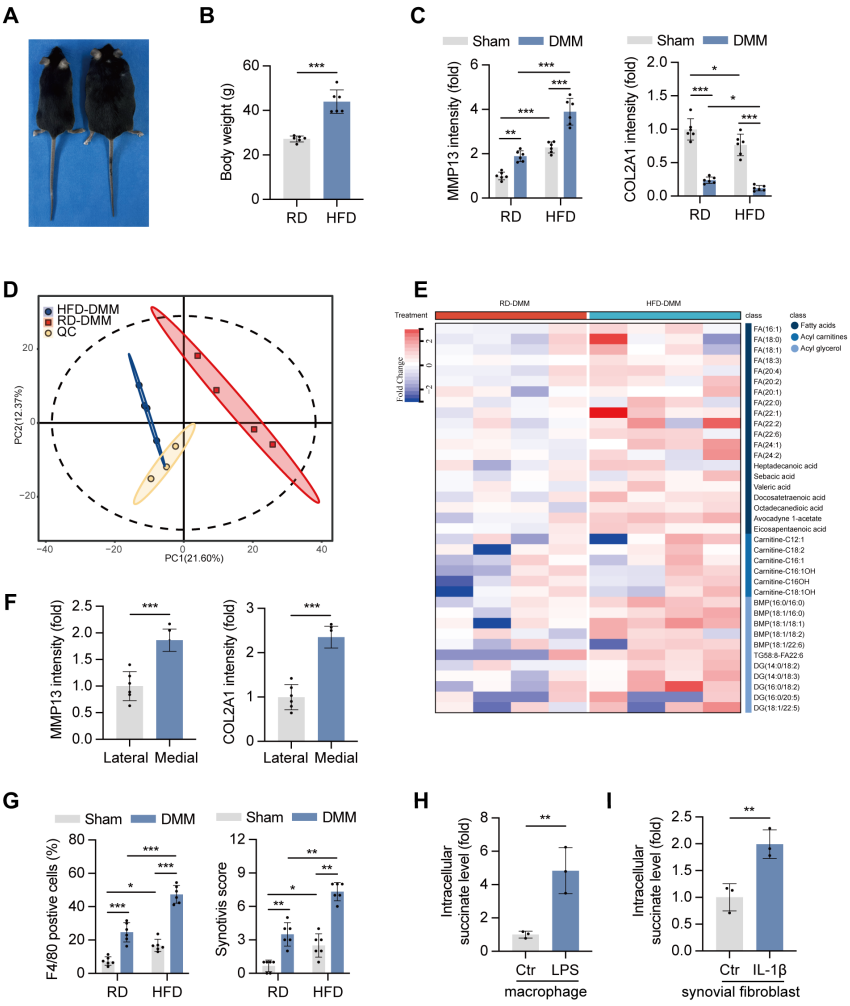
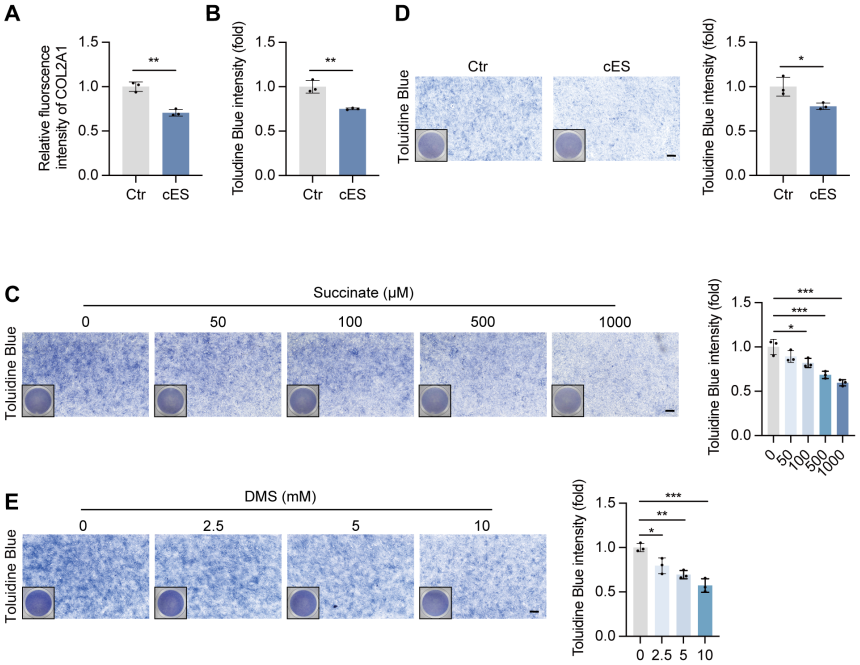
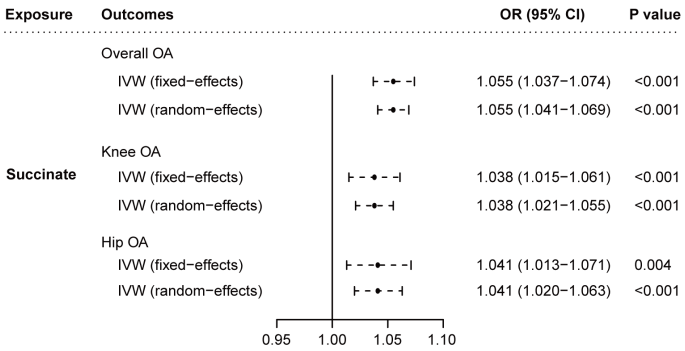


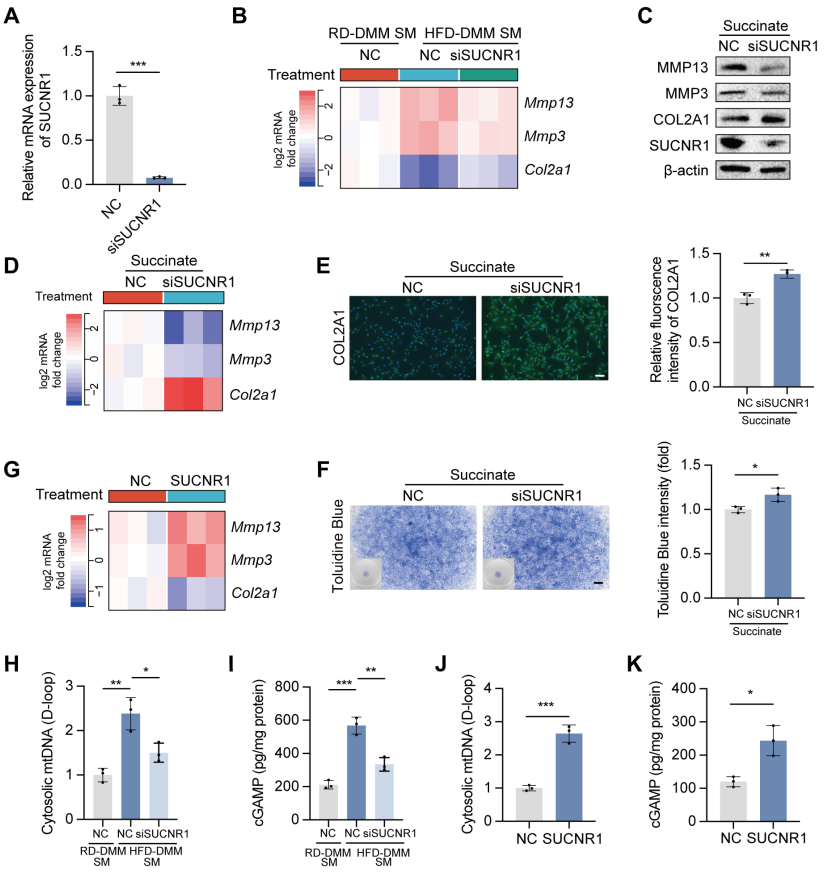
Supplementary Fig. 1. HFD-fed mice and metabolomics. (A) A general view of RD and HFD mice. (B) Body weight of RD and HFD mice (n = 6). (C) Quantification of MMP13 and COL2A1 staining intensity in Fig. 1A (n = 6). (D) PCA analysis of the HFD-DMM group (n = 4), the RD-DMM group (n = 4), and the quality control (QC, n = 3). (E) Heatmap showing the relative levels of lipid-related metabolites in the indicated groups. (F) Quantification of COL2A1 and MMP13 staining intensity in Fig. 1G (n = 6). (G) Quantification of F4/80 and synovitis score in Fig. 1K (n = 6). (H) Quantification of succinate levels in the cell lysis of macrophages treated with 1 μg/mL LPS for 24 h (n = 3). (I) Quantification of succinate levels in the cell lysis of synovial fibroblasts treated with 10 ng/ml IL-1β for 24 h (n = 3). Data are mean ± SD, as determined via unpaired two-tailed t-test (B, F, H, and I), one-way ANOVA with Tukey's test (C and G left) or nonparametric Mann–Whitney test (G right).



