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Supplemental Information

MiR-34a Promotes Osteogenic Differentiation of Human Adipose-Derived Stem Cells via the *RBP2/NOTCH1/CYCLIN D1* Coregulatory Network

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Supplemental Figures and Legends

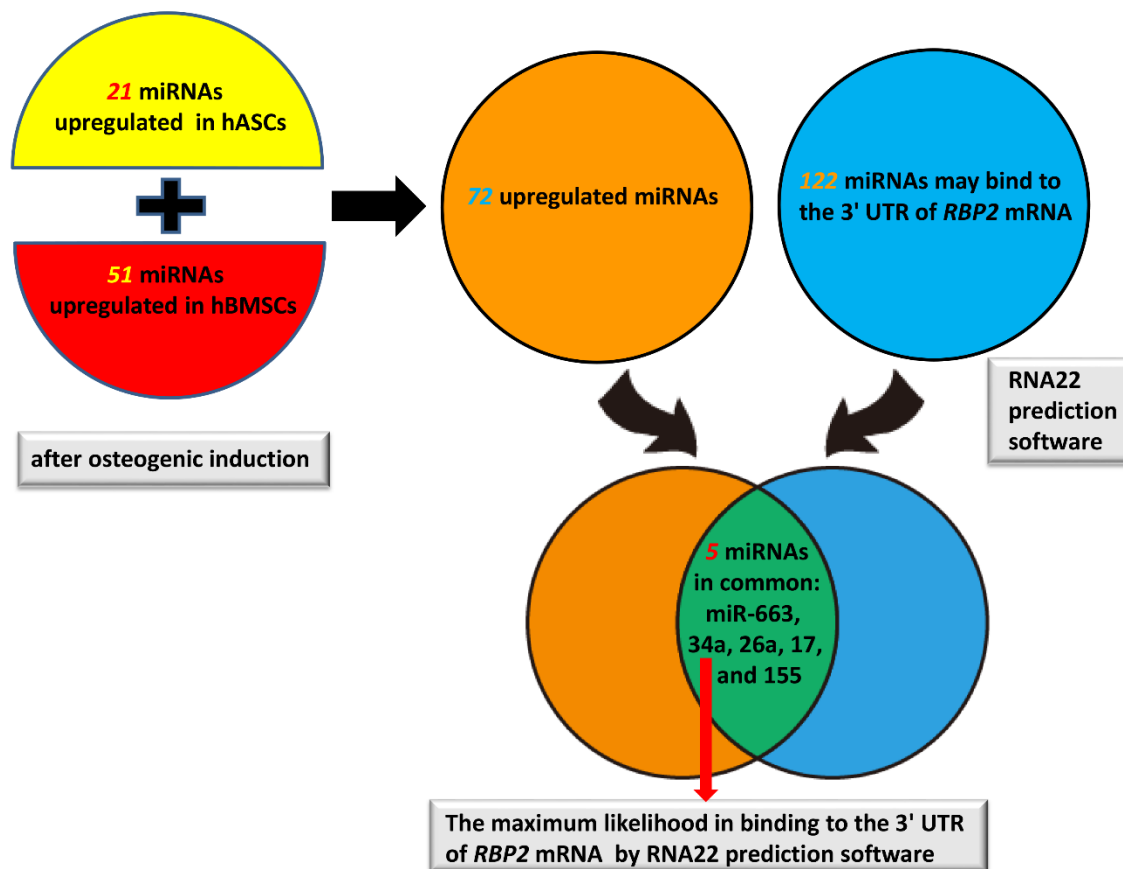


Figure S1 (related to introduction). The procedure for screening and selecting miR-34a for research. 21 and 51 miRNAs were upregulated in hASCs (miRNA expression profile during osteogenic differentiation of hASCs done by Zhang et al, J Cell Biochem, 2012, 113: 888–898) and bone marrow derived MSCs (miRNA expression profile during osteogenic differentiation of hBMSCs done by Gao et al, J Cell Biochem, 2011, 112: 1844–1856), respectively. RNA22 prediction software indicated 122 miRNAs might bind to the 3' UTR of *RBP2* mRNA. By combining and comparing the miRNAs from these two screening methods, an intersection of five

miRNAs were obtained: miR-663, 34a, 26a, 17, and 155. The RNA22 prediction software indicated that miR-34a possessed the maximum likelihood for binding to the 3' UTR of *RBP2* mRNA; therefore, we selected miR-34a for research.

