Supporting Information

Li *et al*. 10.1073/pnas.0804438105

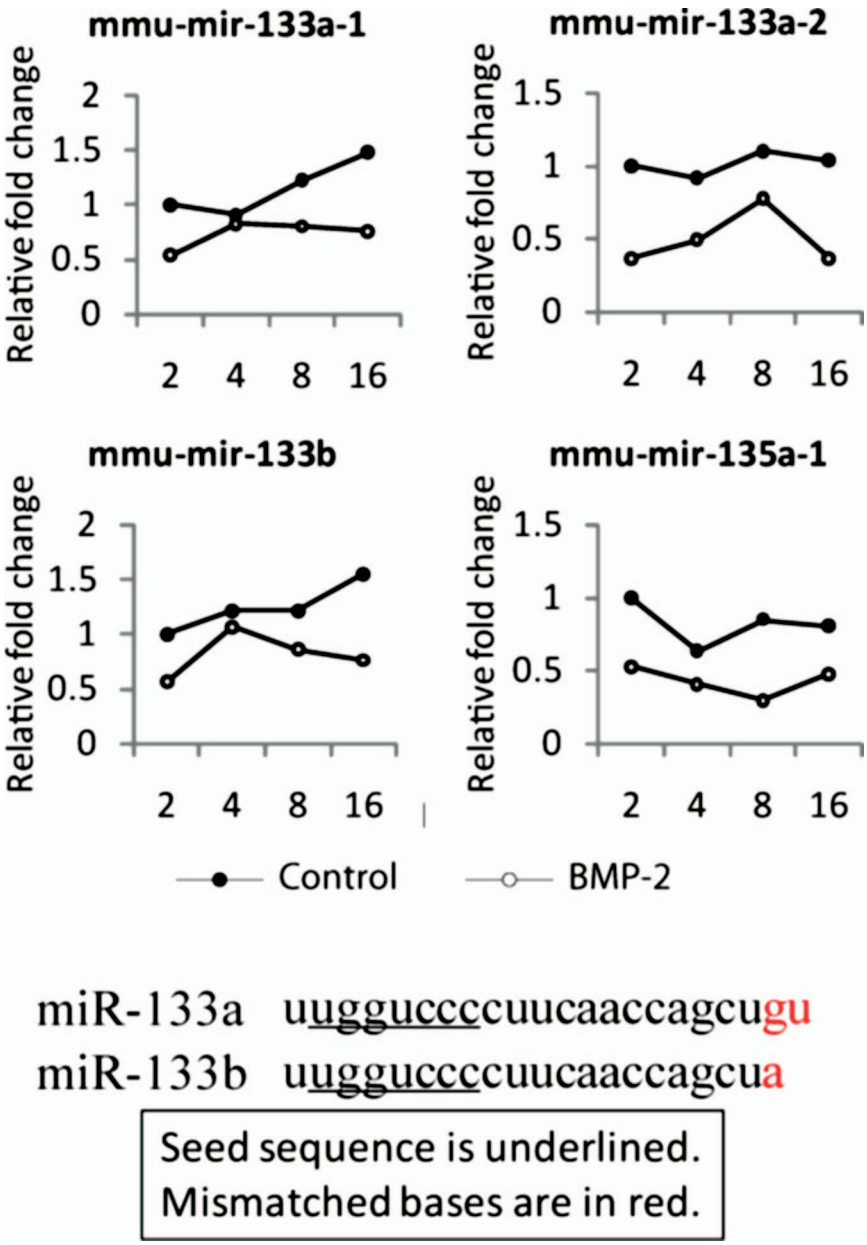

Fig. S1. Expression profile of miR-133 precursors (mmu-mir-133a-1, mmu-mir-133a-2 and mmu-mir-133b) and miR-135a precursor (mmu-mir-135a-1) during BMP2-induced C2C12 osteogenic differentiation. For each time point, the relative fold change of each miRNA precursor was gotten by normalizing 2 power value of fluorescence with 2 power value of fluorescence at 2h in Control. See *Materials and Methods* for miRNA microarray analysis. We also note that miR-133a-1 and miR-133a-2 generate the same mature miR-133a. Both miR-133a and miR-133b have the same seed sequence and differ by a two-nucleotide change outside seed region.

