MicroRNA-216a inhibits NF-κB-mediated inflammatory cytokine production in teleost fish by modulating p65

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Table S1 PCR primer sequence information in this study.

Primers	Sequences (5'-3')
Real-time PCR	
miR-216a-qRT-F	CAGTAATCTCTGCAGGCAAC
miR-216a-qRT-R	GTCCAGTTTTTTTTTTTTTTTCACAG
5.8S rRNA-qRT-F	AACTCTTAGCGGTGGATCA
5.8SrRNA-qRT-R	GTTTTTTTTTTTTTTTGCCGAGTG
p65-qRT-F	CGCTGTTCTTCAGGGACGACT
p65-qRT-R	CCTGCTGCTTCACCTCCACAT
IL-8-qRT-F	AGCAGCAGAGTCTTCGT
IL-8-qRT-R	TCTTCGCAGTGGGAGTT
TNFa-qRT-F	GTTTGCTTGGTACTGGAATGG
TNFa-qRT-R	TGTGGGATGATGATCTGGTTG
IL1β-qRT-F	CATAAGGATGGGGACAACGAG
IL1β-qRT-R	TAGGGGACGGACACAAGGGTA
IL6-qRT-F	GCGGTAAAGGCATGGATAT
IL6-qRT-R	GTTGTAGTTGGAAGGGCAG
β-actin-qRT-F	GAGCCGCACGCTTCTTT
β-actin-qRT-R	CTGCTGTAGCCGAGGAC
Vector construction	
pre-miR-216a-KpnIF	CGCGAGCTCCTGATGTCCGCTGATGAG
pre-miR-216a-XbaI R	TGCTCTAGATCATCTCCAAAGCACATT-
pre-miR-216a-Mut-F	ACTGGGTAtgcTCTGCAGGCAACTGTGATGGT
pre-miR-216a-Mut- R	TGCAGAgcaTACCCAGTCAGACCAACATACAAAC
p65-3'UTR-Wt-SacIF	CGCGAGCTCCTGATGTCCGCTGATGAG
p65-3'UTR-Wt-XbaIR	TGCTCTAGATCATCTCCAAAGCACATT
p65-3'UTR-Mut-F	CAGATTTGACGTATCGGGTTATCCACCAACAAAC
p65-3'UTR-Mut-R	CCCGATACGTCAAATCTGGCTGACACAGTGGTA
*Lc*p65-3'UTR-Wt-SacIF	CGCGAGCTCATCCCATTGCCCTTGTTA
*Lc*p65-3'UTR-Wt- XbaIR	TGCTCTAGAGTTTGCATAGCCTTACCG
*Lc*p65-3'UTR-Mut-F	GTCAGCCAGATTTGcttTTATCGGGTTATCAACCAACAAAC
*Lc*p65-3'UTR-Mut-R	aagCAAATCTGGCTGACACAGTGGTGGAAAAT
*Dr*p65-3'UTR-Wt-SacIF	CGCGAGCTCGAGAATACTGTCCTGCACCC
*Dr*p65-3'UTR-Wt-XbaIR	TGCTCTAGAGCTCCGATGTAATCTGATGT
*Dr*p65-3'UTR-Mut-F	GATtcaTACATCGGAGCGATCAGCTTGTTATG
*Dr*p65-3'UTR-Mut-R	GCTCCGATGTAtgaATCTGATGTTGTTTTGAACATTATGGG
p65-BamHIF	CGCGGATCCATGGCGGATGTGTACGGA
p65-XbaIR	AACTCTGGATCATCTCCAAAGCACATT
GFP- p65-3'UTR-Wt-HindIIIF	CCCAAGCTTGCTAGCTGATGTCCGCTGATGAG
GFP-p65-3'UTR- Wt-BamHIR	CGCGGATCCTCATCTCCAAAGCACATT
GFP- p65-3'UTR-Mut-HindIIIF	GATTTGAtcaTATCGGGTTATCCACCAACAAAC
GFP-p65-3'UTR-Mut-BamHIR	CCCGATAtgaTCAAATCTGGCTGACACAGTGG

