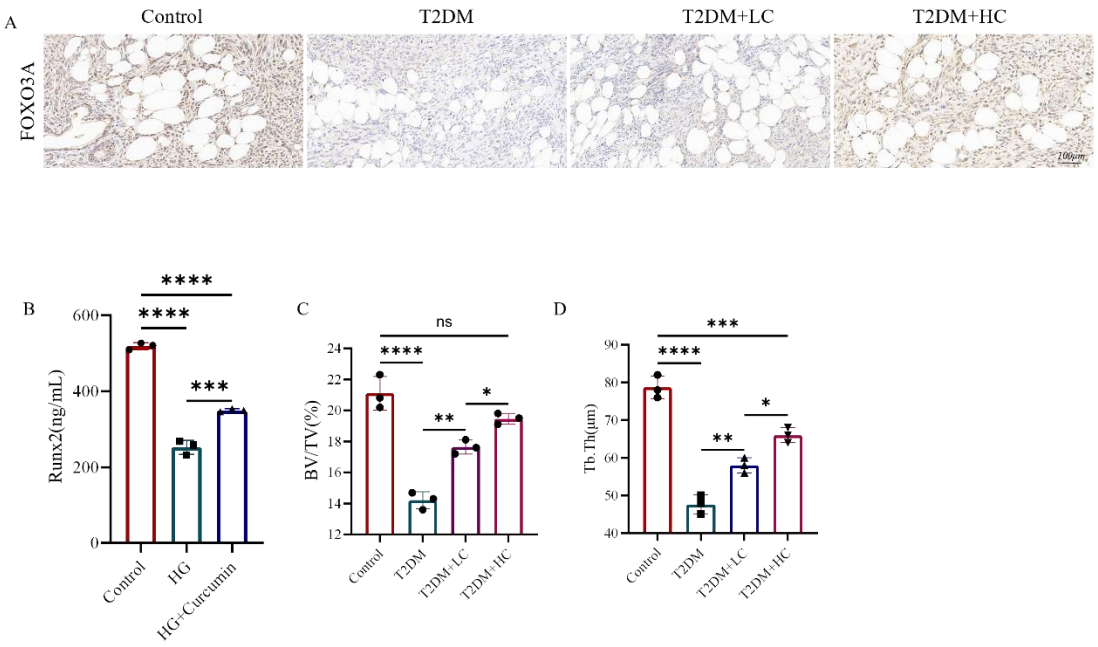
Substrate Reaction & Termination:Wells were washed 5× with PBST, then incubated with 100 μL/well of TMB substrate for 15 min at 25°C. The reaction was stopped by adding 50 μL/well of 2 M H_2SO_4.

Absorbance Measurement:Optical density (OD) was measured at 450 nm (reference: 570 nm) using a microplate reader (BioTek Synergy H1).

Quantification:Standard curves were generated for each target protein using four-parameter logistic (4PL) regression. Target protein concentrations were expressed as pg per mg total protein, normalized to BCA-quantified total protein.

Cell alizarin red staining

Cell samples must be processed to the mineralization stage. After discarding the culture medium, gently wash three times with pre-cooled PBS, each time for 5 minutes, to remove any residual culture medium. Fix with 4% neutral formaldehyde at room temperature for 15 minutes. Prolonged fixation time may cause damage to cell structure. After fixation, rinse three times with PBS for 3 minutes each to completely remove formaldehyde residues. During staining, evenly cover the cell surface with prepared alizarin red staining solution and incubate at 37°C in the dark for 30 minutes. Insufficient staining time may result in inadequate color development, while exceeding 40 minutes may cause non-specific staining. After staining, immediately rinse three times with deionized water, ensuring gentle handling to avoid dislodging mineralized nodules. After rinsing, invert the culture plate onto absorbent paper to drain excess moisture, then air-dry at room temperature or dry in a 37°C incubator for 15 minutes. Results are observed using an inverted optical microscope.



Supplementary Figure 2: A:Expression of FOXO3A in bone tissue samples.B: The control group, high-sugar group, and curcumin-treated group were analyzed using ELISA to measure Runx2 levels in MC3T3-E1 cells. C: Statistical analysis of bone mass scores in each group. D: Statistical analysis of trabecular thickness in each group. *p <0.05, ***p <0.001, ****p <0.0001