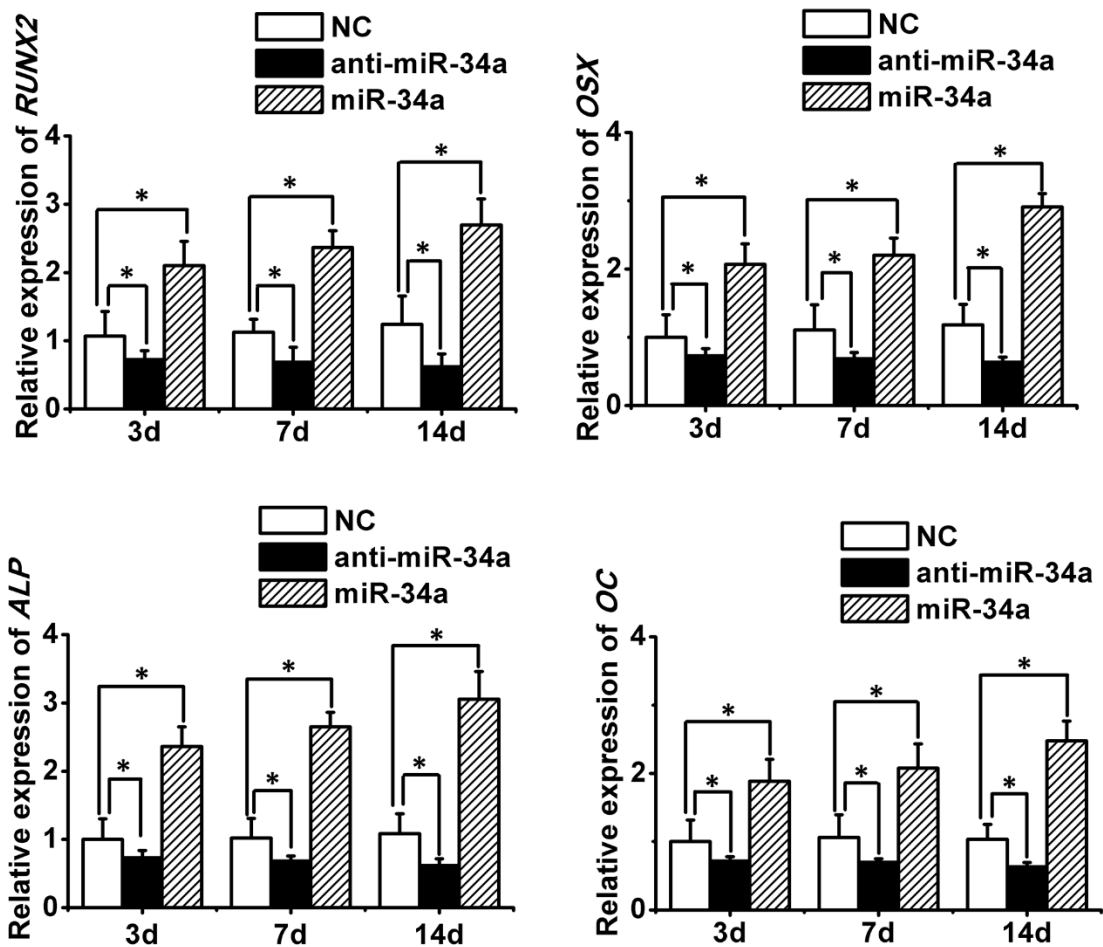


Figure S2 (related to main figure 2F). The effect of miR-34a on *RUNX2*, *OSX*, *ALP* and *OC* mRNA expression in hASCs cultured in PM.

(*RUNX2*, runt-related transcription factor 2; *OSX*, osterix; *ALP*, alkaline phosphatase; *OC*, osteocalcin; *PM*, proliferation medium).