TSS-1359-F	tcttacgcgtgctagcccgggATGAGTAGAATTGTCTTTTATTCTTCCTTAA
TSS-1230-F	tcttacgcgtgctagcccgggAGCTCAGTGGGACCCGAAGC
TSS-636-F	tcttacgcgtgctagcccgggTGAGTCTTAACCACCAGGACTTCTT
TSS-270-F	tcttacgcgtgctagcccgggGTCCCAAGTGAGAGGCCTGG
TSS-117-F	tcttacgcgtgctagcccgggTAGGGTCAAAGCTTGGTCTCT
TSS-Universal R	cagtaccggaatgccaagcttCAGAGATTACCCAGTCAGACCAACA
Ap1 mut1 F	CCGCTCGAGCCCGGGCTAGCACGCGTAAGA
Ap1 mut1 R	CCGCTCGAGCCACCAGGACTTCTTATATTG
Sp1 mut1 F	CCGCTCGAGCAAGGTGACAAAATTTTACAC
Sp1 mut1 R	CCGCTCGAGGTTTCAGTGTTTGTTGAGGTC
Sp1 mut2 F	CCGCTCGAGCACCACTCACCAGCCTTCAGC
Sp1 mut2 R	CCGCTCGAGGTGACATCTGACCTCTAACTG
Ap1 mut2 F	CCGCTCGAGAGTTAGAGGTCAGATGTCACC
Ap1 mut2 R	CCGCTCGAGCCCTGGAGGATACGGCTCAAC
Sp1 mut3 F	CCGCTCGAGTGAGCCGTATCCTCCAGGGTC
Sp1 mut3 R	CCGCTCGAGTCACCATGGTAACACAGGACC
Sp1 mut4 F	CCGCTCGAGGTCCTGTGTTACCATGGTGAT
Sp1 mut4 R	CCGCTCGAGTAGGGTCAAAGCTTGGTCTCT

Table S2 Comparison of the differentially expressed miRNAs. miRNAs with |log2 (IN/CO)| ≥ 1 and P-value < 0.01 were defined as the significantly differential ones. Experiment group (IN) and control group (CO).

miR_name	Sequence (5'-3')	CG-std	IG-std	Fold-change (log2 IG/CG)	P-value (IG/CG)
miR-132-1-3p	UAACAGUCUACAGCCAUGGUCG	54.66	7.03	-2.96	1.03E-70
miR-217-5p	UACUGCAUCAGGAACUGAUUGGC	9.46	1.55	-2.61	6.65E-12
miR-8159-5p	UCAGUAACUGGAAUCUGUCCCUG	15.68	2.81	-2.48	6.58E-18
novel-mir-176-3p	UACCAUGACGUCAUCUUCCACG	5.65	1.83	-1.63	9.38E-05
miR-129-1-3p	AAGCCCUUACCCCAAAAAGCAU	8.19	2.81	-1.54	5.56E-06
novel-mir-63-5p	ACGCAUGGGGACGGGAAGUAUGG	3.57	1.41	-1.35	7.42E-03
miR-2188-3p	GCUGUGUGAGGUCAGACCUAUC	4.27	1.83	-1.22	6.40E-03
miR-150-5p	ACUCCCAAUCCUUGUACCAGUG	149.91	73.29	-1.03	5.72E-47
miR-2188-5p	AAGGUCCAACCUCACAUGUCCU	1238.03	614.56	-1.01	0.00E+00
novel-mir-153-5p	UCUGUACUGUGAGGACAUGU	16.95	40.51	1.26	7.09E-19
miR-15b-2-3p	CGAACCAUUAUUUGCUGCUUUA	57.20	148.26	1.37	4.59E-74
novel-mir-19-5p	UGCAAGAAGUAGAUAGACACCU	1.15	3.66	1.67	1.08E-03
miR-216a-2-5p	UAAUCUCUGCAGGCAACUGUGA	9.57	33.90	1.82	8.96E-27

Fig. S1 pre-miR-216a targets the 3'UTR of miiuy croaker p65 gene. (A) The construction of the pre-miR-216a plasmid. (B) The construction of the mutant type of pre-miR-216a plasmid. (C) HEK293 cells were cotransfected with the wild type of p65-3'UTR (Wt-UTR) or the mutant type of p65-3'UTR (Mut-UTR), together with pcDNA6.2 or the mutant type of pre-miR-216a plasmid. pcDNA6.2 were used to control the same amount of molecules for transfections. The luciferase activity was determined and normalized by Renilla luciferase activity. Data are presented as the means ± SE from at least three independent triplicated experiments. **, $p < 0.01$ versus the controls.