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**Supplemental Information**

**Promotion Effects of miR-375 on the Osteogenic Differentiation of Human Adipose-Derived Mesenchymal Stem Cells**

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Supplemental Figures:

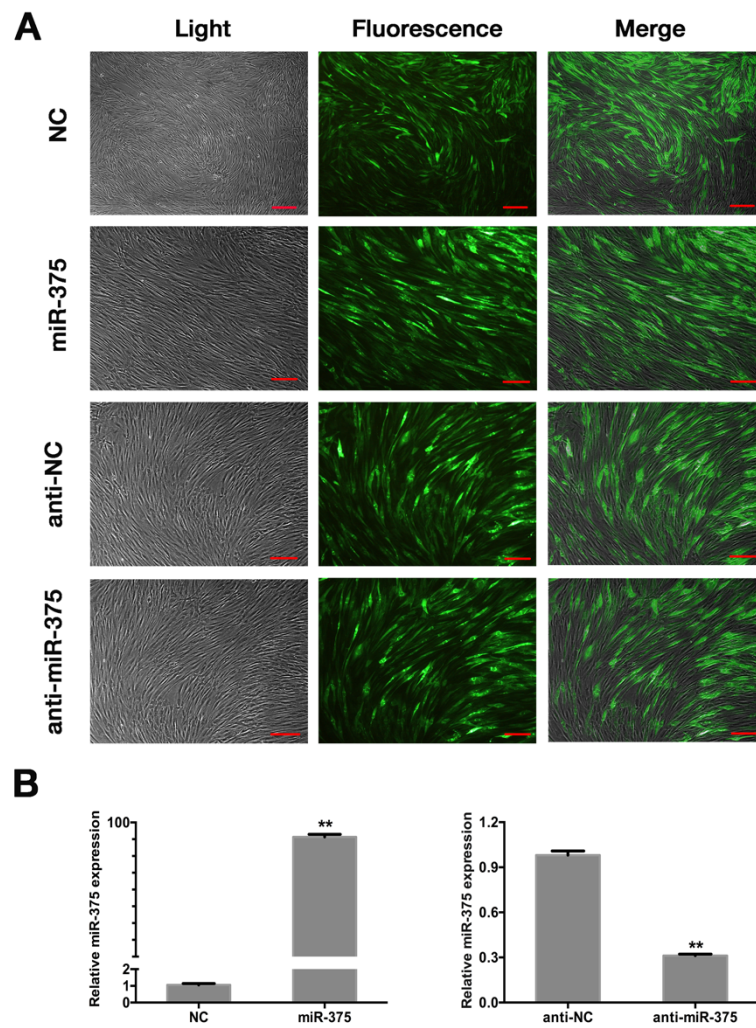


Figure S1 (related to main figure 2). Transduction efficiency of lentivirus containing miR-375, anti-miR-375, or control vectors. (A) Micrographs of GFP-positive hASCs under ordinary and fluorescent light. Scale bars: 500  $\mu$ m. (B) Left: relative miR-375 expression in miR-375 and NC groups as determined by qRT-PCR. Right: relative miR-375 expression in anti-miR-375 and anti-NC groups as determined by qRT-PCR. U6 was used for normalization. Data are represented as mean  $\pm$  SD; \* $p < 0.05$ , \*\* $p < 0.01$  ( $n = 3$  independent experiments).

