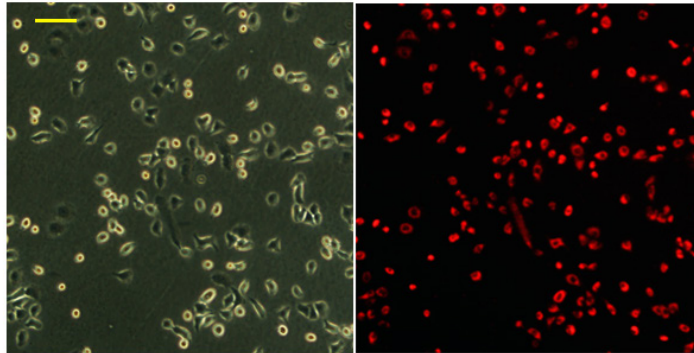


Figure S1 Multipotent differentiation capacity of mouse bone marrow stromal cells (BMSCs). (A) BMSCs were cultured in DMEM media containing 10% fetal bovine serum, 1% penicillin-streptomycin and osteogenesis-inducing fluid (50 $\mu\text{g/ml}$ ascorbic acid 10 mM β -glycerophosphate and 0.1 μM dexamethasone) and stained with Alizarin Red S at day 21; (B) BMSCs were induced by Adipogenic liquid (0.1 μM dexamethasone, 10 mg/ml insulin and 0.45 mM 3-isobutyl-1-methyl-xanthinel) and stained with Oil Red at day 21. (Scale bar: 50 μm).

Table S1 Primers used for mRNA amplification

Gene	Acc. No	Primer sequence	Size (bp)
SP7	NM_130458.3	F:5'- GCGGCAAGGTGTACGGCAAGG-3' R:5'-GGAACAGAGCAGGCAGGTGAACTTC-3'	179
ALP	NM_007431.2	F: 5'-ATCTTTGGTCTGGCTCCCATG-3' R: 5'-TTTCCCGTTCACCGTCCAC-3'	179
Runx2	NM_001145920.1	F: 5'-CGCCCCTCCCTGAACTCT-3' R: 5'-TGCCTGCCTGGGATCTGTA-3'	106
BMP2	NM_007553.2	F:5'-AGCGTCAAGCCAAACACAAACAG-3' R:5'-GGTTAGTGGAGTTCAGGTGGTCAG-3'	75
GAPDH	NM_008084.2	F: 5'-ACCACAGTCCATGCCATCAC-3' R: 5'-TCCACCACCCTGTTGCTGTA-3'	183

A 50nM



B 100nM

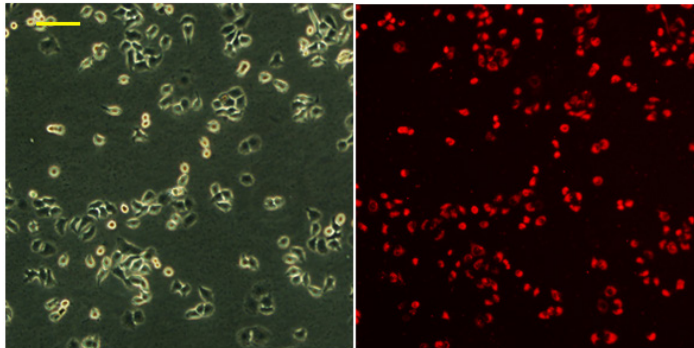


Figure S2 Synthetic miRNA were transfected in MC3T3-E1 cells effectively. Cy-3 miRNA mimics labeled red fluorescence were synthesized and transfected into MC3T3-E1 cells to monitor transfection efficiency 12h after transfection. The grey scale images show the phase contrast images and the red visual fields show the Cy-3 fluorescence of the cells. Medium was replaced with fresh culture medium before calculation. The transfection efficiency was determined as $E = \text{Cy-3-positive cell number} / \text{cell number in phase contrast}$, was ~90%. (A) 50 nM Cy-3 labeled miRNA mimics were transfected into cells; (B) 100 nM Cy-3 labeled miRNA mimics were transfected into cells. Magnification of 400 \times . Scale bar: 50 μm .

