



Supplementary Materials

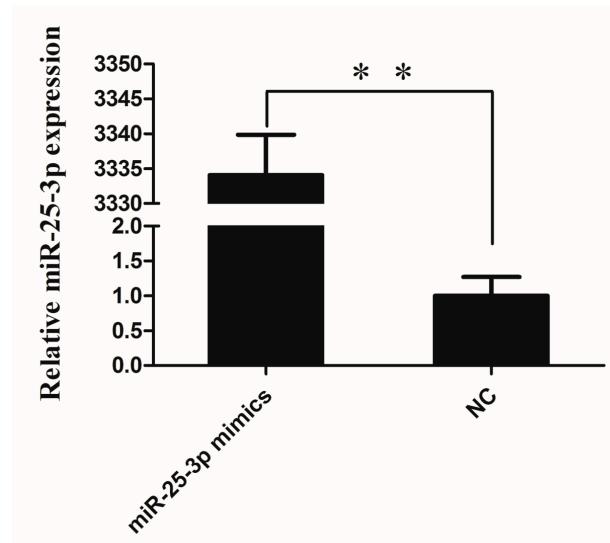


Figure S1. The mRNA expression of miR-25-3p was detected by qRT-PCR. NC, negative control (miR-239b-5p of *caenorhabditis elegans*). Data were presented as means \pm SD ($n = 3$), ** $p < 0.01$.

