

Supplemental Materials: miR-218 Inhibits Erythroid Differentiation and Alters Iron Metabolism by Targeting *ALAS2* in K562 Cells

Yanming Li, Shuge Liu, Hongying Sun, Yadong Yang, Heyuan Qi, Nan Ding, Jiawen Zheng, Xunong Dong, Hongzhu Qu, Zhaojun Zhang and Xiangdong Fang

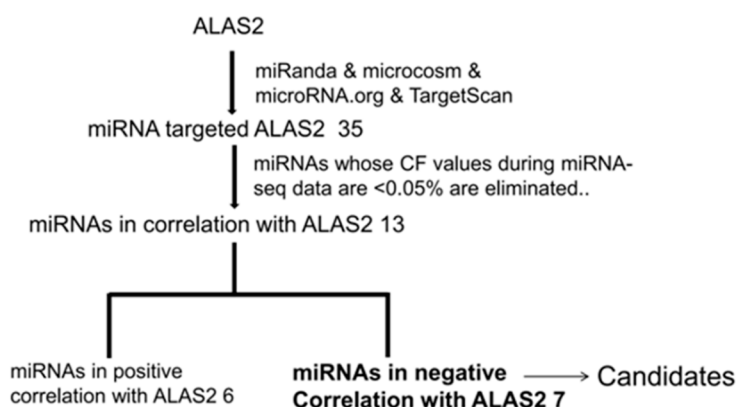


Figure S1. Methodology of screening candidate miRNAs.

Table S1. Expression (CF) pattern of miRNAs which may targeting *ALAS2* with inverse correlation with *ALAS2*.

miRNA	HESC	ESER	FLER	PBER
hsa-miR-124	75.226	0.147	3.074×10^{-4}	0.032
hsa-miR-206	0.500	9.198×10^{-4}	2.043×10^{-6}	0
hsa-miR-218	0.051	1.004×10^{-4}	2.101×10^{-7}	0
hsa-miR-222	119.028	0.232	4.864×10^{-4}	4.200
hsa-miR-330-3p	13.583	0.026	5.551×10^{-5}	5.818
hsa-miR-342-5p	0.288	5.627×10^{-4}	1.178×10^{-6}	0.243
hsa-miR-518d-5p	0.081	1.579×10^{-4}	3.310×10^{-7}	0

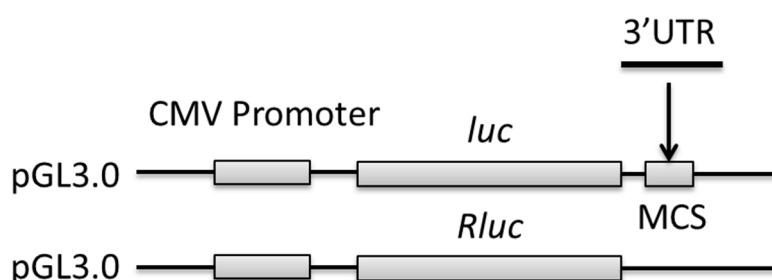


Figure S2. Vectors applied in dual-luciferase reporter assay.

Table S2. Primers sequences for ampification of primary miRNAs.

Traget	Primer Sequence (5'-3')
Pri-miR-124	GGGCTCGAGCTGTAAATGGCATGGAGATATA GGGGCGGCCGCGGAGATTTGCCTGCGGATTT
Pri-miR-206	GGGCTCGAGCTGCCATTCTCACAACAGA GGGGCGGCCGCTGAGGGAGTCATTTGCTG
Pri-miR-218	GGGCTCGAGCAGTTCTGAGGAATGGTGGA GGG GCGGCCGCTCTAGCCTACTTCACAGCTA
Pri-miR-222	GGGCTCGAGAGCCCCAGCTGATAATGTTGGACT GGGGCGGCCGCATGTCACTCAGTCAGTATCTGTTGG
Pri-miR-330	GGGCTCGAGCCACTCACCCACACTGAAGA GGGGCGGCCGCGTTTTCTCCCTCTGCTTGACG
Pri-miR-342	GGGCTCGAGGGGCAGGTGCTGTTTCTGT GGGGCGGCCGCTCACAGATGGCGAAACTGAG
Pri-miR-518	GGGCTCGAGAAAACAGGCAAACAGGACCA GGGGCGGCCGCGCACAGAACCCCGATATCAT

Table S3. Primers for ampification of 3'UTR or mutated 3'UTR.

Traget	Primer Sequence (5'-3')
VIM-3'UTR	GGGGCTAGCCAGCATCACGATGACCTT GGGCTCGAGGAAGTGAGGTCTATCAAAAATG
ALAS2-3'UTR	GGGCTCGAGCATGCTGTGGTTTGTGCTTT GGGGCTAGCGTATGTCACCACCTATGCCT
ALAS2-3'UTR-M1	GTGCCTCTAGCTGAATTGAGCCTAAAAATACTCCACAAACCACAGCATG CATGCTGTGGTTTGTGGAGTATTTTTAGGCTCAATTCAGCTAGAGGCAC
ALAS2-3'UTR-M2	CCTCTAGCTGAATTGAGCCTAAAAATAAAGCAGCCACCACAGCATGTGAAGC GCTTCACATGCTGTGGTGCCTGCTTTATTTTTAGGCTCAATTCAGCTAGAGG
ALAS2-3'UTR-M4	TCTTCTGCTTTGTTGTGTGCCTCTAGCTGAATTGAGCCTA AAAATAGGATGTGAACCACAGCATGTGAAGCCTTTTA TAAAAGGCTTCACATGCTGTGGTTCACATCCTATTTTTAG GCTCAATTCAGCTAGAGGCACACAACAAAGCAGAAGA

Table S4. Primer sequences of quantitative real-time PCR.

Traget	Primer Sequence (5'-3')
<i>GAPDH</i>	TGTTGCCATCAATGACCCCTT CTCCACGACGTACTIONCAGCG
<i>β-actin</i>	ATAGCACAGCCTGGATAGCAACGTAC CACCTTCTACAATGAGCTGCGTGTG
<i>ALAS2</i>	CTACCCAAGGACCAAACCTGTTC TGCCTTCTGCACAATCTTGCT
<i>IRP2</i>	TCGATGTATCTAAACTTGGCACC GCCATCACAATTCGTACAGCAG
<i>Ferritin</i>	CAGCCTGGTCAATTTGTACCT GCCAATTCGCGGAAGAAGTG
<i>TFR1</i>	ACCATTGTATATACCCGGTTC CAATAGCCCAAGTAGCCAATCAT
<i>Transferrin</i>	GGTGGCAGAGTTCTATGGGTC ACAGTAAAGTAAGCCTATGGGGA
<i>HBA1</i>	CAGGAACTTGTCCAGGGAGG GGACCCGGTCAACTTCAA
<i>HBZ</i>	AGTGCGGGAAGTAGGTCTTG TGACCAAGACTGAGAGGACCA
<i>HBE</i>	GGTGCATTTTACTGCTGAGGA CCCATGCATTGAGAACCAA
<i>HBG</i>	GCCATGGGTCATTTACAG TGGTCACCAGAGCCTACCTT
<i>HBB</i>	ATCAGAAAGTGGTGGCTGGT GATGCTCAAGGCCCTTCATA
<i>GATA-1</i>	CCAGACGACCACCACGACA TCCTTCGGCTGCTCCTGTG
<i>KLF1</i>	TTGCGGCAAGAGCTACACC GTCAGAGCGCGAAAAAGCAC