miR-203 inhibits the traumatic heterotopic ossification by targeting Runx2

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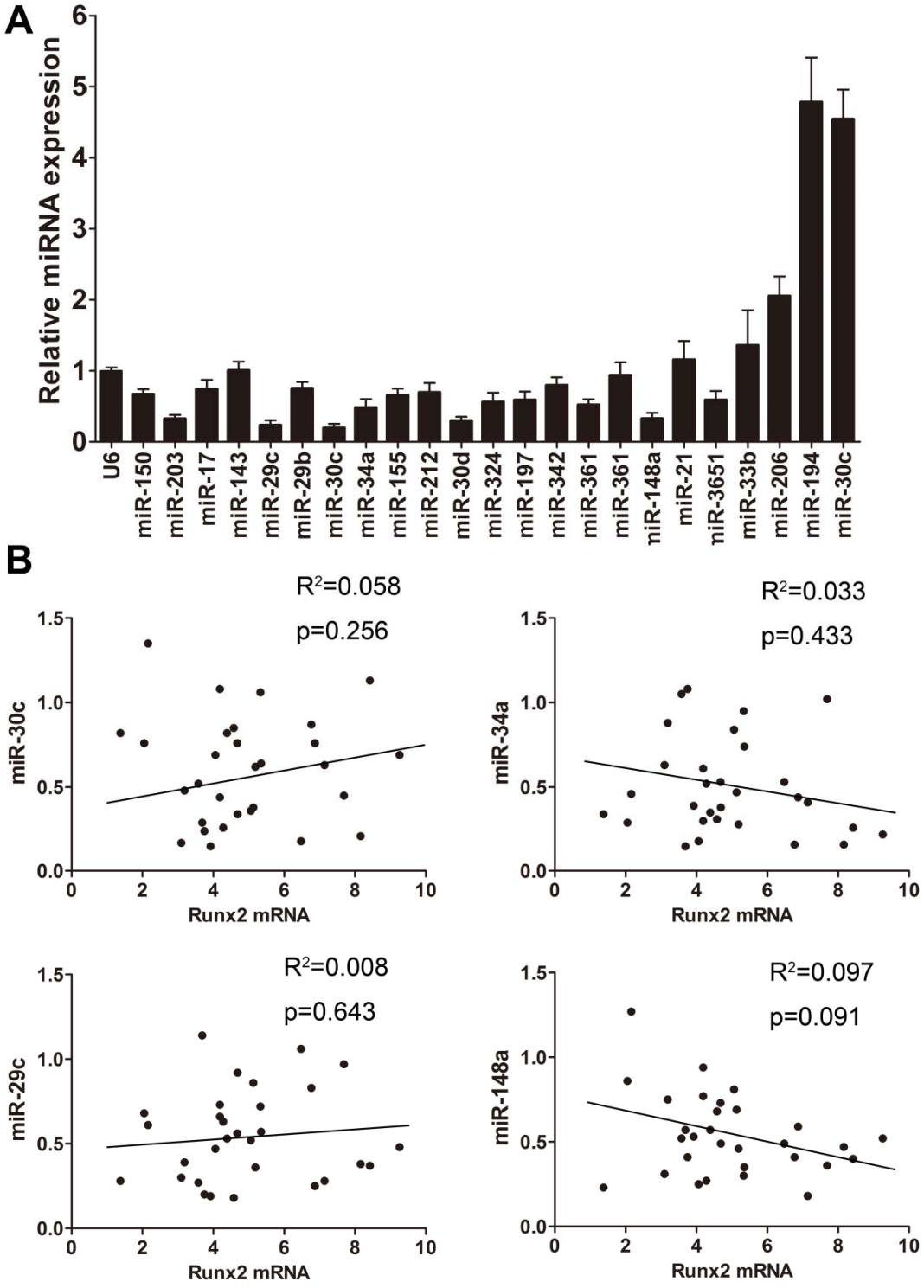
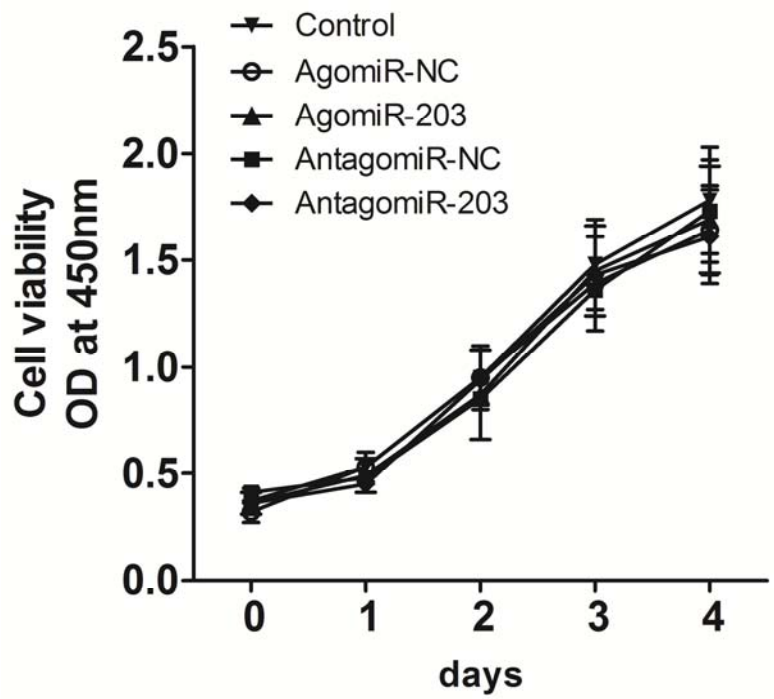
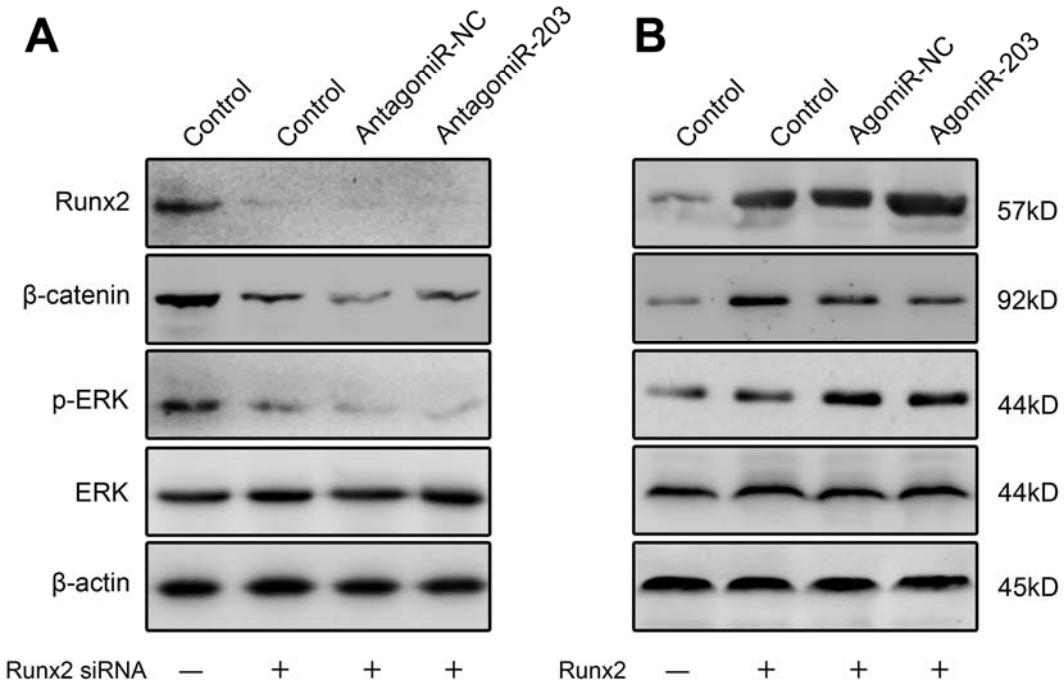
Supplementary Information



