

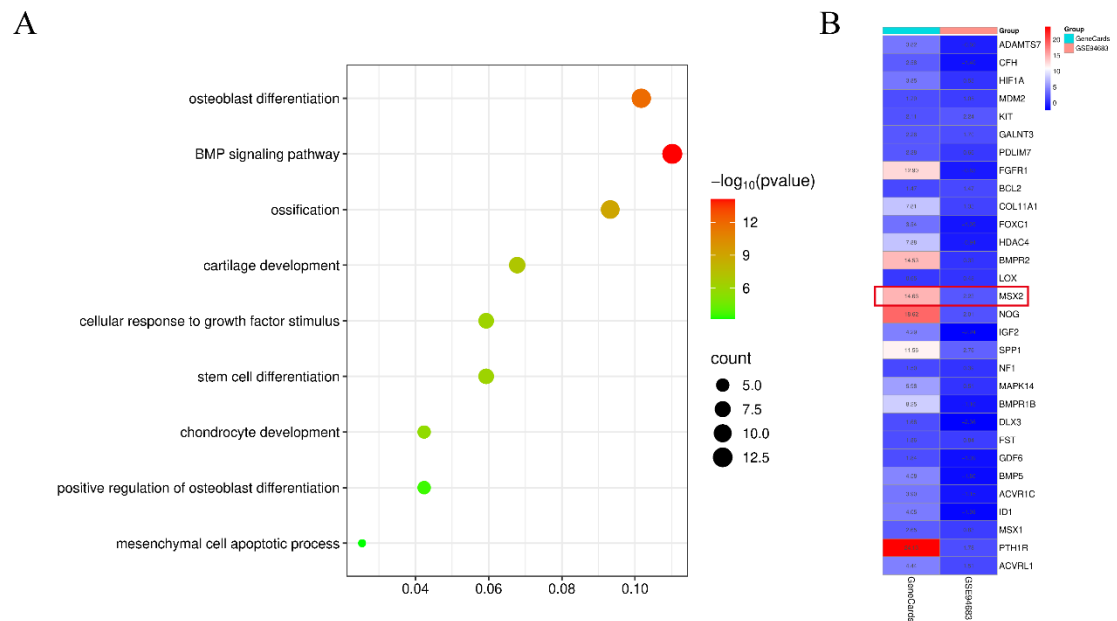


Fig S1 MSX2 screening bioinformatics analysis. **A** Biological process enriched by Gene Ontology analysis. **B** Heat map of DEGs involved in these biological process pathways, GeneCards refers to the relevance score, while the GSE94683 dataset refers to the log2 fold change.

