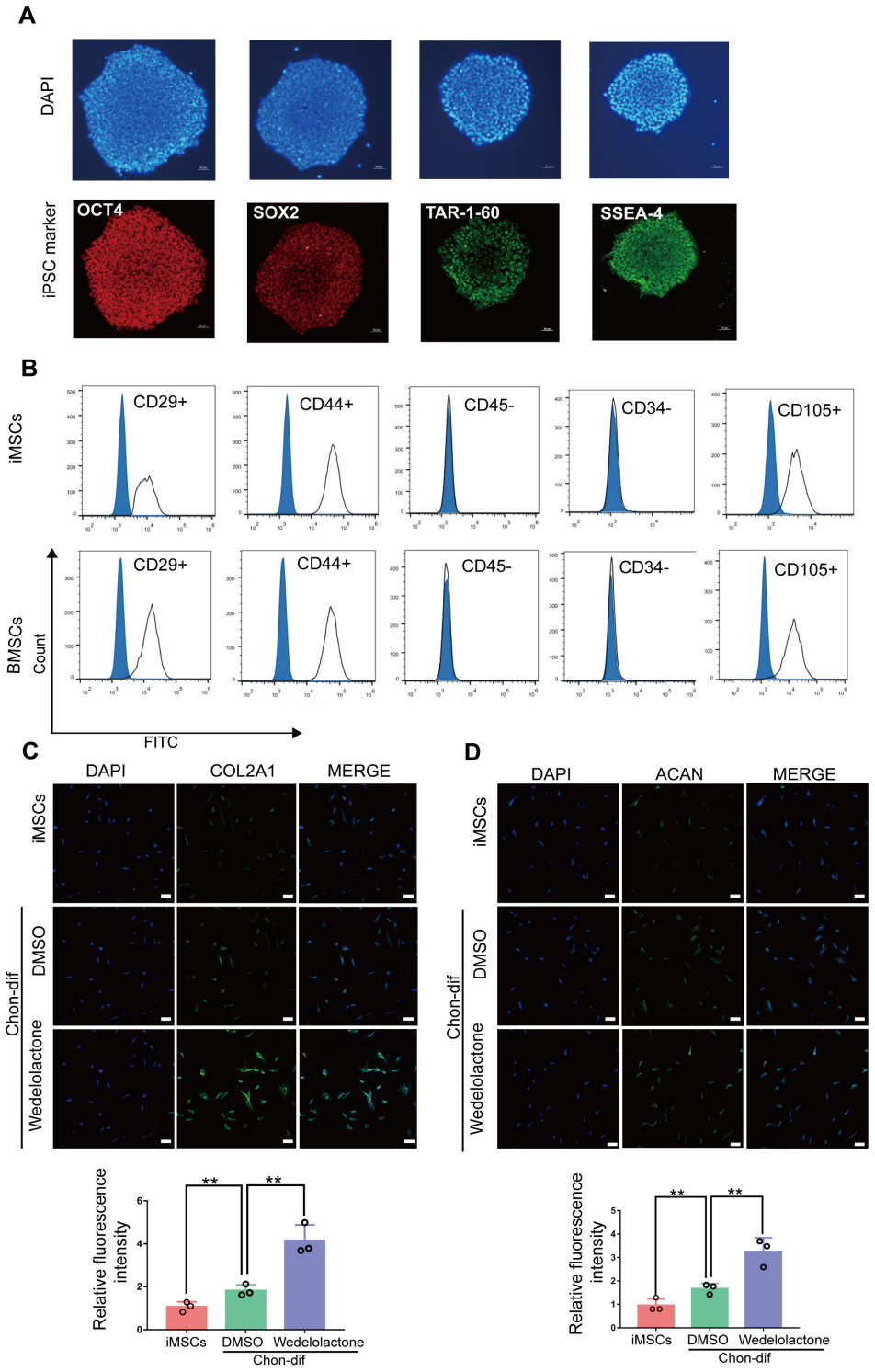

Fig. S1. Wedelolactone promotes the differentiation of hiPSC-derived MSCs to chondrocytes *in vitro*. Related to Figure 1. (A) Immunofluorescence image of the iPSCs markers OCT4, SOX2, TAR-1-60, and SSEA4. Scale bars = 50 μm. (B) Flow cytometry analysis of surface marker expression of human iPSC-derived MSCs and BMSCs. (C, D) Immunofluorescence image of human iPSC-derived MSCs under chondrogenic differentiation stained with COL2A1, and ACAN. Scale bars = 100 μm. Relative fluorescence intensity was used to quantify the expression of COL2A1 and ACAN. Data are expressed as Mean ± SD (n = 3). Statistics differences were analyzed using one-way ANOVA followed by Dunnett's test: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

