miR-128 is implicated in stress responses by targeting MAFG in skeletal muscle cells

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SUPPLEMENTARY MATERIAL

Supplemental Table S1. List of putative redox-related targets of miR-128 predicted in human by *in silico* computational analysis

Gene symbol	Gene name	Accession number*	Prediction programs (conserved site/s among mammals)
NFE2L	nuclear factor, erythroid 2-like 2	ENST00000397063.4	TargetScan, MiRanda RNAhybrid (conserved site)
MAFG	musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	ENST00000357736.4HM	TargetScan, MiRanda RNAhybrid (conserved site)
MAFK	musculoaponeurotic fibrosarcoma oncogene homolog K (avian)	ENST00000343242.4	RNAhybrid
MAFF	musculoaponeurotic fibrosarcoma oncogene homolog F(avian)	ENST00000338483.2	MiRanda, RNAhybrid
BACH1	BTB Domain and CNC Homolog 1	ENST00000286800.3	TargetScan, MiRanda RNAhybrid
KEAP1	Kelch-like ECH-associated protein 1	ENST00000171111.5	
HMOX1	heme oxygenase (decycling) 1	ENST00000216117.8	TargetScan, MiRanda, RNAhybrid
HMOX2	heme oxygenase (decycling) 2	ENST00000570646.1	RNAhybrid
GSTA1	Glutathione-S-Transferase A1	ENST00000334575.5	
NQO1	NAD(P)H dehydrogenase, quinone 1	ENST00000379047.3	
X-CT	solute carrier family 7, (cationic amino acid transporter, y+ system) member 11	ENST00000280612.5	TargetScan, MiRanda, RNAhybrid (conserved site)
GCLM	glutamate-cysteine ligase, modifier subunit	ENST00000370238.3	TargetScan, RNAhybrid
GCLC	glutamate-cysteine ligase, catalitic subunit	ENSG00000001084.6	
SQSTM1	sequestosome 1	ENST00000389805.4	

* accession numbers correspond to those reported in TargetScan 7.1

Supplemental Tables S1

Bioinformatic analysis. For the identification of miR-128 putative targets, three different algorithms were used, namely TargetScan release 7.1 (http://www.targetscan.org), miRanda (http://www.microrna.org) and RNAhybrid ((http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/welcome.html). The intersection of these programs indicated MAFG and Nrf2 as potential targets for miR-128.

Supplemental Table S2: Sequences of oligonucleotides used for this study

HUMAN OLIGOS

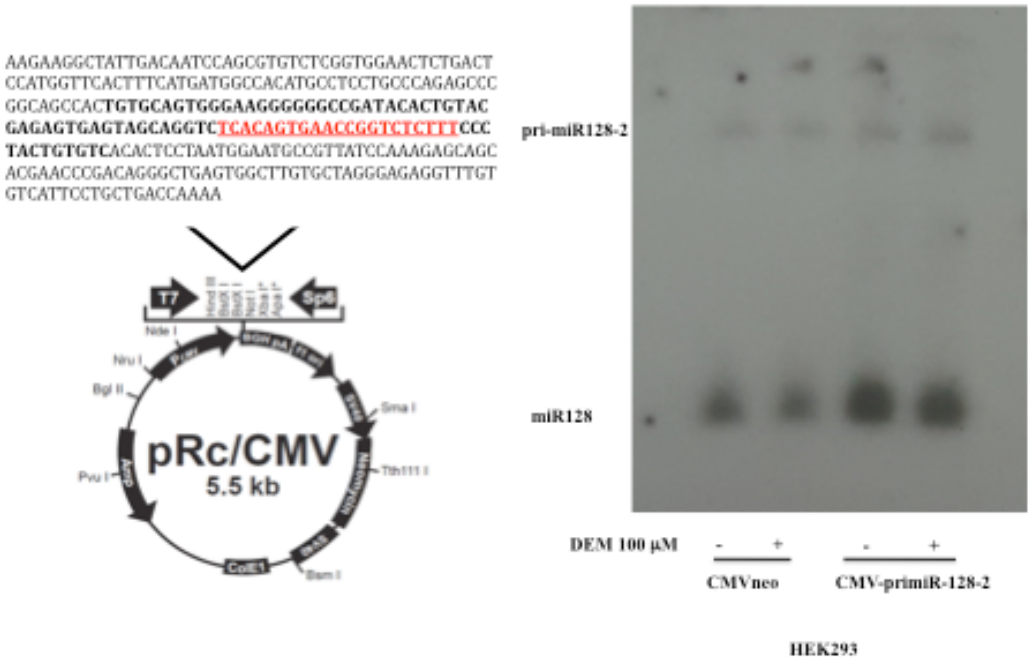
F GSTA-1	5'-GGGCTGACATTCACCTGGTG-3'
R GSTA-1	5'-TTCACTGTGGGCAGGTTACTGA-3'
F HMOX1	5'-GGTGACCCGAGACGGCTT-3'
R HMOX1	5'-GCGAAGACTGGGCTCTCCT-3'
F NQO1	5'-CAGCTCACCGAGAGCCTAGT-3'
R NQO1	5'-TAGAGGTCCGACTCCACCAC-3'
F NRF2	5'-AACTACTCCCAGGTTGCCCAC-3'
R NRF2	5'- GACCGGGAATATCAGGAACAAG-3'
F $p21^{WAF1}$	5'-CTGGAGACTCTCAGGGTCGAA-3'
R $p21^{WAF1}$	5'-CGGCGTTTGGAGTGGTAGAA-3'
F x-CT	5'-TGAAATCCCTGAACTTGCGAT-3'
R c-CT	5'-TCTGGATCCGGGCGCT-3'
F β2-microglobulin	5'-CCGTGGCCTTAGCTGTGCT-3'
R β2-microglobulin	5'-TCGGATGGATGAAACCCAGA-3'
F c-ABL	5′-TGGAGATAACACTCTAAGCATAACTAAAGGT-3′
R c-ABL	5′-GATGTAGTTGCTTGGGACCCA-3′
F AKR1D1	5'-TTGAAGTACCCATGGCCTTT-3'
R AKR1D1	5'-TGAAATACGGATGGCACTCA-3'
F ALDH3A1	5'-GGAAGAGTCCCTGCTACGTG-3'
R ALDH3A1	5'-CCATAGTCCCGGGATTTCTT-3'
F CCDC53	5'-CACAAATGGAGCACATCCTG-3'
R CCDC53	5'-AGCATCTGGCCTCTCAAGAA-3'
F PCBD2	5'-CCTTAAAGCAGCAGGATGGT-3'
R PCBD2	5'-TAGGGCAACTCGGGACATAA-3'
F UCHL1	5'-CCAGCATGAGAACTTCAGGA-3'
R UCHL1	5'-CACAGGAATTCCCAATGGTC-3'