Table S2 Functional analysis of differentially regulated microRNAs closely related to the BMP2 signaling pathway

miRNA	Fold change (miRNA array)	Putative targets involved in BMP signaling
miR-106b	-3.5483	BMP2, FZD1, FZD4, FZD7, SMAD5
miR-19b	-5.4860	TGFBRII, SMAD3, Smurf1, BAMBI, CRIM1
miR-199a-3p	-5.6432	TGIF2, CRIM1, ACVR2A, ACVR2B, ACVR2C
miR-20a	-7.5456	TGFBR2, PPAR γ , BAMBI, CRIM1, SMAD4, SMAD6
miR-21	-3.7843	BMPRII, TGFB1, TGIF2, SMAD7, ACVR2A
miR-134	3.8784	HDAC5, SMAD6
miR-135a	-3.6902	BMPRII, SMAD4, SMAD5, TGFBRII, TGFBRII, PPAR γ
miR-34a	-8.3139	PPAR γ , EphA5, TGFBRII, ACVR2B
miR-34c	-4.2184	PPAR γ , EphA5, TGFBRII, ACVR2B
miR-140	-6.3280	HDAC4, ADAMTS5, BMP2, ACVR2B
miR-200b	-5.1462	HDAC4, ACVR2A, ACVR2B, HDAC4

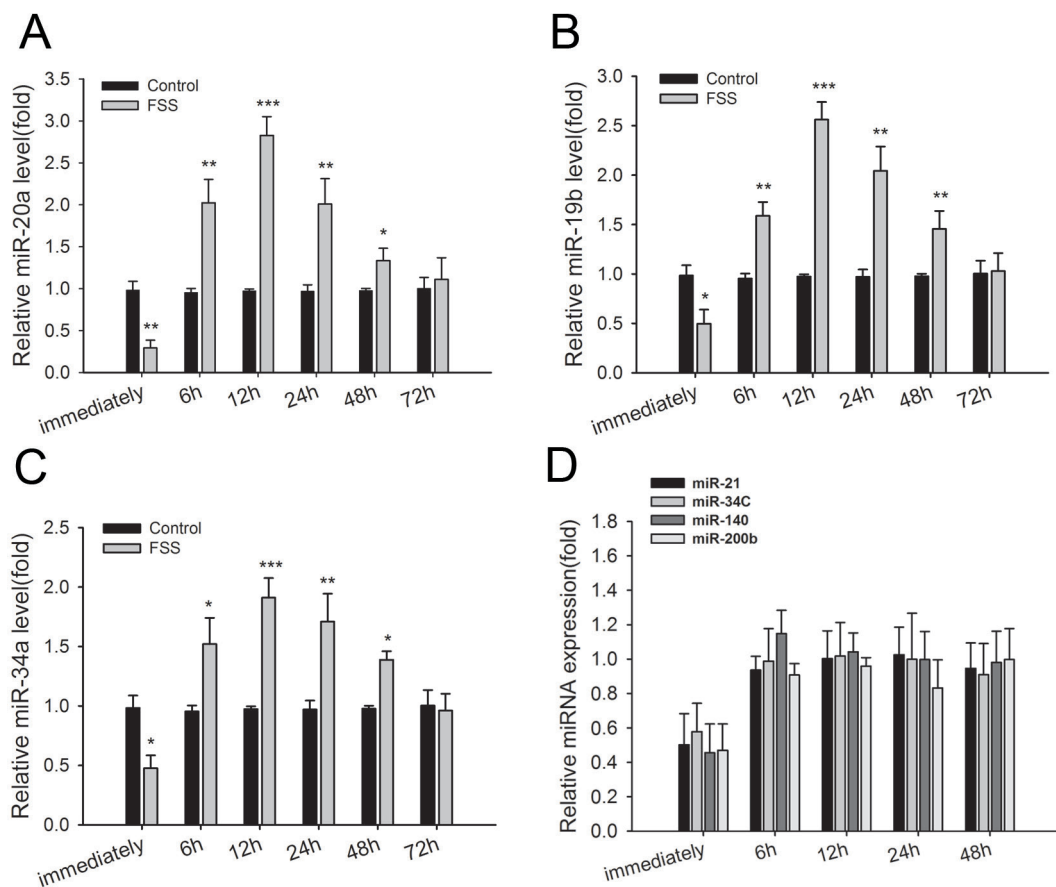


Figure S3 Spatiotemporal expression patterns of miR-20a, miR-19b, and miR-34a in FSS-induced osteogenic differentiation. After treating MC3T3-E1 cells with 12 dyn/cm² FSS for 1 h, qRT-PCR analysis showed that the expression levels of several miRNAs decreased immediately for a short time, but quickly increased at 6 h, then peaked at 12 h and remained higher than control until 72h. (A) miR-20a; (B) miR-19b; (C) miR-34a; (D) miR-21, miR-34C, miR-140, miR-200b; (Data are shown as mean \pm SD. n=3; *P<0.05; **P<0.01; ***P<0.001 vs. control group). FSS, fluid shear stress; qRT-PCR, real-time quantitative polymerase chain reaction.