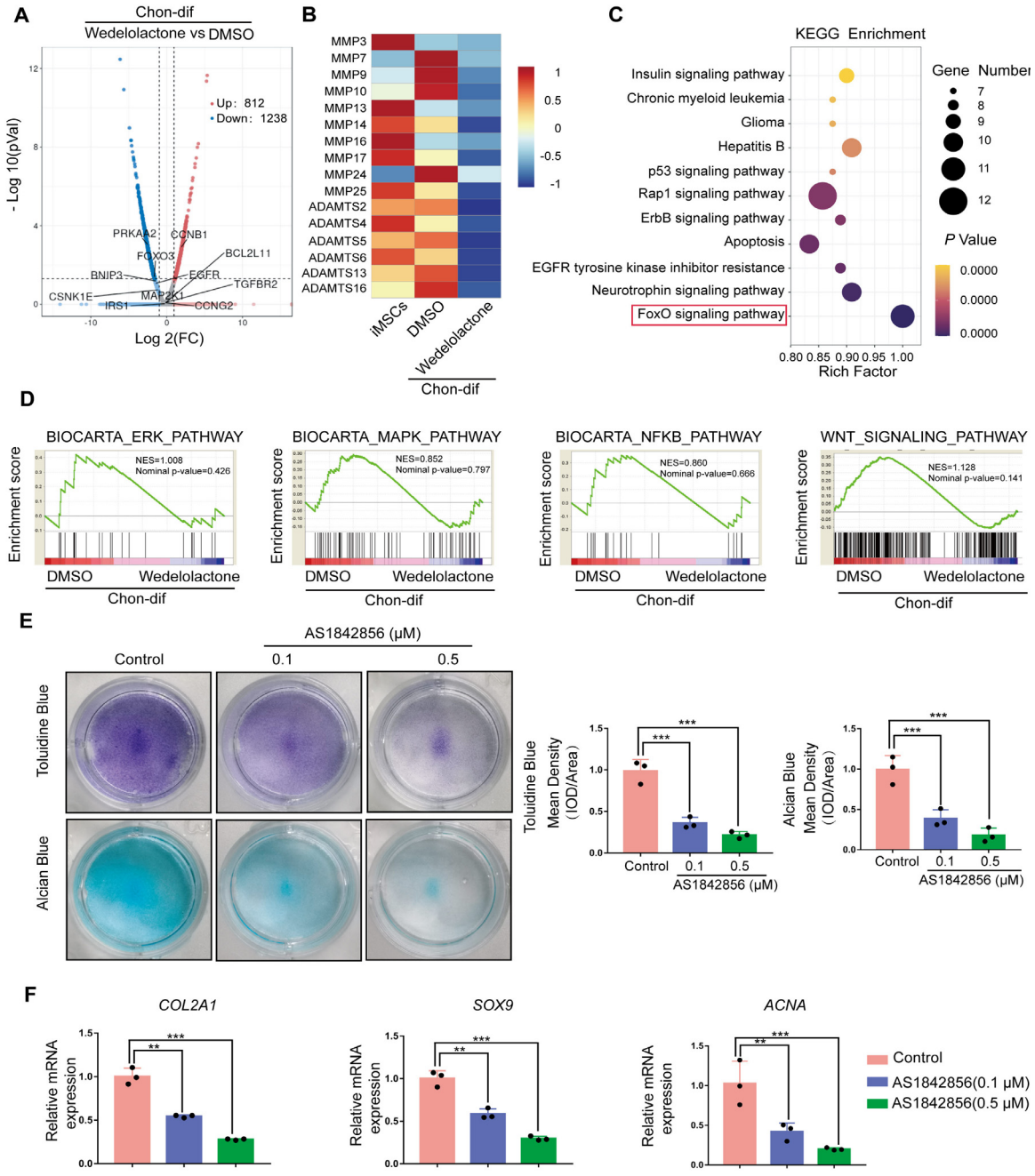

Fig. S2. Wedelolactone promotes the chondrogenic differentiation of MSCs by activating the FOXO pathway. Related to Figure 3. (A) Volcanic map shows differently expressed proteins in the chondrogenic pellet differentiated from human iPSC-derived MSCs treated with DMSO or Wedelolactone. (B) Heatmap of the RNA-analysis showing differently expressed RNA associated with chondrogenic markers and hypertrophic markers. (C) The Kyoto Encyclopedia of Genes and Genomes enrichment analysis of differentially expressed genes in the chondrogenic pellet differentiated from human iPSC-derived MSCs treated with DMSO or Wedelolactone. (D) Gene Set Enrichment Analysis of differentially expressed genes in the chondrogenic pellet differentiated from human iPSC-derived MSCs treated with DMSO or