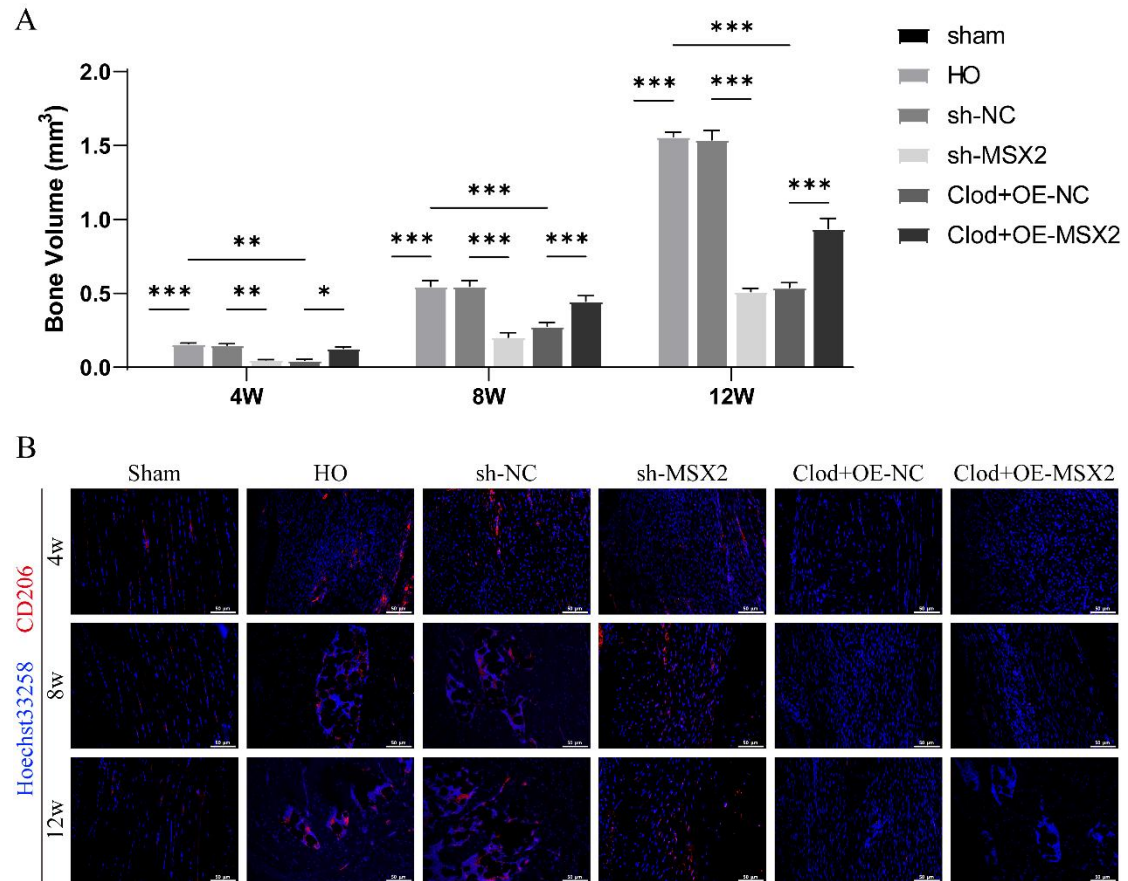


Fig S2 Elevated MSX2 promotes the progression of HO slowed by macrophage clearance. **A** Micro-CT quantitative analysis of bone volume. **B** Immunofluorescence staining of CD206 in different groups of mice. Scale bar: 50µm. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

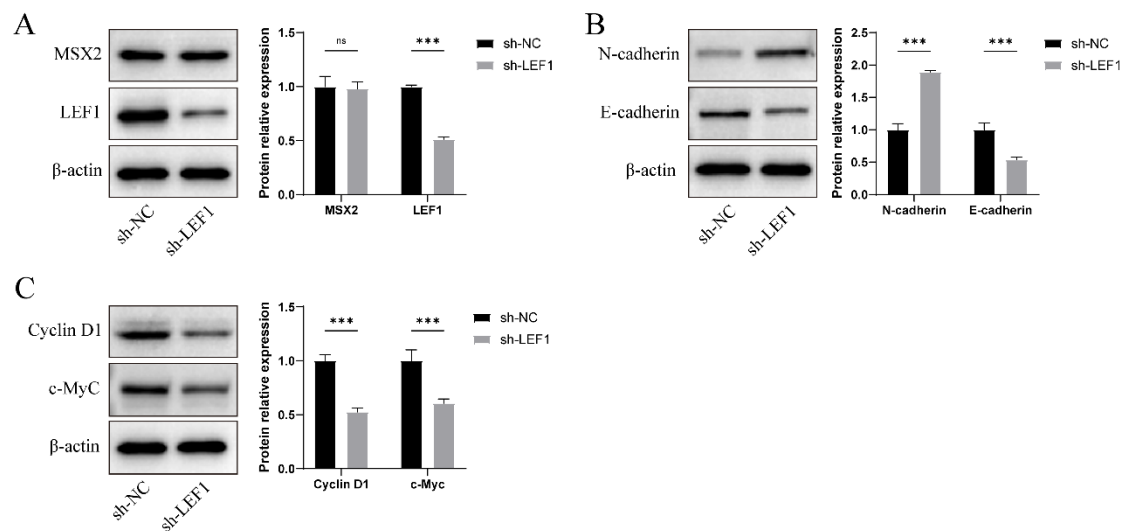


Fig S3 Changes in protein expression following LEF1 knockdown. **A** The protein expression of LEF1 and MSX2 in MAOECs by Western blot. **B** The protein expression of E-cadherin and N-cadherin in MAOECs by Western blot. **C** The protein expression of c-Myc, and Cyclin D1 in MAOECs by Western blot. *** $P < 0.001$, ns indicates no statistical difference.