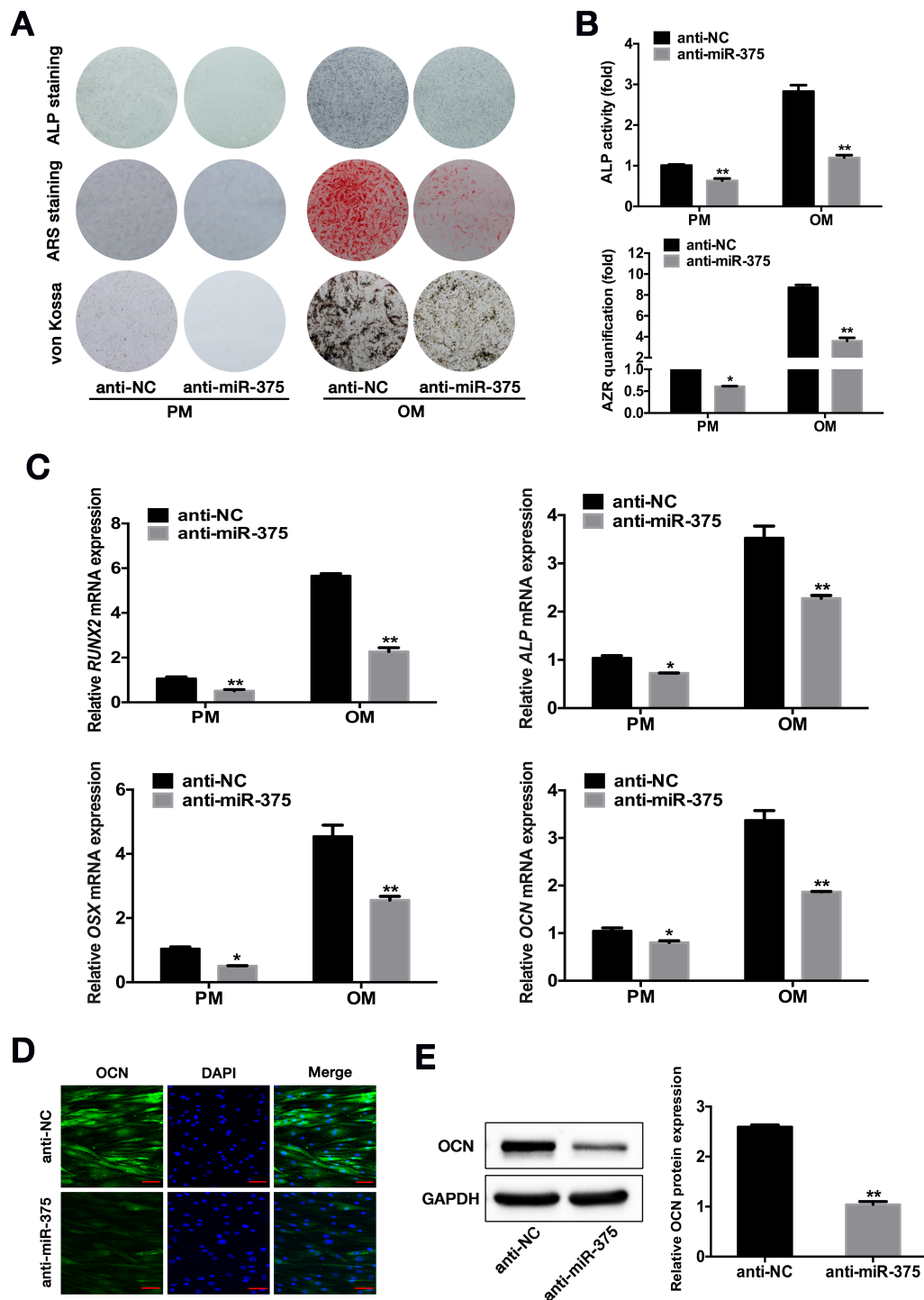
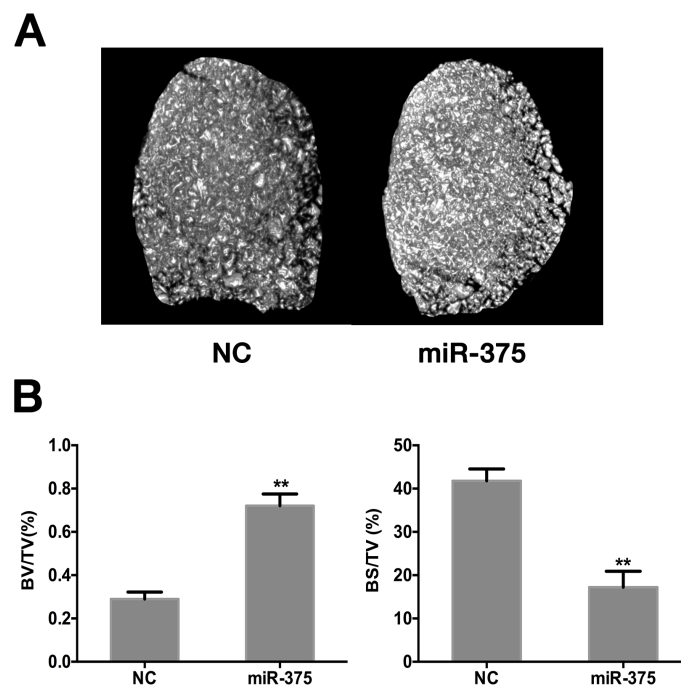