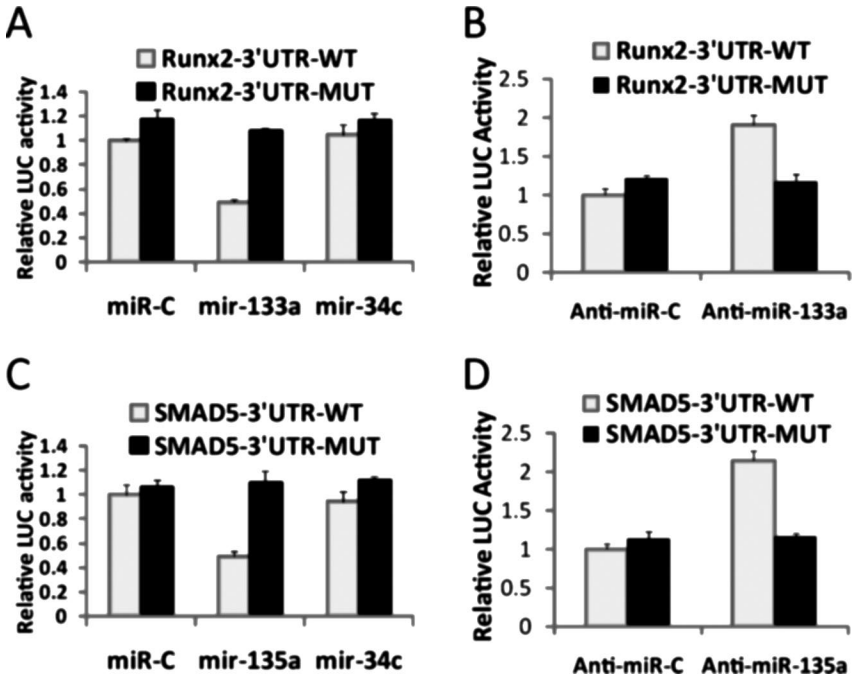

Fig. S2. Effect of pre-miRs and Anti-miRs on 3′ UTR reporter activities of targets in C2C12 cells. (*A*) C2C12 cells were co-transfected with the luciferase reporters carrying wild-type Runx2 3′ UTR or mutated Runx2 3′ UTR, phRL-null and 100 nM RNA oligonucleotides of miR-Control (miR-C), the miR-133a or miR-34c as an irrelevant control. Effects of miR-133a and control miRNAs on the reporter constructs were shown after 36 h. The ratio of reporter (*Firefly*) to control phRL-null plasmid (*Renilla*) in relative luminescence units was plotted. Error bars represent the standard error for $n = 3$. (*B*) C2C12 cells were co-transfected with the luciferase reporters, phRL-null and 100 nM RNA oligonucleotides of Anti-miR-Control (miR-C) or the Anti-miR-133a. Functional activity of the luciferase reporter plasmid was assessed as described above in panel A. Error bars represent the standard error for $n = 3$. (*C*) Functional activity of the luciferase reporter plasmid carrying wild-type or mutated Smad5 3′ UTR was assessed as described above in panel A. Error bars represent the standard error for $n = 3$. (*D*) Functional activity of the luciferase reporter plasmid carrying wild-type or mutated Smad5 3′ UTR was assessed as described above in panel B. Error bars represent the standard error for $n = 3$.

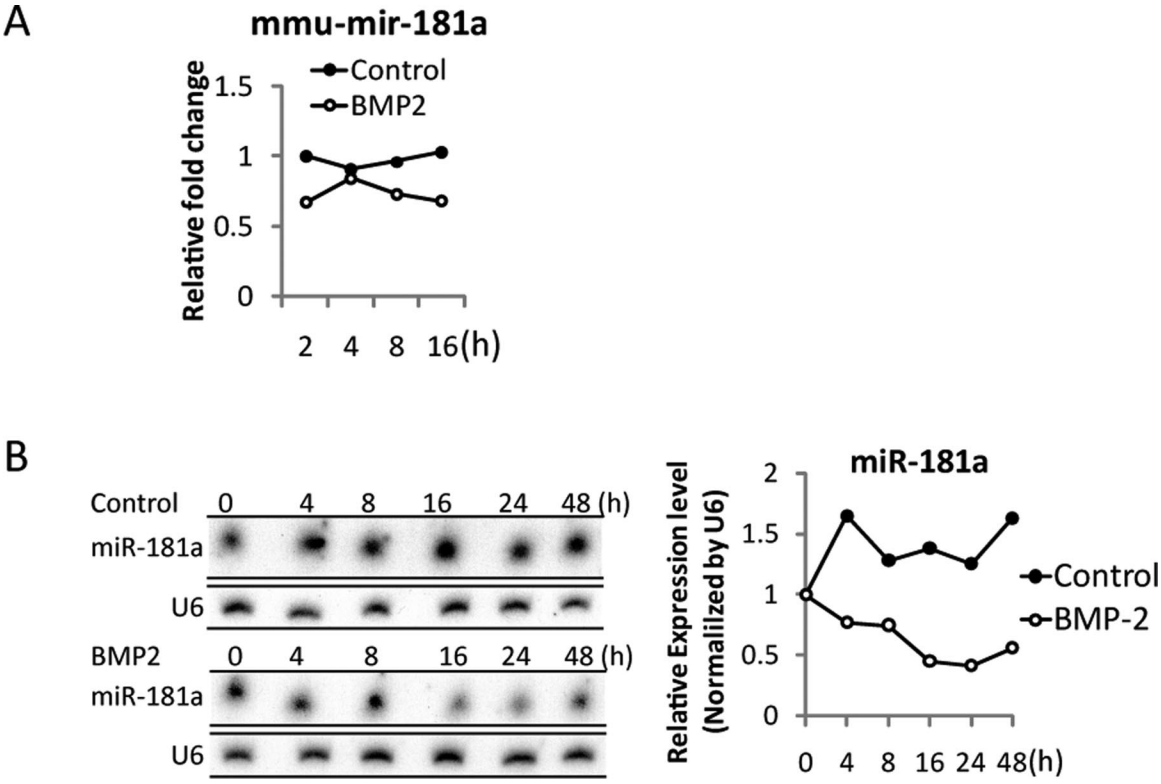

Fig. S3. miR-181a expression during BMP2-induced C2C12 osteogenic differentiation. (*A*) Expression profile of mmu-miR-181a precursor, the relative fold change was gotten as described in supplement 1. (*B*) Northern blot analysis of miR-181a expression in Control or BMP2 treatment for 0, 4, 8, 16, 24 and 48 h. U6 snRNA was used as a loading control and miR-181a expression normalized by U6 after densitometric quantitation was plotted.