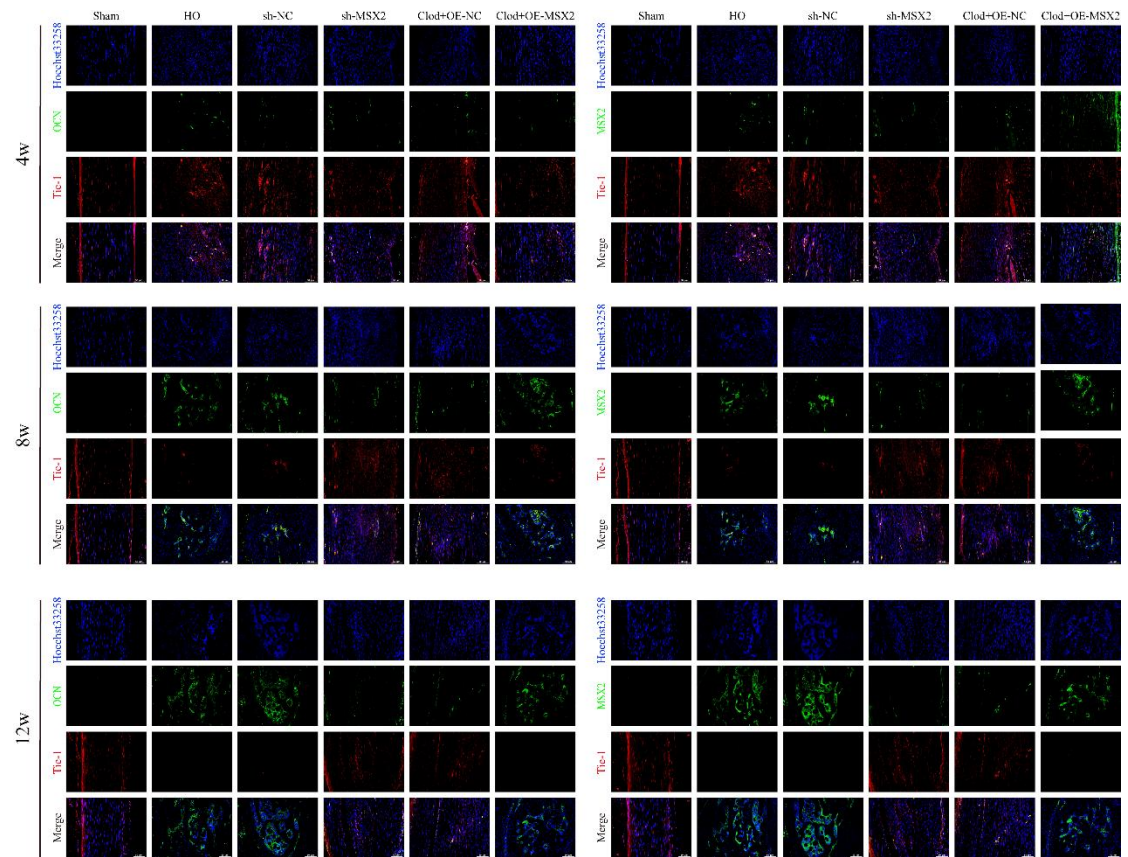


Fig S4 Tie-1, OCN, and MSX2 expression in mouse ectopic bone at 4, 8, and 12 weeks. Scale bar: 50 μm.

Table 1 Primer sequences for qPCR

Gene Name	Forward primer (5'-3')	Reverse primer (5'-3')
MSX2	CTAAAGGCGGTGACTTGTTTTCG	CGGCTTCTTGTCGGACATGAG
E-cadherin	CAGTTCCGAGGTCTACACCTT	TGAATCGGGAGTCTTCCGAAAA
Occludin	TGAAAGTCCACCTCCTTACAGA	CCGGATAAAAAGAGTACGCTGG
N-cadherin	AGGCTTCTGGTGAAATTGCAT	GTCCACCTTGAAATCTGCTGG
Vimentin	CGTCCACACGCACCTACAG	GGGGGATGAGGAATAGAGGCT
OPN	ATCTCACCATTTCGGATGAGTCT	TGTAGGGACGATTGGAGTGAAA
OCN	CCTCAGTCCCCAGCCCAGAT	ATAGTGATACCGTAGATGCGTTTGT
Runx2	ATGCTTCATTCGCCTCACAAA	GCACTCACTGACTCGGTGTTGG
SOX9	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT
SP7	GGAAAGGAGGCACAAAGAAGC	CCCCTTAGGCACTAGGAGC
LEF1	GCCACCGATGAGATGATCCC	TTGATGTCGGCTAAGTCGCC
β -actin	GTCCCTCACCTCCCAAAAG	GCTGCCTCAACACCTCAACCC

Table S2. List of antibodies

Antibodies	Source	Identifier
F4/80	Invitrogen	MA1-91124
CD86	Proteintech	65165-1-Ig
CD206	Abcam	ab64693
Tie-1	Abcam	ab201986
CD90	Abcam	ab133350
SOX9	Abcam	ab185966
OCN	Abcam	ab198228
MSX2	Invitrogen	PA5-106670
MSX2	Proteintech	68550-1-Ig
β -actin	Servicebio	GB11001
Vimentin	Proteintech	60330-1-Ig
N-cdherin	Proteintech	22018-1-AP
Occludin	Abcam	ab216327
E-cdherin	Proteintech	20874-1-AP
RUNX2	Proteintech	20700-1-AP
OCN	Abcam	ab13418
OPN	Proteintech	25715-1-AP
SP7	Abcam	ab209484
CD68	Abcam	ab125212
Tie-1	Proteintech	19329-1-AP
OCN	Servicebio	GB120012
MSX2	Santa Cruz	sc-365232
LEF1	Invitrogen	MA1-12420
c-Myc	Affinity	AF6054
Cyclin D1	Proteintech	26939-1-AP