MOUSE OLIGOS

F ATROGIN	5'-CAGCAGCCTGAACTACGACG-3'
R ATROGIN	5'-GGCAGTCGAGAAGTCCAGTC-3'
F MURF1	5'-ACCTGCTGGTGGAAAACATC-3'
R MURF1	5'-CTTCGTGTTCCTTGCACATC-3'
F GAPDH	5'-AACATCAAATGGGGTGAGGCC-3'
R GAPDH	5'-GTTGTCATGGATGACCTTGGC-3'
F GCLC	5'-TGCGAAAAAAGTGCCCGT-3'
R GLCL	5'-TGCATTCCAAAACATCTGGAAA-3'
F GSTA-1	5′-CAGGTGGCTCCTAGCTGCA-3′
R GSTA-1	5′-GGTCTGCGCCAGCTTCA-3′
F HMOX1	5'-AGGATTTGTCTGAGGCCTTG-3'
R HMOX1	5'-AGGAAGCCATCACCAGCTTA-3'
F MAFG	5'-TGTGAGTGCCTGCTCACTGT-3'
R MAFG	5'-GTCAAGCTGGTGCCATTCTC-3'
F NQO1	5′-CCCTCAACATCTGGAGCCAT-3′
R NQO1	5′-GCGTAGTTGAATGATGTCTTCTCTGA-3′
F NRF2	5'-GGCCCAGCATATCCAGACA-3'