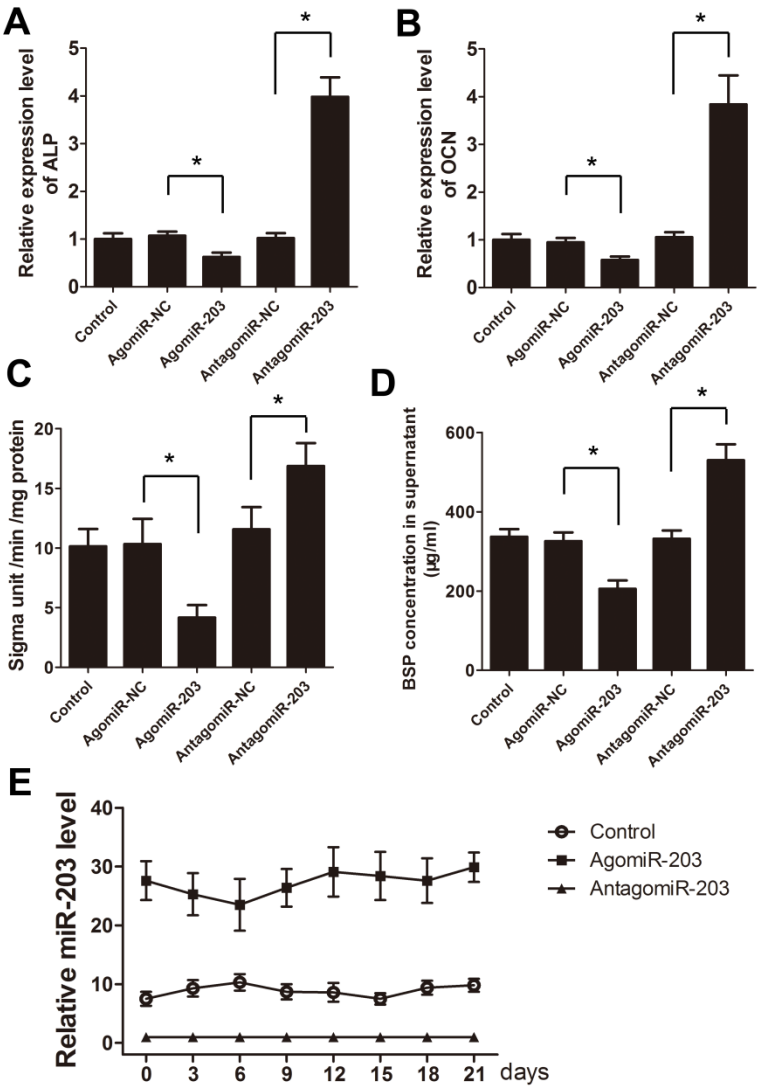
Supplementary Figure 1. miRNA screening and correlation analysis with Runx2 expression in human HO specimens. (A) Realtime-PCR analysis of miRNA expression (normalized by U6 in normal bones) in human HO. (B) Correlation analysis between miR-30c, miR-34a, miR-29c and miR-148a levels and Runx2 mRNA levels in human HO specimens.