Wedelolactone. (E) Alcian blue staining and toluidine blue staining images of chondrogenic differentiation of human iPSC-derived MSCs after FOXO1 inhibitor intervention. quantification of mean intensity of alcian blue staining and toluidine blue staining. (F) Gene expression analysis of chondrogenic differentiation markers (COL2A1, SOX9, ACAN) after FOXO1 inhibitor intervention. Data are expressed as Mean±SD (n=3). Statistics differences were analyzed using one-way ANOVA followed by Dunnett's test: *p<0.05, **p<0.01, ***p<0.001.

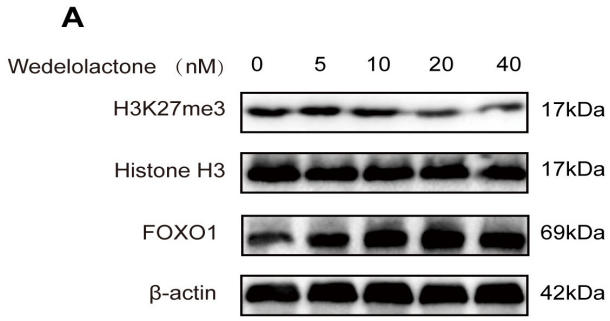

Fig. S3. Wedelolactone decreases EZH2-dependent trimethylation of H3K27 on the promoter region of *FOXO1*. Related to Figure 5. (A) western blot analysis of FOXO1 and H3K27me3 in human iPSC-derived MSCs after wedelolactone intervention during chondrogenic differentiation.