■ AliBaba2.1

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seq( 1860.. 1919)      tagctgagcgccccaggctccatttgtttatggggaggctagtggagttcagaagggtc
Segments:
2.3.1.0    1854 1863  1===
2.3.1.0    1867 1877  ===YY1===
1.6.1.0    1868 1877  =AP-2alph=
2.3.2.3    1868 1877  ===BRF1===
1.1.3.0    1882 1891  =C/EBPalpha=
1.6.1.0    1890 1899  =AP-2alph=
```

■ Genomatix

Matrix	Start position	End position	Core sim.	Matrix sim.	Sequence
Activator protein 2 alpha (AP2)	1908	1922	0.906	0.942	agcGCCCcaggctcc

Figure S2. Transcription factor AP-2 α binding sites in the core promoter of mouse miR-25-3p were predicted by AliBaba 2.1 and Genomatix softwares programs.

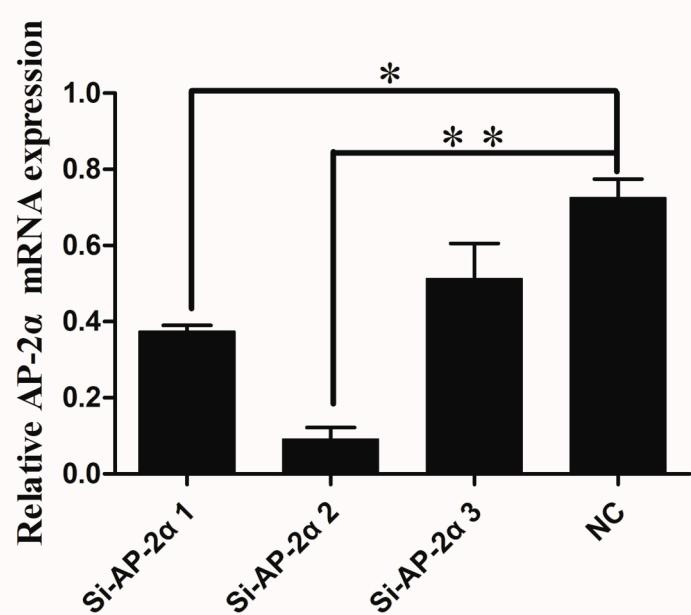


Figure S3. The mRNA expression of AP-2 α was detected by qRT-PCR. Data were presented as means \pm SD ($n = 3$), * $p < 0.05$; ** $p < 0.01$.

Table S1. Oligonucleotide sequences of miR-25-3p and AP-2 α siRNAs.

Name	Sequence (5'-3')
miR-25-3p mimics	CAUUGCACUUGUCUCGGUCUGA UCAGACCGAGACAAGUGCAAUG
miR-25-3p NC	UUUGUACUACACAAAAGUACUG CAGUACUUUUGUGUAGUACAAA
miR-25-3p inhibitor	UCAGACCGAGACAAGUGCAAUG
miR-25-3p inhibitor-NC	CAGUACUUUUGUGUAGUACAAA
siAP-2 α -1	F: GGAGAGCGAAGCUUAAGAATT R: UUCUUAGACUUUCGUCUCCTT
siAP-2 α -2	F: CCAAAGCAGUAGCAGAAUUTT R: AAUUCUGCUACUGCUUUGGTT
siAP-2 α -3	F: GCAUCCUUCGAGCAAAUATT R: UUUAUUGCUCGAAGGAUGCTT

Note: Italics bases represent protective bases.

Table S2. Primers for amplification of *Akt1* 3' UTR and its mutated fragment.

Name	Primer sequence (5'-3')
Akt1	F: <u>GGGT</u> TTAACGCCAGGTCA <u>GG</u> GAGGAAACTA R: <u>CCG</u> CTCGAG <u>CTT</u> CTGGTGC <u>CTT</u> CTGCTG
	Mut-F: GAC <u>CG</u> GACC <u>AT</u> G <u>T</u> GG <u>GG</u> CT <u>AT</u> G
	Mut-R: CATGAG <u>CC</u> CAC <u>AA</u> ACT <u>AG</u> CATGGTC <u>CG</u> GT <u>C</u>

Note: Italics, protective bases; underlined, enzyme loci; shadow, mutation sites

Table S3. Primers for amplification of miR-25-3p promoters and AP-2 α transcription factor binding sites mutated fragment.

Name	Primer sequence (5'-3')	Size (bp)	Tm (°C)
miR-25-3p-P1	F: <u>GGGT</u> TAC <u>CA</u> CTGACT <u>TT</u> AG <u>GGG</u> CGGTG R: CCA <u>AG</u> CTT <u>GT</u> CAG <u>AC</u> CGAG <u>AC</u> GTGC	2034	61
miR-25-3p-P2	F: <u>GGGT</u> TAC <u>CG</u> G <u>TT</u> CT <u>CG</u> ATT <u>CG</u> AC <u>CT</u> CCT R: CCA <u>AG</u> CTT <u>GT</u> CAG <u>AC</u> CGAG <u>AC</u> GTGC	1779	60
miR-25-3p-P3	F: <u>GGGT</u> TAC <u>CG</u> CAG <u>AC</u> AC <u>CT</u> AA <u>AC</u> AC <u>CT</u> CC	1623	60

	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P4	F: <u>GGGGTACCC</u> CATCACCTATGTCCACCAGCA	1425	60
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P5	F: <u>GGGGTACCC</u> CTGGGTCTCTGTAGCCA	1218	60
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P6	F: <u>GGGGTACCC</u> CTCCGCTTCCCCTGAACC	782	60
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P7	F: <u>GGGGTACCC</u> CTCCGACTTTCCACTGCTC	602	59
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P8	F: <u>GGGGTACCC</u> AGGCTGCTTGCTGCTTGAAT	383	59
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
miR-25-3p-P9	F: <u>GGGGTACCC</u> CCCCCAGGACACAACCTCTG	263	59
	R: CCAAGCTTGTCAAGACCGAGACAAGTGC		
AP-2α-mut	F: TAGCTGAGGCCCTGTACTCCATTGTTGA R: TCAAACAAATGGAGTACAGGGCGCTCAGCTA		

Note: Italics, protective bases; underlined, enzyme loci.

Table S4. Primers for ChIP assays.

Name	Primer sequence (5'-3')	Size (bp)	Tm (°C)
ChIP-AP-2α	F: TGCTGAGCTAGCACTTCCC R: CTGTCCTGTGAGGGAGACCA	132	60

Table S5. Primers for qRT-PCR.

Primer	Primer sequence (5'-3')	Size(bp)	Tm (°C)
miR-25-3p Loop	CTCAACTGGTGTGAGTCGGCAATTCA GAGTCAGACCG	66	60
miR-25-3p	F: CTGGTAGGC ATTGCACTTGTCT R: TCAACTGGTGTGAGTCGGAG		
U6 Loop	CTCAACTGGTGTGAGTCGGCAATTCA GAGAAAAATATGGAACGCT		
U6	F: CTGGTAGG TGCTCGCTCGCAG R: TCAACTGGTGTGAGTCGGAG	150	60
Akt1	F: GATGGAGGCCAGGGTACAAA R: GCAGCGACACCAACAAAAATGA	176	59
PI3K	F: GAAATCTCCTGGGATGTGTCGT R: ATCTGGTGGCTCTGGAGTAA	223	59
β-actin	F: CAAGAGAGGTATCCTGACCCT R: TGATCTGGGTCATCTTTCAC	188	58

Note: red, loop sequences; yellow, universal sequences; bold bases, protective bases.