

Supporting Information

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Human and Mouse miR-23a~27a~24-2 Cluster Promoter

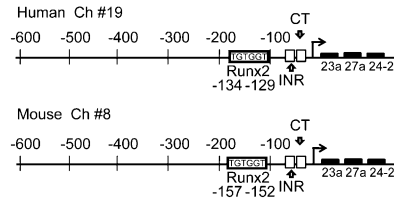


Fig. S1. Schematic of the human and mouse miR-23a~27a~24-2 cluster promoter. Representation of the -0.639 -kb miR-23a~27a~24-2 cluster promoter fragment and chromosome location for human and mouse. Transcription factor (TRANSFAC; TESS) analysis of the proximal promoter identified a Runx binding site (TGTGGT; -129 to -134 for mouse, -152 to -157 for human) which is bound by Runx2 in osteoblasts. Two other important motifs, the INR (Initiator; CCCACCTCC) motif and the CT motif in sequence at -56 to -34 , were characterized in the proximal miR-23a~27a~24-2 cluster promoter (1, 2).

1. Lee Y, et al. (2004) MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 23:4051–4060.
2. Zhou X, Ruan J, Wang G, Zhang W (2007) Characterization and identification of microRNA core promoters in four model species. *PLoS Comput Biol* 3:e37.

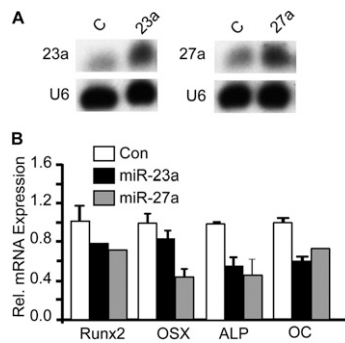


Fig. S3. Expression of cluster miRNAs 23a and 27a inhibit differentiation of MC3T3-E1 cells. (A) Northern blot showing levels of lentiviral-mediated over-expressed miR-23a and -27a in preosteoblast MC3T3-E1 cells at day 7. (B) The mRNA expression of osteoblast-specific marker genes. Transcription factors Runx2 and Osterix, ALP, and osteocalcin are represented as markers of early and late osteoblastogenesis.

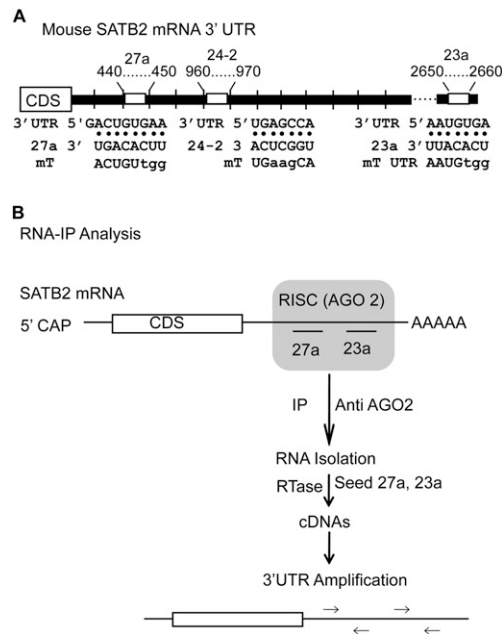


Fig. S4. RNP-IP analysis for in vivo cellular binding of the miR-23a and -27a to SATB2. (A) Schematic illustration of the 3' UTR of SATB2 mRNA with target sequences of each miR. Three putative target sites of miR-23a, -27a, and -24-2 were predicted in SATB2 mRNA by TargetScan, HOCTAR, and miRanda programs. Lowercase nucleotides indicate the mutations in the seed sequence of respective miR binding. (B) SATB2 3' UTR and RNP-IP strategy. The miR-silencing complex from MC3T3-E1 polysomal extract was immunoprecipitated with anti-argonaute (Ago2) antibody. cDNAs were made from immunoprecipitated RNA using miR-23a and -27a seed sequences. The miR-23a and -27a binding to SATB2 3' UTR were validated by PCR using primers (arrows) derived from the upstream and downstream sequences of the binding site (Table S1).

Table S1. Nucleotide sequences of primers and probes used for qPCR, ChIP, EMSA, and Northern blot

Type	Real-time qPCR primers (5'-3')
Bone marker genes	
Runx2	F: CGG CCC TCC CTG AAC TCT R: TGC CTG CCT GGG ATC TGT A
ALP	F: CCA ACT CTT TTG TGC CAG AGA R: GGC TAC ATT GGT GTT GAG CTT TT
BSP	F: CAG GGA GGC AGT GAC TCT TC R: AGT GTG GAA AGT GTG GCG TT
OC	F: CTG ACA AAG CCT TCA TGT CCA A R:GCG CCG GAG TCT GTT CAC TA
OSX	F: ATG GCG TCC TCT CTG CTT G R: TGA AAG GTC AGC GTA TGG CTT
Col1A1	F: GCT CCT CTT AGG GGC CAC T R: CCT TTGTCA GAA TAC TGA GCA GC
Precursor miRNA	
miR-23a	F: TTT GAT GCC AGT CAC AAA TCA CAT TG
miR-27a	F: GTC GTG TTC ACA GTG GCT AAG
miR-24-2	F: CAC TGG CTC AGT TCA GCA GG
Mature miRNA	
miR-23a	ATC ACA TTG CCA GGG ATT TCC
miR-27a	TTC ACA GTG GCT AAG TTC CGC
miR-24-2	TGG CTC AGT TCA GCA GGA ACA G
Chromatin IP	
Murine promoter	miR cluster ChIP primers (5'-3') F: TAG AGG AGG GCT AGG GTG TG R: GCT TGC CTG CCT ATC TTG AC
Rat promoter	F: CCT CCC GAT CTC ACT TTC CT R: GCA CAG GGT TCA GTT GGA AAT
EMSA (Runx2 site in miR promoter)	
WT	Gel shift primers (5'-3') CTT AAA CTG TGt gtg gtG AGGTGT ACC
miR MT	CTT AAA CTG TGt gta ctG AGGTGT ACC
Control	
GAPDH	Control primers (5'-3') F: AGG TCG GTG TGA ACG GAT TTG R: TGT AGA CCA TGT AGT TGA GGT CA
U6	F: CGC TTC GGC AGC ACA TAT AC R: AAA ATA TGG AAC GCT TCA CGA
Northern probes	
Mouse/rat miR-23a	Primers (5'-3') GGAAATCCCTGGCAATGTGAT
Mouse/rat miR-27a	GCGGAACTTAGCCACTGTGAA
Mouse/rat miR-24-2	CTGTTCCCTGCTGAACTGAGCCA
RIP-ChIP primers	
Mmu satb2 3'UTR (23a)	F: CTTGCCAGATCTTTGCGAAT R: GGGAAAATTGTGCTTTGTCAAG
Mmu satb2 3'UTR (27a)	F: TCGACCATAGTAATTCTAGTCACG R: CACACTTGACAGTTCCTTCA
miR-23a SEED	CAATGTGAA
miR-27a SEED	ACTGTGAA
siRNAs (Qiagen)	
Mouse Runx2 siRNA	5'-r(UGC CUC UGC UGU UUG AAA) d(TT)-3')
NS siRNA	5'-r(UUC UCC GAA CGU GUC ACG U) dTdT-3'