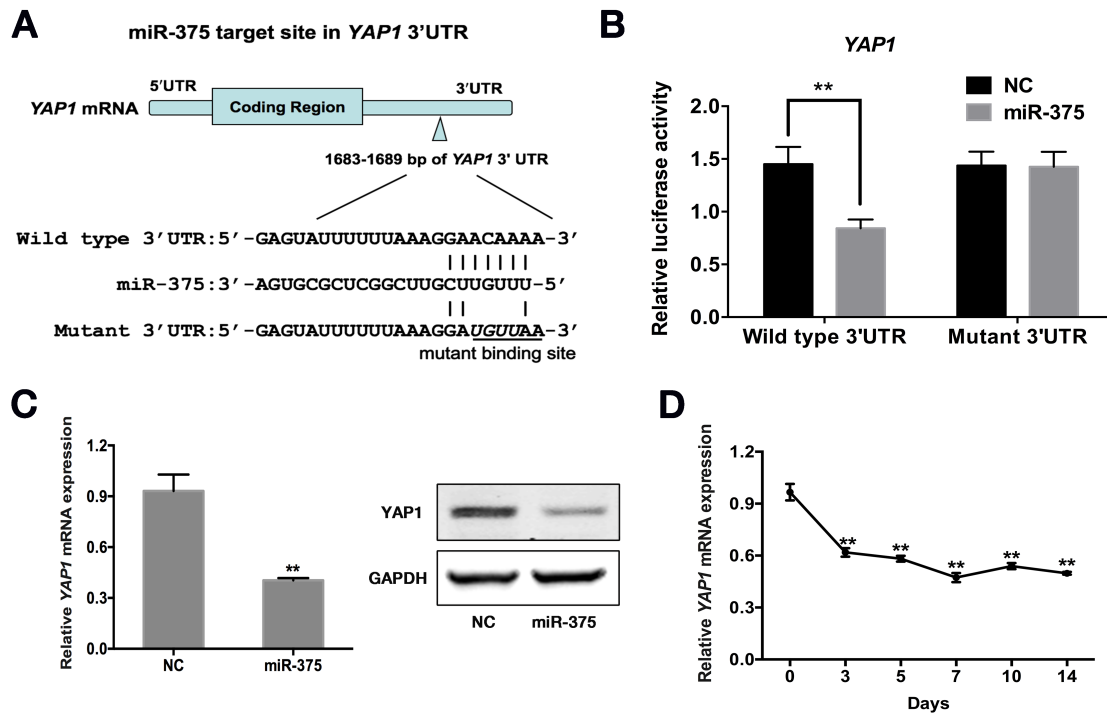