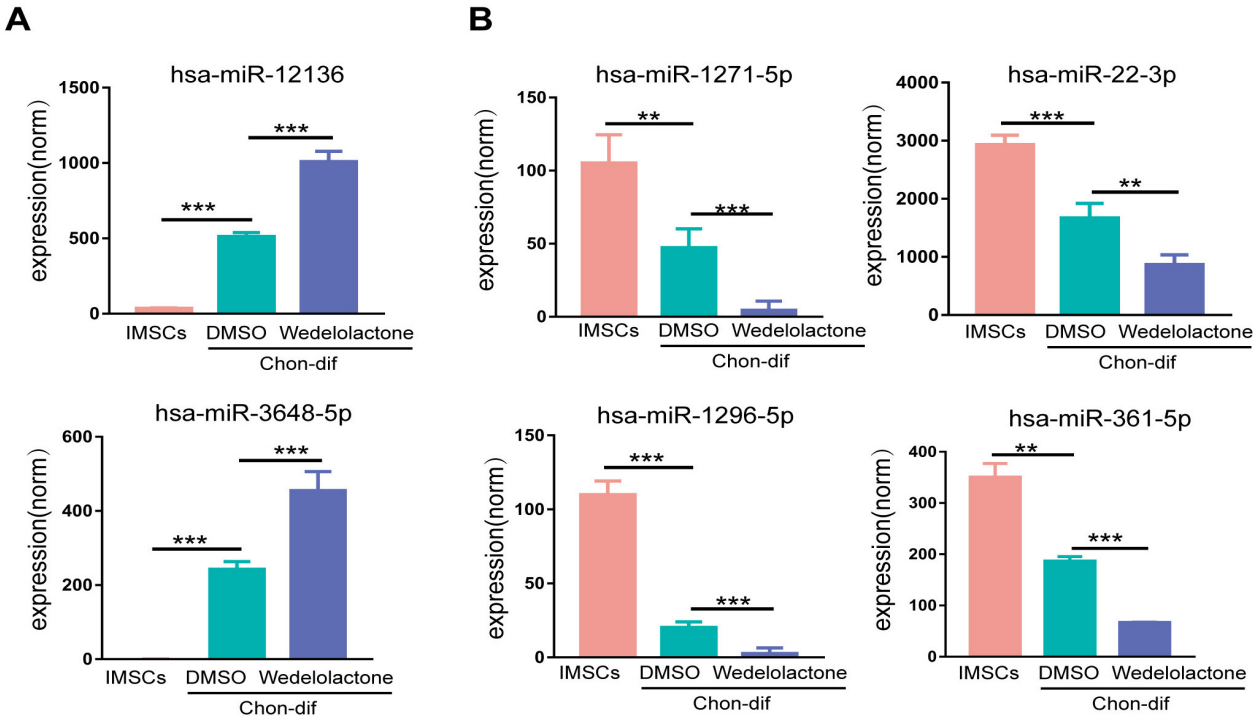

Fig. S4. miR-1271-5P interferes with *FOXO1* expression during chondrogenic differentiation. Related to Figure 6. (A) Upregulated miRNAs in the chondrogenic pellet differentiated from human iPSC-derived MSCs, and human iPSC-derived MSCs treated with DMSO or Wedelolactone. (B) Downregulated miRNAs in the chondrogenic pellet differentiated from human iPSC-derived MSCs, and human iPSC-derived MSCs treated with DMSO or Wedelolactone. Differences were analyzed using one-way ANOVA followed by Dunnett's test: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

Table S1. shRNA sequences used for experiments in this study

Names	Sequences
Control shRNA	CAACAAGATGAAGAGCACCAA
FOXO1 shRNA-1	GCGGGCTGGAAGAATTCAATT
FOXO1 shRNA-2	GCGGGCTGGAAGAATTCAATT
EZH2 shRNA-1	TTGGGACAGTAAAAATGTGTC
EZH2 shRNA-2	GTTTAGAGTCAAAGAATCTAG

Table S2. Sequences of primers used for experiments in this study

Names	Sequences
Human *FOXO1*-forward	GTCCTACGCCGACCTCATC
Human *FOXO1*reverse	CTGTTGCTGTCACCCTTATCC
Human COL2A1-forward	ACCAAAGGGACAGAAAGGAG
Human *SOX9*-forward	AGGTGCTCAAAGGCTACGACTG
Human *SOX9*-reverse	AGGTGCTCAAAGGCTACGACTG
Human *ACAN*-forward	GAAGGAGGTAGTGCTGCTGG
Human *ACAN*-reverse	CCTGTCAAAGTCGAGGGTGT
Rat- *Col2a1* -forward	GCCAGGATGCCCGAAAATTAG
Rat- *Col2a1* -reverse	GGCTCCGGGAATACCATCAG
Rat- *Sox9* -forward	TCCCCGCAACAGATCTCCTA
Rat- *Sox9* -reverse	TCCCCGCAACAGATCTCCTA
Human *EZH2*-forward	ACGGCTTCCCAATAACAG
Human *EZH2*-reverse	TGAGGCTTCAGCACCACT
hsa-miR-1271-5P-forward	GCGCTTGGCACCTAGCAAG
hsa-miR-1271-5P-reverse	GCGCTTGGCACCTAGCAAG
U6-forward	CTCGCTTCGGCAGCACA
U6-reverse	CTCGCTTCGGCAGCACA
FOXO1 ChIP-qPCR-forward	GGGGTAGTGGGGTGTTTTTC
FOXO1 ChIP-qPCR-reverse	AGTACTCGGCTCTGCTGCTC