Supplementary Fig. 2. AgomiR-203 and antagomiR-203 have no effects on the proliferation of osteoblast cells. The hFOB1.19 osteoblast cells were transfected with agomiR-203 or antagomiR-203. The cell viability was examined by CCK-8 assay after 4 days. (n=3, $p>0.05$).



Supplementary Fig. 3. miR-203 inhibits the ERK and β-catenin signaling in a Runx2-dependent manner. (A) hFOB1.19 cells were co-transfected with Runx2 siRNA and antagomiR-203. Western blot analysis of the relative levels of Runx2, β-catenin, p-ERK and ERK protein expression at 72h. (B) hFOB1.19 cells were co-transfected with Runx2 overexpression plasmid and agomiR-203. Western blot analysis of the relative levels of Runx2, β-catenin, p-ERK and ERK protein expression at 72h.



Supplementary Fig. 4. Effect of miR-203 on the activity of hFOB1.19 osteoblasts. (A) RT-PCR analysis of the miR-203 level in hFOB1.19 cells after treatment with 200 μM agomir-203, antagomir-203 or their NCs for 48 h. (B) RT-PCR analysis of OCN mRNA levels in hFOB1.19 cells after treatment with agomir-203, antagomir-203 or their NCs for 48 h. (C) ALP activity was examined in hFOB1.19 cells after treatment with agomir-203, antagomir-203 or their NCs for 48 h. (D) ELISA analysis of the BSP protein level in the supernatant of hFOB1.19 cells after treatment with agomir-203, antagomir-203

or their NCs for 48 h. (E) Time course of changes in the level of miR-203 as determined by RT-PCR analysis in hFOB1.19 cells after treatment with agomir-203 or antagomir-203 in OM. The data are representative of 3 independent experiments. The data are shown as the means ± SD. *$p<0.01$.

SUPPLEMENTAL TABLES:

Supplementary table 1. The RT-PCR primer sequences

Genes	Primer sequences
Runx2	forward 5'- CCGCCTCAGTGATTTAGGGC-3'
	reverse 5'- GGGTCTGTAATCTGACTCTGTCC-3'
β -catenin (CTNNB1)	forward 5'-AGCTTCCAGACACGCTATCAT-3'
	reverse 5'- CGGTACAACGAGCTGTTTCTAC-3'
ERK (MAPK1)	forward 5'-TCTGGAGCAGTATTACGACCC -3'
	reverse 5'- CTGGCTGGAATCTAGCAGTCT -3'
ALP	forward 5'- TGAGGGTGTGGCTTACCAG-3'
	reverse 5'- GATGGACGTGTAGGCTTTGCT-3'
OCN	forward 5'- CCTCACACTCCTCGCCCTATT-3'
	reverse 5'- CCCTCCTGCTTGGACACAAA-3'
GAPDH	forward 5'- ATGGGGAAGGTGAAGGTCG-3'
	reverse 5'- GGGGTCATTGATGGCAACAATA-3'
miR-203	forward 5'- GGGGTGAAATGTTTAGGAC-3'
	reverse 5'- CAGTGCGTGTCGTGGAGT-3'
U6	forward 5'-CTCGCTTCGGCAGCACA-3'
	reverse 5'-AACGCTTCACGAATTTGCGT-3'

Supplementary table 2. The primers used for cloning the pGL3-Runx2 3' UTR.

Gene	Primer sequence
Runx2 3'UTR-P1	forward 5'- TGCGTTTGGCTATGTGTTGT-3'
	reverse 5'- GGCAAAGCTGTGTTAGAGGC-3'
Runx2 3'UTR-P2	forward 5'- TTTGTAGGCCACCCAGCATT-3'
	reverse 5'- GCATCCCTAAAGTCACTCGGT-3'

Runx2 3’UTR-P3	forward 5’- GCAGCAACCCAGAAACACTT-3’
	reverse 5’- TGCTACAAAGAGAACCACGCT-3’
Runx2 3’UTR-P4	forward 5’- TGCACTGGGTCATGTGTTTG-3’
	reverse 5’- TCAAGGTTTGGAAGAAGTGTCT-3’