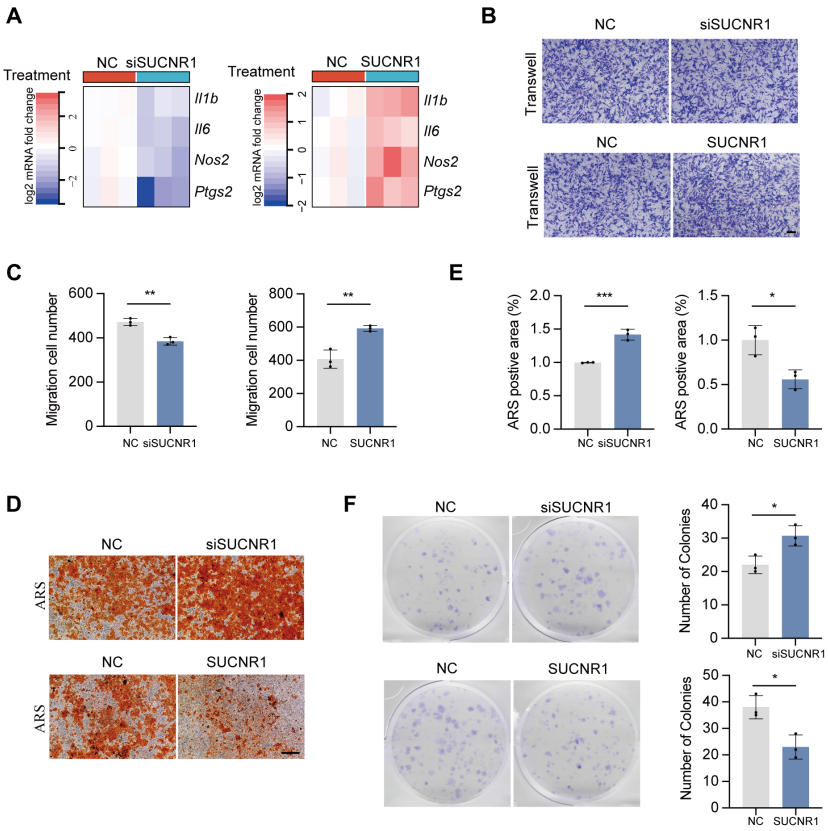
Supplementary Fig. 2. Succinate modulated ECM metabolism. (A) Quantification of fluorescence intensity of COL2A1 in Fig. 2G (n = 3). (B) Quantification of toluidine blue intensity in Fig. 2H (n = 3). (C) Toluidine Blue staining of micromass of ATDC5 cells treated with succinate for 48 h (n = 3). Scale bars, 250 μm. (D) Toluidine Blue staining of micromass of ATDC5 cells treated with 50 μM cES for 48 h (n = 3). Scale bars, 250 μm. (E) Toluidine Blue staining of micromass of ATDC5 cells treated with DMS for 48 h (n = 3). Scale bars, 250 μm. Data are mean ± SD, as determined via unpaired two-tailed t-test (A, B, and D) or one-way ANOVA with Tukey's test (C and E).