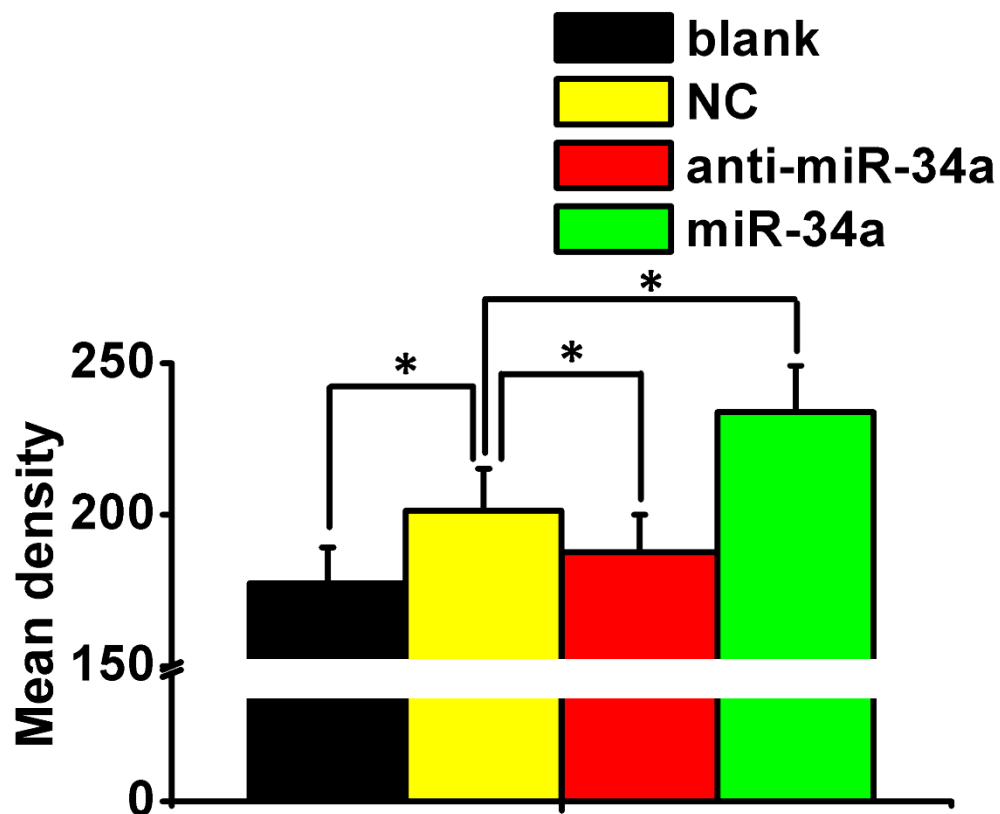


Figure S3 (related to main figure 3D-b). Quantification of HE staining. Data was represented as the mean \pm SD of three independent areas in each group.

(*HE, hematoxylin and eosin; Blank, scaffolds without hASCs; NC/anti-miR-34a/miR-34a, scaffolds seeded with hASCs transfected by lentivirus negative control/antisense miR-34a/miR-34a mimics*).

NOTCH1

| miRNA identifier | leftmost position of predicted target site | cDNA region | folding energy (in -Kcal/mol) includes contribution from linker | base pairs in putative heteroduplex | span of target | p-value |
|------------------|--|-------------|---|-------------------------------------|----------------|----------|
| hsa_miR_34a | 8237 | 3'UTR | -17.70 | 14 | 20 | 0.003420 |
| hsa_miR_34a | 8353 | 3'UTR | -17.50 | 15 | 21 | 0.265000 |
| hsa_miR_34a | 8638 | 3'UTR | -19.90 | 13 | 23 | 0.047700 |
| hsa_miR_34a | 9059 | 3'UTR | -16.50 | 16 | 20 | 0.329000 |

CYCLIN D1

| miRNA identifier | leftmost position of predicted target site | cDNA region | folding energy (in -Kcal/mol) includes contribution from linker | base pairs in putative heteroduplex | span of target | p-value |
|------------------|--|-------------|---|-------------------------------------|----------------|----------|
| hsa_miR_34a | 1910 | 3'UTR | -12.10 | 14 | 21 | 0.380000 |
| hsa_miR_34a | 2059 | 3'UTR | -12.10 | 17 | 24 | 0.051800 |
| hsa_miR_34a | 3179 | 3'UTR | -14.20 | 16 | 19 | 0.081000 |
| hsa_miR_34a | 3808 | 3'UTR | -19.20 | 15 | 20 | 0.026100 |
| hsa_miR_34a | 3886 | 3'UTR | -18.60 | 14 | 22 | 0.186000 |

Figure S4 (related to main figure 5A, 5B). The potential miR-34a target sites in *NOTCH1* and *CYCLIN D1* transcripts were predicted by RNA22 software.