Table S3 Computational prediction of targets of miR-20a involved in BMP2 signaling pathway

miRNA	Target	function	Method
miR-20a	BAMBI	BMP2 false receptor, binding to BMP2, blocking BMP2 signaling	Tag and Pic
	PPAR γ	Suppressing Runx2, negatively regulating BMP2 signaling pathway	Tag
	SMAD6	Suppressing Smad1/5/8-Smad4 complex, negatively regulating BMP2 signaling pathway	Tag and Pic

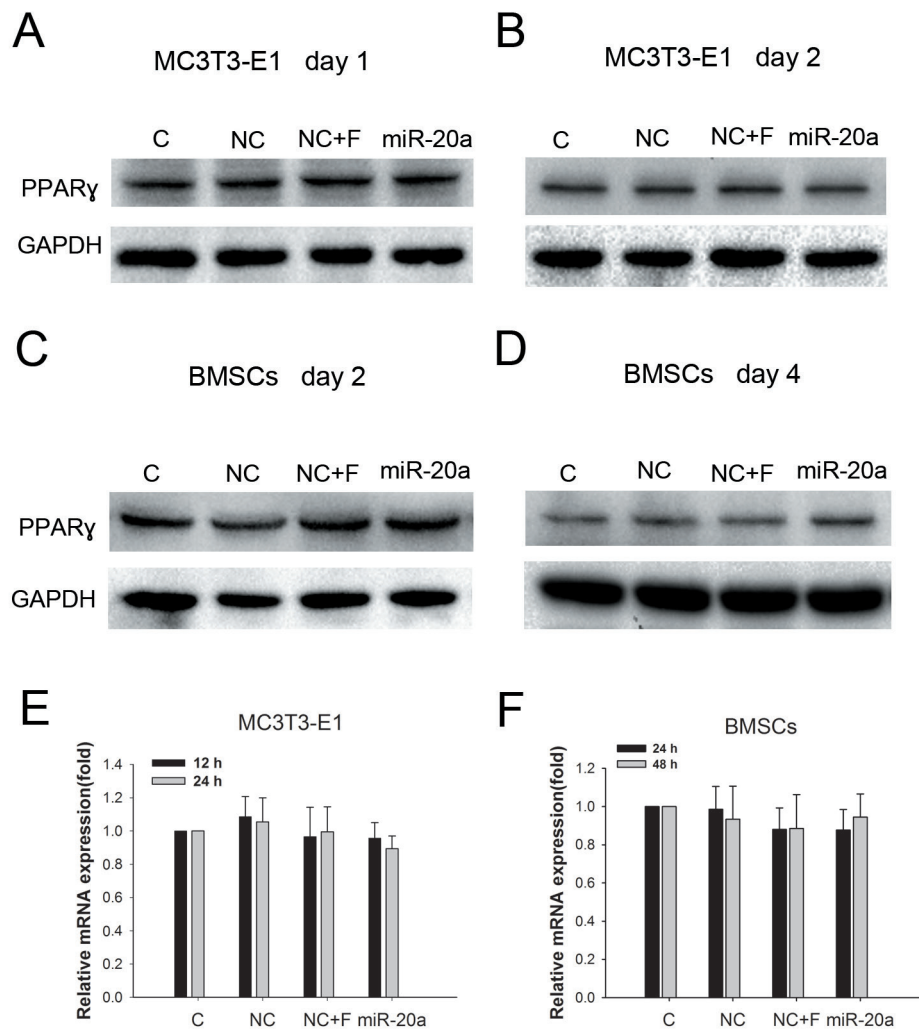


Figure S4 mRNA and protein levels of PPAR γ in MC3T3-E1 cells and mouse BMSCs post-FSS treatment. Immunoblot analysis of PPAR γ protein at day 1 and day 2 pf. in MC3T3-E1 cells(A, B) and mouse BMSCs (C, D) at day 2 and day 4 pf.. qRT-PCR analysis of PPAR γ mRNA expression at 12 h and 24 h pf. in MC3T3-E1 (E) and mouse BMSCs at 24 h and 48 h (F). qRT-PCR, real-time quantitative polymerase chain reaction; BMSCs, bone marrow stromal cells; C, transfection reagent only group; NC, miRNA negative control group; NC+F, negative control plus fluid shear stress group; miR-20a, miR-20a mimics group.