Table S1. Antibodies used for Western blot analysis

Antibody	Source	Dilution for application	Company
Runx2	Mouse monoclonal	1:2,000	R & D Systems
SMAD5	Goat polyclonal	1:2,000	Santa Cruz Biotechnology, Inc.
p-Smad1/5	Rabbit monoclonal	1:1,000	Cell Signaling Technology, Inc.
p27	Mouse monoclonal	1:2,000	Santa Cruz Biotechnology, Inc.
MyoD	Rabbit polyclonal	1:2,000	Santa Cruz Biotechnology, Inc.
Lamin B1	Mouse monoclonal	1:5,000	Zymo Research Corp.
β-actin	Goat polyclonal	1:5,000	Santa Cruz Biotechnology, Inc.

Table S2. Nucleotide sequences of primers used for quantitative RT-PCR detection

Gene	Primer Sequences
Runx2	5′-CGCCCCTCCCTGAACTCT-3′ (Forward)
	5′-TGCCTGCCTGGGATCTGTA-3′ (Reverse)
Alkaline phosphatase	5′-TTGTGCGAGAGAAAGGAGA-3′ (Forward)
	5′-GTTTCAGGGCATTTTTCAAGGT-3′ (Reverse)
Osteocalcin	5′-CTGACAAAGCCTTCATGTCCAA-3′ (Forward)
	5′-GCGCCGGAGTCTGTTCACTA-3′ (Reverse)
Fibromodulin	5′-CCTCCTGTCAACACCAACCT-3′ (Forward)
	5′-GAAGTTCATGACGTCCACCA-3′ (Reverse)
Myogenin	5′-AGTGAATGCAACTCCCACAG-3′ (Forward)
	5′-ACGATGGACGTAAGGGAGTG-3′ (Reverse)
Cadherin 15	5′-CCCATCTGACATTGCCAACT-3′ (Forward)
	5′-CAGAGCCATCTCCCTCGTAG-3′ (Reverse)
Hist2H4*	5′-CCAGCTGGTGTTTCAGATTACA-3′ (Forward)
	5′-ACCCTTGCCTAGACCCTTTC-3′ (Reverse)
SMAD5	5′-AGATGG CCCCAGATAATTCC-3′ (Forward)
	5′-ACCAGTGTTTGGGCTCCTC-3′ (Reverse)
Hoxa10	5′-CTCTCCGGAGAAGGACTC-3′ (Forward)
	5′-TCTTTGCTGTGAGCCAGTTG-3′ (Reverse)
HPRT	5′-CAGGCCAGACTTTGTTGGAT-3′ (Forward)
	5′-TTGCGCTCATCTTAGGCTTT-3′ (Reverse)

*Mouse homologue of the human H4/n histone gene [Braastad CD *et al*. (2004) Functional characterization of a human histone gene cluster duplication. *Proc Natl Acad Sci USA* 342:35–40].

Table S3. Nucleotide sequences of probes for Northern blot analysis

miRNA	Probe sequence
miR-133	5′-ACAGCTGGTTGAAGGGGACCAA-3′
miR-135	5′-ACAGCTGGTTGAAGGGGACCAA-3′
miR-181	5′-ACTCACCGACAGCGTTGAATGTT-3′
U6	5′-ACAGCTGGTTGAAGGGGACCAA-3′

Table S4. Nucleotide sequences of primers for construct and mutation of plasmids

Gene	Primer sequences
WT Runx2 sense	5′-GCTCTAGAGCCCAGAATGATGGTGTTGACG-3′
WT Runx2 anti-sense	5′-GGCCGGCCCTGCCTCTTGTCCCTTTCTG-3′
Mutagenic Runx2 sense	5′-GGCCCAGTGGCATGGTCGACACATCCCGCATGTG-3′
Mutagenic Runx2 anti-sense	5′-CACATGCGGGATGTGTCGACCATGCCACTGGGCC-3′
WT Smad5 sense	5′-GCTCTAGAGCTAAAGCATGGGGAGCCTTAG-3′
WT Smad5 anti-sense	5′-GGCCGGCCTTTTAAGCAGATTTTGAGGTTTATT-3′
Mutagenic Smad5 sense	5′-GGTTTTACTGTATTGTGCTCTCACAGGCCTAACTCTTAAGAAATTTGG-3′
Mutagenic Smad5 anti-sense	5′-CCAAATTTCTTAAGAGTTAGGCCTGTGAGAGCACAATACAGTAAAACC-3′