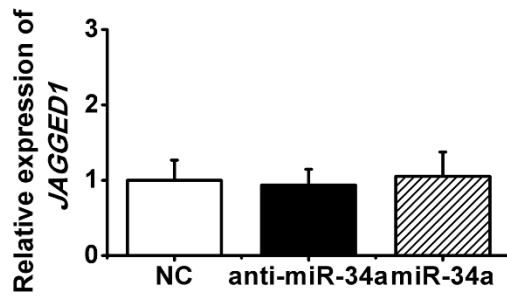
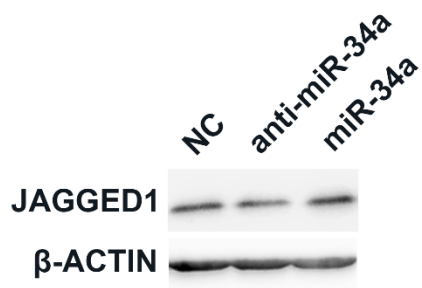
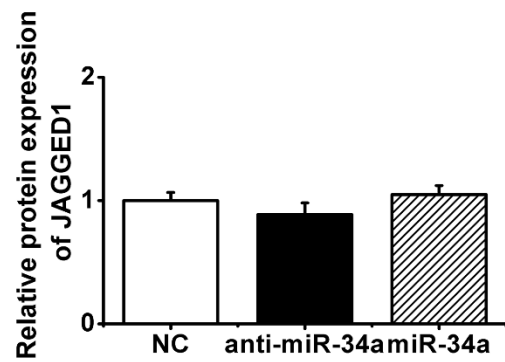
A**B****C**

Figure S5 (related to discussion). qRT-PCR (A), Western blotting and quantitative analysis (B, C) of *JAGGED1* expression in hASCs with overexpression or knock-down of miR-34a.

Supplemental Table

Table S1. Sequences of the primers used for qRT-PCR.

| | Forward primer (5' to 3') | Reverse primer (5' to 3') |
|-------------------------|----------------------------------|----------------------------------|
| <i>RBP2</i> | GTCCAGCGCCTGAATGAACTT | GCAACAATCTTGCTCAAAGCATA |
| <i>NOTCH1</i> | CGCTGACGGAGTACAAGTG | GTAGGAGCCGACCTCGTTG |
| <i>P27</i> | ATGTCAAACGTGCGAGTGTCTAA | TTACGTTTGACGTCTTCTGAGG |
| <i>CYCLIN D1</i> | GTGCTGCGAAGTGGAACC | ATCCAGGTGGCGACGATCT |
| <i>RUNX2</i> | CCGCCTCAGTGATTTAGGGC | GGGTCTGTAATCTGACTCTGTCC |
| <i>OSX</i> | CCTCTGCGGGACTCAACAAC | TAAAGGGGGCTGGATAAGCAT |
| <i>ALP</i> | ATGGGATGGGTGTCTCCACA | CCACGAAGGGGAACCTTGTC |
| <i>OC</i> | CACTCCTCGCCCTATTGGC | CCCTCCTGCTTGGACACAAAG |
| <i>GAPDH</i> | GAAGGTGAAGGTCGGAGTC | GAAGATGGTGATGGGATTTC |
| <i>U6</i> | CTCGCTTCGGCAGCACA | AACGCTTCACGAATTTGCGT |

Abbreviations: RBP2, retinoblastoma binding protein 2; Runx2, runt-related transcription factor 2; OSX, osterix; ALP, alkaline phosphatase; OC, osteocalcin.

Supplemental References

Gao, J., Yang, T., Han, J., Yan, K., Qiu, X., Zhou, Y., Fan, Q., and Ma, B. (2011). MicroRNA expression during osteogenic differentiation of human multipotent mesenchymal stromal cells from bone marrow. *J Cell Biochem* 112, 1844–1856.

Zhang, Z.J., Zhang, H., Kang, Y., Sheng, P.Y., Ma, Y.C., Yang, Z.B., Zhang, Z.Q., Fu, M., He, A.S., and Liao, W.M. (2012). MiRNA expression profile during osteogenic differentiation of human adipose-derived stem cells. *J Cell Biochem* 113, 888–898.