Figure S2 (related to main figure 2). miR-375 knockdown inhibits the osteogenic differentiation of hASCs. hASCs were transfected with lentivirus expressing anti-miR-375 or control vector (anti-NC), and cultured in proliferation medium (PM) or osteogenic medium (OM). (A) Alkaline phosphatase (ALP) staining on day 7, Alizarin Red S (ARS) staining on day 14, and von Kossa (VK) staining on day 21 after osteogenic induction. (B) ALP activity on day 7 and ARS mineralization assay on day 14 after osteogenic induction. (C) Relative mRNA levels of *RUNX2* and *ALP* measured by qRT-PCR on day 7 of osteogenic induction. Relative mRNA levels of *OSX* and *OCN* measured by qRT-PCR on day 14 of osteogenic induction. *GAPDH* was used for normalization. (D) Confocal microscopy of OCN with DAPI counterstaining on day 14 after osteogenic induction. Scale bars:

200  $\mu$ m. (E) Left: western blot of OCN protein level on day 14 after osteogenic induction. GAPDH was used as the internal control. Right: quantification of band intensities. Data are represented as mean  $\pm$  SD; \* $p < 0.05$ , \*\* $p < 0.01$  ( $n = 3$  independent experiments).





1) Figure S4 (related to main figure 7). miR-375 suppresses *YAP1* via directly targeting its 3'UTR. (A) Binding site of miR-375 in the 3'UTR of *YAP1*-WT mRNA (mutated bases in the 3'UTR of *YAP1*-MT are underlined). (B) Luciferase activity of cells with miR-375 overexpression in the *YAP1*-WT or *YAP1*-MT groups. (C) Relative mRNA and protein levels of *YAP1* in miR-375 and NC groups as determined by qRT-PCR and western blot. *GAPDH* was used for normalization and the internal control. (D) Relative mRNA levels of *YAP1* at various time points during the osteogenic differentiation of hASCs as determined by qRT-PCR. *GAPDH* was used for normalization. Data are represented as mean  $\pm$  SD; \* $p < 0.05$ , \*\* $p < 0.01$  ( $n = 3$  independent experiments).

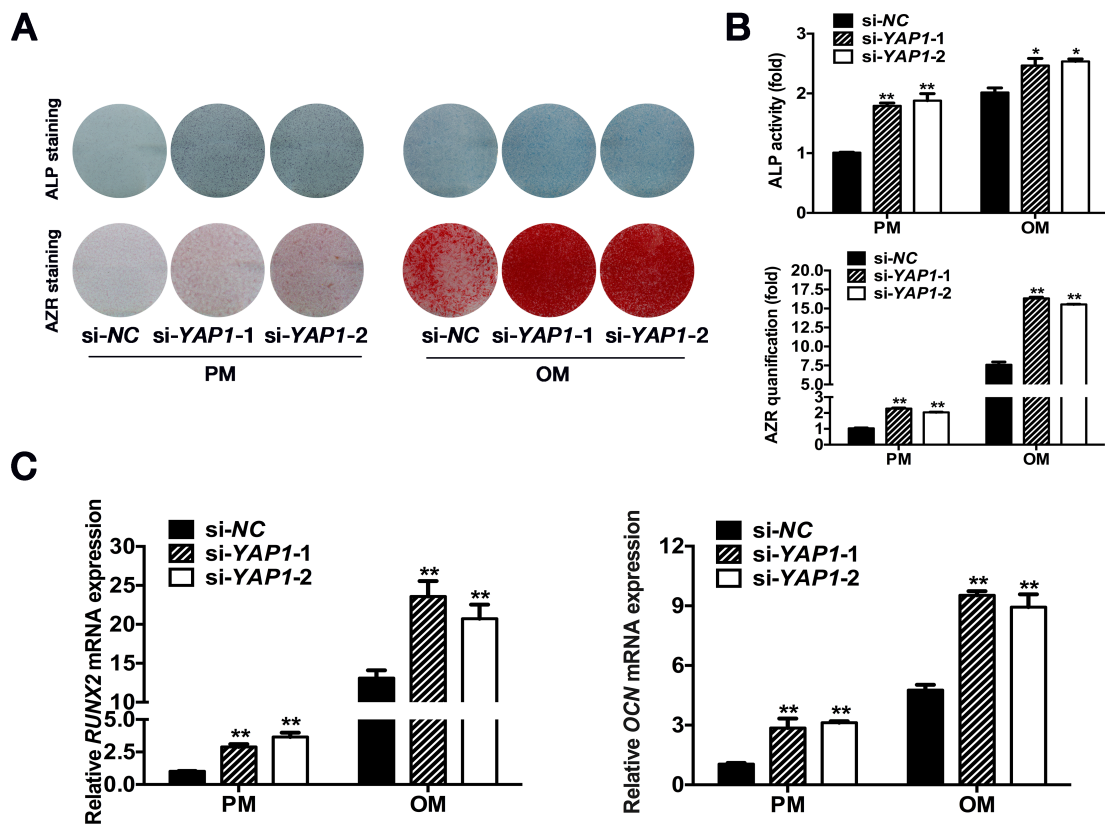


Figure S5 (related to main figure 7). Knockdown of *YAP1* enhanced the osteogenic differentiation of hASCs. (A) Alkaline phosphatase (ALP) staining on day 7 and Alizarin Red S (ARS) staining on day 14 after osteogenic induction. (B) ALP activity on day 7 and ARS mineralization assay on day 14 after osteogenic induction. (C) Relative mRNA levels of *RUNX2* and *OCN* as determined by qRT-PCR on day 14 of osteogenic induction. *GAPDH* was used for normalization. Data are represented as mean  $\pm$  SD; \* $p < 0.05$ , \*\* $p < 0.01$  ( $n = 3$  independent experiments).

### Supplemental Tables

**Table S1.** Gene expression in the osteogenic differentiation of hASCs transfected with miR-375 by microarray analysis ( $p < 0.001$ ).