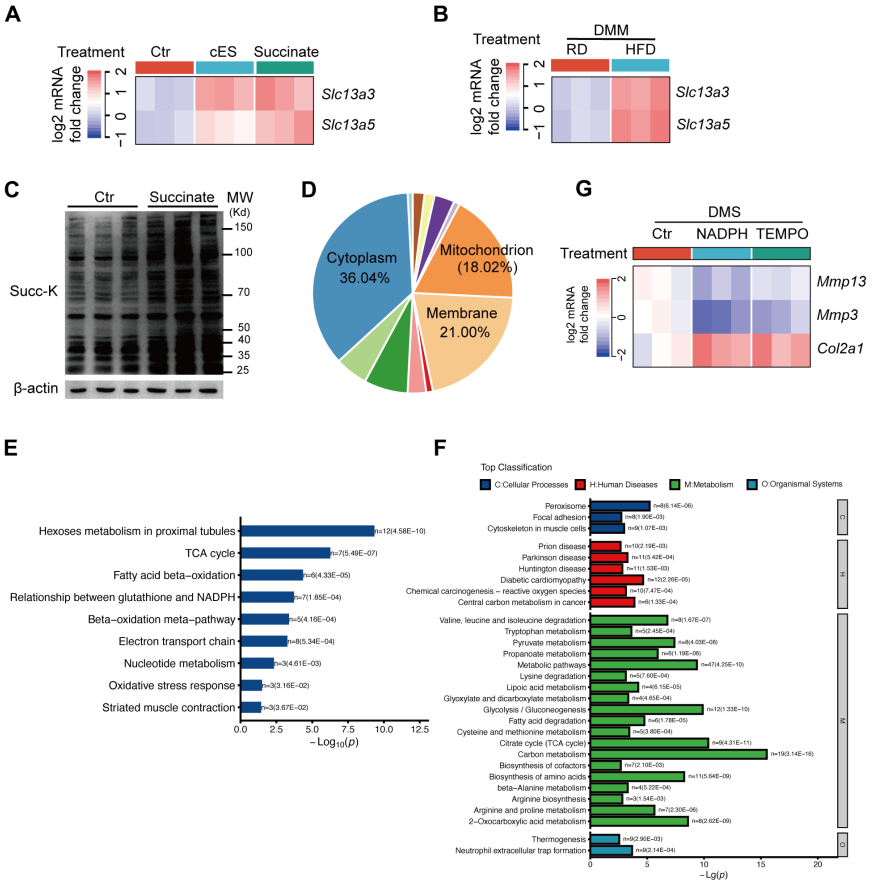
Supplementary Fig. 3. Two-sample Mendelian randomization analysis for the potential causal association between serum succinate levels and the risk of osteoarthritis.



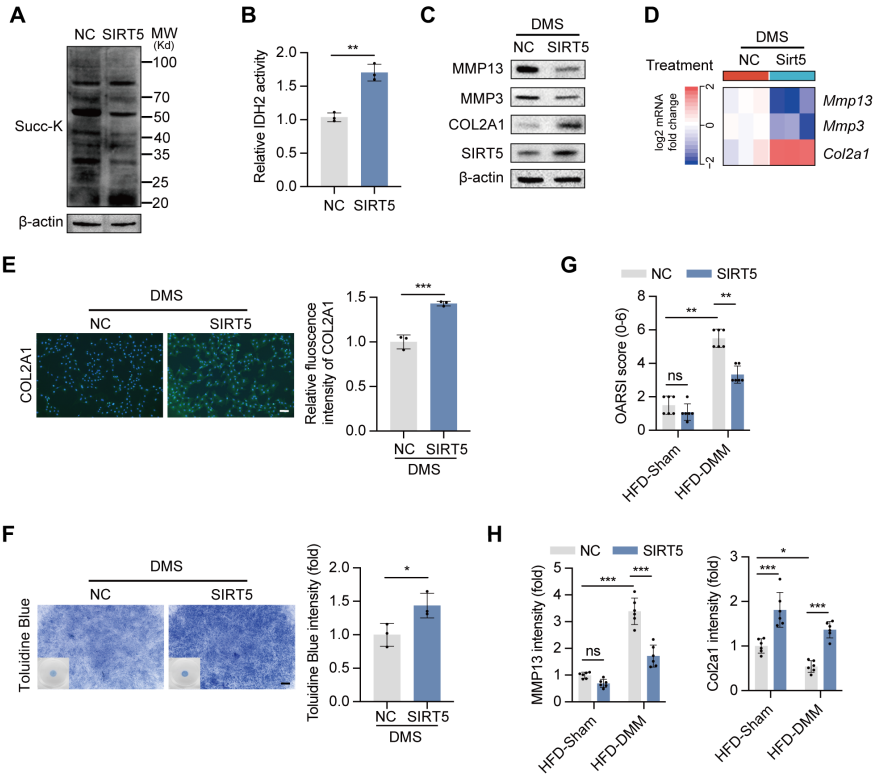
Supplementary Fig. 4. Succinate modulated ECM metabolism in chondrocytes through SUCNR1. (A) Relative mRNA expression of SUCNR1 in primary chondrocytes transfected with the negative control or siSUCNR1 (n = 3). (B) Heatmap showing relative mRNA expression of *Mmp13*, *Mmp3*, and *Col2a1* in co-cultured chondrocytes transfected with the negative control or SUCNR1 siRNA (n = 3). (C) Western blotting of MMP13, MMP3, and COL2A1 in primary chondrocytes transfected with the negative control or SUCNR1 siRNA in the presence of 500 μM succinate for 48 h (n = 3). (D) Heatmap showing relative mRNA expression of *Mmp13*, *Mmp3*, and *Col2a1* in the indicated groups (n = 3). (E) Immunofluorescence of COL2A1 in primary chondrocytes in the indicated groups (n = 3). Scale bars, 100 μm. (F) Toluidine Blue staining of micromass in the indicated groups (n = 3). Scale bars, 250 μm. (G) Heatmap showing relative mRNA expression of *Mmp13*, *Mmp3*, and *Col2a1* in primary chondrocytes transduced with lv-NC or lv-SUCNR1 (n = 3). (H) qPCR quantification of the levels of mtDNA (D-loop region) present in the cytosolic fraction in the indicated groups (n = 3). (I) Quantification of intracellular cGAMP levels by ELISA in the indicated groups (n = 3). (J) qPCR quantification of the levels of mtDNA (D-loop region) present in the cytosolic fraction in the indicated groups (n = 3). (K) Quantification of intracellular cGAMP levels by ELISA in the indicated groups (n = 3). Data are mean ± SD, as determined via unpaired two-tailed t-test (A, E, F, J and K) or one-way ANOVA with Tukey's test (H and I).



Supplementary Fig. 5. Effect of SUCNR1 on macrophages, fibroblasts, and BMSCs. (A) Heatmap showing relative mRNA expression of *Il1b*, *Il6*, *Nos2*, and *Ptgs2* in macrophages treated with 1 μg/mL LPS for 24 h in the indicated groups (n = 3). (B-C) Migration assay of synovial fibroblasts in the indicated groups (n = 3). Scale bar = 100 μm. (D-E) ARS staining of BMSCs after osteogenic differentiation (n = 3). Scale bar, 100 μm. (F) Clone formation assay of BMSCs in the indicated groups (n = 3). Data are mean ± SD, as determined via unpaired two-tailed t-test.