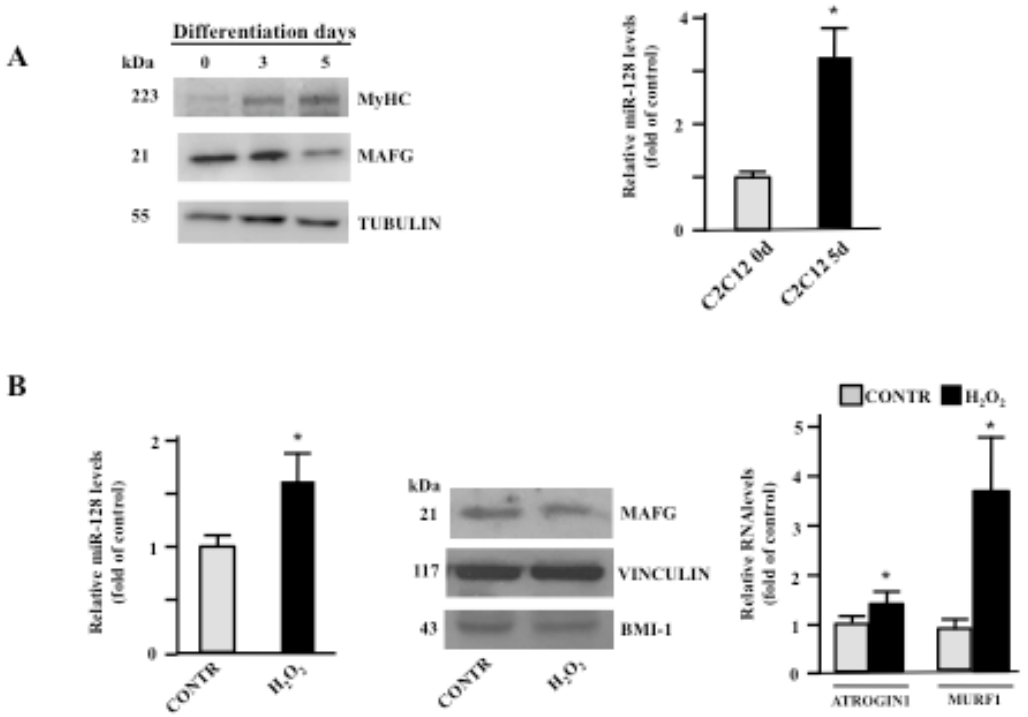
R NRF2	5’-CCAGGGCAAGCGACTCAT-3’
F $p21^{WAF1}$	5’-CCACAGCGATATCCAGACATTC-3’
R $p21^{WAF1}$	5’-CGAAGAGACAACGGCACACTT-3’
F SQSTM1	5’-AGAATGTGGGGGAGAGTGTG-3’
R SQSTM1	5’-TTTCTGGGGTAGTGGGTGTC-3’
F x-CT	5′-TACCTCAACTTTATTACTGAAGAAGTAGACAA-3′
R x-CT	5′-TGTCAGTACGTAGCCCACTGTGA-3′
F c-ABL	5′-GGTATGAAGGGAGGGTGTACCA-3′
R c-ABL	5′-GTGAACTAACTCAGCCAGAGTGTTGA-3′
F AKR1D1	5’-ATGGCGCCTATGTTTACCAC-3’
R AKR1D1	5’-CATTGATGGGACATGCTCTG-3’
F ALDH3A1	5’-CATCTGACCCCTGTCACCTT-3’
R ALDH3A1	5’-CCCATAGTCATGGGACTGCT-3’
F CCDC53	5’-AAAAGAACCGTGGCCTTTCT-3’
R CCDC53	5’-ATTTGCTGAATCCGGAGAGA-3’
F PCBD2	5’-TTCAGATGCACAGTGGTTGA-3’
R PCBD2	5’-CATAAAGCCAAACGCCTGAT-3’
F UCHL1	5’-GACCATCGGAAACTCCTGTG-3’
R UCHL1	5’-GGACAGCTTCTCCGTTTCAG-3’

Supplementary FIGURE S1



Northern blot analysis of miR-128 expression. Total RNA from HEK293 cells transfected for 48 h with pCMV-miR-128 or pCMVneo was isolated using TRIzol Reagent (ThermoFisher SCIENTIFIC) following manufacturer's instructions. Twenty micrograms of RNA were resolved on 15% polyacrylamide gel with urea 6M and transferred overnight to Hybond N^+ membrane (GE Healthcare Life Sciences). RNA was fixed by UV exposure (250 nm for 5'). After prehybridization, the membrane was incubated overnight with a probe generated using an oligonucleotide (AAAgAgaCCggTTCACTgTgA**ggACAgAg**) containing the mature anti–miR-128 and an extra-sequence complementary for annealing to the OprobAAA (5'-TTTTTTTTTTCTCTgTCC 3') that was elongated with Klenow Polymerase (3'-5'exo$^-$) (New England BioLabs) using α(^{32}P)-dATP (PerkinElmer Inc.) to produce a radioactive adenine tail.

Supplementary FIGURE S2



Expression of MAFG under physiological/pathological conditions. (A) Mouse C2C12 cells were cultured in DMEM supplemented with 20% FBS. To induce differentiation, medium was replaced to near-confluent cultures (about 80%) with DMEM containing 2% horse serum and cells cultured up to 5 days. Western Blotting analysis of MAFG protein levels was performed on total protein extracts; MyHC (Abcam, USA) was used as control of differentiation and Tubulin as a loading control. Analyses of miR-128 expression levels in control (C2C12 0d) and in 5 days-differentiated C2C12 cells (C2C12 5d) were performed by RT-qPCR as described in the Materials and Methods section of main text. (B) 4 days-differentiated C2C12 cells were treated or not with H_2O_2 (100 μM) for 24 h. RT-qPCR experiments for miR-128 levels and Western Blotting analyses for MAFG and BMI-1 protein levels were performed as in (A); Vinculin was used as a loading control. RT-qPCR analyses of ATROGIN1 and MURF1 mRNAs were used as controls of atrophic stimulation in differentiated cells. RT-qPCR assays were performed as (A) and GAPDH was used as internal control. The data are expressed as the mean ± standard error and are representative of 3 independent experiments. *$p<0.05$.