Upregulation		Downregulation	
Gene Name	Fold Change	Gene Name	Fold Change
<i>MMP1</i>	8.0583	<b><i>DEPTOR</i></b>	<b>0.3794</b>
<i>MMP3</i>	5.8323	<i>ERG1</i>	0.399
<i>COL10A2</i>	3.633	<i>MGARP</i>	0.404
<i>PARRES2</i>	2.6751	<i>CTSC</i>	0.4061
<i>ADRA2A</i>	2.5316	<i>MEST</i>	0.4194
<i>CXCL5</i>	2.391	<i>FRZB</i>	0.4449
<i>COL10A1</i>	2.2842	<i>INMT</i>	0.4455
<i>AKR1C1</i>	2.2155	<i>SERPINB2</i>	0.4468
<i>CD163</i>	2.1417	<i>FHL1</i>	0.4661
<i>G0S2</i>	2.0636	<i>HSPB7</i>	0.4697
		<i>FAM43A</i>	0.4709
		<i>EFHD1</i>	0.473
		<i>KPTAP1-5</i>	0.4809
		<i>ELN</i>	0.4836

Table 1 shows the fold changes of gene expression:  $\geq 2$  indicates upregulation and  $\leq 0.5$  indicates downregulation. ***DEPTOR*** decreased most among the downregulated genes with miR-375 overexpression in hASCs.

**Table S2.** Sequences of RNA and DNA oligonucleotides.

Name	Sense Strand/Sense Primer (5'-3')	Antisense Strand/Antisense Primer (5'-3')
<b>siRNA</b>		
<i>DEPTOR1</i>	GUCAUCAUCUCAAGACCUATT	UAGGUCUUGAGAUGAUGACTT
<i>DEPTOR2</i>	GUCUGUCAGUUUGUCGUCUTT	AGACGACAAACUGACAGACTT
<i>YAPI-1</i>	GGUGAUACUAUCAACCAAATT	UUUGGUUGAUAGUAUCACCTT
<i>YAPI-2</i>	GACGACCAAUAGCUCAGAUTT	AUCUGAGCUAUUGGUCGUCTT
<i>NC</i>	UUCUCCGAACGUGUCACGUTT	ACGUGACACGUUCGGAGAATT
<b>Primers for qRT-PCR</b>		
miR-375 RT primer	GCTGTCAACGATACGCTACCTAACGGCATGACAGTGTCAGCCTA	
miR-375	GTGCAGGGTCCGAGGT	AGCCGTTTGTTCGTTCCGGCT
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
<i>RUNX2</i>	CCGCCTCAGTGATTTAGGGC	GGGTCTGTAATCTGACTCTGTCC
<i>ALP</i>	ATGGGATGGGTGTCTCCACA	CCACGAAGGGGAAGTTGTC
<i>OSX</i>	CCTCTGCGGGACTCAACAAC	TAAAGGGGCTGGATAAGCAT
<i>OCN</i>	CACTCCTCGCCCTATTGGC	CCCTCCTGCTTGGACACAAAG
<i>DEPTOR</i>	TTTGTGGTGCAGGAAGTAA	CATTGCTTTGTGTCATTCTGG
<i>YAPI</i>	ACCCTCGTTTTGCCATGAAC	TTGTTCAACCGCAGTCTCTC
<i>GAPDH</i>	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTC
<b>Primers for ChIP-qPCR</b>		
<i>Ch-1</i>	CCAGTAGCACTACATTTGGGTAA	CTGTGGCAGCCATGACATTC
<i>Ch-2</i>	CGTGGGCAACATAGCAAGAC	GCACCTCAGCCTCAAAGTAA
<i>Ch-3</i>	GGTGCCGTACTTCCGCCAATT	CTCCTCCAACCTTTCTTCTCCTCTTC
<i>Ch-4</i>	GTGGTGCCACTTCCCTACCGA	CCCTGCCATCCTTCCCTCT
<i>Ch-5</i>	GGTCGAGGTCACCACTGGATT	AGGGAGGAGCCGAGAAGCA

Abbreviation: *DEPTOR*, dep-domain containing mTOR-interacting protein; *YAPI*, Yes-associated protein 1. *RUNX2*, runt-related transcription factor 2; *ALP*, alkaline phosphatase; *OSX*, osterix; *OCN*, osteocalcin.