Supplementary Fig. 6. Succinylation modification omics. (A) Heatmap showing relative mRNA expression of *Slc13a3* and *Slc13a5* in primary chondrocytes treated with 50 μM cES or 500 μM succinate for 48 h (n = 3). (B) Heatmap showing relative mRNA expression of *Slc13a3* and *Slc13a5* in whole cartilage lysate obtained from RD-DMM and HFD-DMM mice (n = 3). (C) Western blot analysis of succinyl-lysine from primary chondrocytes treated with 500 μM succinate for 48 h (n = 3). (D) Distribution of subcellular compartments of proteins with succinylation sites. Wiki pathway enrichment analysis (E) and GO enrichment analysis (F) of proteins with altered succinylation sites in primary chondrocytes treated with 2.5 mM DMS for 48 h. (G) Heatmap showing relative mRNA expression of *Mmp13*, *Mmp3*, and *Col2a1* in chondrocytes treated with 100 μM NADPH or 10 μM TEMPO for 48 h (n = 3).



Supplementary Fig. 7. SIRT5 mitigated cartilage degeneration induced by DMS. (A) Western blot analysis of succinyl-lysine from primary chondrocytes transduced with lv-NC or lv-SIRT5 (n = 3). (B) Measurement of the enzymatic activity of IDH2 in primary chondrocytes transduced with lv-NC or lv-SIRT5 (n = 3). (C) Western blotting of MMP13, MMP3, COL2A1 and SIRT5 in primary chondrocytes transduced with lv-NC or lv-SIRT5 in the presence of 2.5 mM DMS (n = 3). (D) Heatmap showing relative mRNA expression of *Mmp13*, *Mmp3*, and *Col2a1* in the indicated groups (n = 3). (E) Immunofluorescence of COL2A1 in the indicated groups. Scale bars, 100 μm. (F) Toluidine Blue staining of micromass in the indicated groups. Scale bars, 250 μm. (G-H) OARSI scores and quantification of COL2A1 and MMP13 staining intensity in Fig. 5O (n = 6). Data are mean ± SD, as determined via unpaired two-tailed t-test (B, E and F), or one-way ANOVA with Tukey's test (H), or nonparametric Mann–Whitney test (G).