

# Supplementary Materials: miR-30-5p Regulates Muscle Differentiation and Alternative Splicing of Muscle-Related Genes by Targeting MBNL

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**Table S1.** Primers for the real-time PCR for miR-30-5p.

Name of Primers	Sequence of Primers (5'-3')	Amplicon Size
Stem-loop RT-miR-30a-5p	gtcgatccaggatgcagggtccgaggtattcgactggatacggacagcttc	
miR-30a-5p-F	GGCGTGTAAACATCCTCGACTG	
miR-30a-5p-R	GTGCAGGGTCCGAGGT	62 bp
Stem-loop RT-miR-30b-5p	gtcgatccaggatgcagggtccgaggtattcgactggatacggacagctga	
miR-30a-5p-F	GGCGTGTAAACATCCTACACTC	
miR-30a-5p-R	GTGCAGGGTCCGAGGT	60 bp
Stem-loop RT-miR-30e-5p	gtcgatccaggatgcagggtccgaggtattcgactggatacggacagcttc	
miR-30e-5p-F	GGCGTGTAAACATCCTTGACTG	
miR-30e-5p-R	GTGCAGGGTCCGAGGT	62 bp
U6-F	GCTTCGGCAGCACATATACTAAAAT	
U6-R	CGCTTCACGAATTGCGTGTAT	107 bp

Stem-loop RT-miR-30a-5p, Stem-loop RT-miR-30a-5p and Stem-loop RT-miR-30a-5p were used to reverse transcription of miR-30a-5p, miR-30b-5p and miR-30e-5p.

**Table S2.** Primers for the constructs.

Name of Primers	Sequence of Primers (5'-3')	Amplicon Size
MBNL1-3' UTR-F	GCTCTAGATCAGCCACAAGACATCCACA	448 bp
MBNL1-3' UTR-R	GCTCTAGATCAGATCCCTCCCTCACCAC	
MBNL2-3' UTR-F	GCTCTAGAGGGTTGTAAGTGAACAGCAT	220 bp
MBNL2-3' UTR-R	GCTCTAGAAATTGTATCGCTATTACCTTGA	
MBNL3-3' UTR-F	GCTCTAGATTCAACCCGCCTAGATAGAT	375 bp
MBNL3-3' UTR-R	AAATACTGTGGAATAACCCCT	
Pre-miR-30a-5p-F	CCCAAGCTTTGGGAGAAGACTTAATGGTGT	319 bp
Pre-miR-30a-5p-R	GGGGTACCTAACGAAATGTAGGGATGGGT	
Pre-miR-30b-5p-F	CCCAAGCTTCATGTCAATCTTGTACCTCCTG	291 bp
Pre-miR-30b-5p-R	GGGGTACCTTGCCATATCCTCTATCCGTGT	
Pre-miR-30e-5p-F	GGGGTACCAAGGAGGAACGTAGGCCGTGGACA	241 bp
Pre-miR-30e-5p-R	CCCAAGCTTGACCCTGCCTGGGGACCTTG	

The red font represents the sequence sites recognized by restriction enzyme.

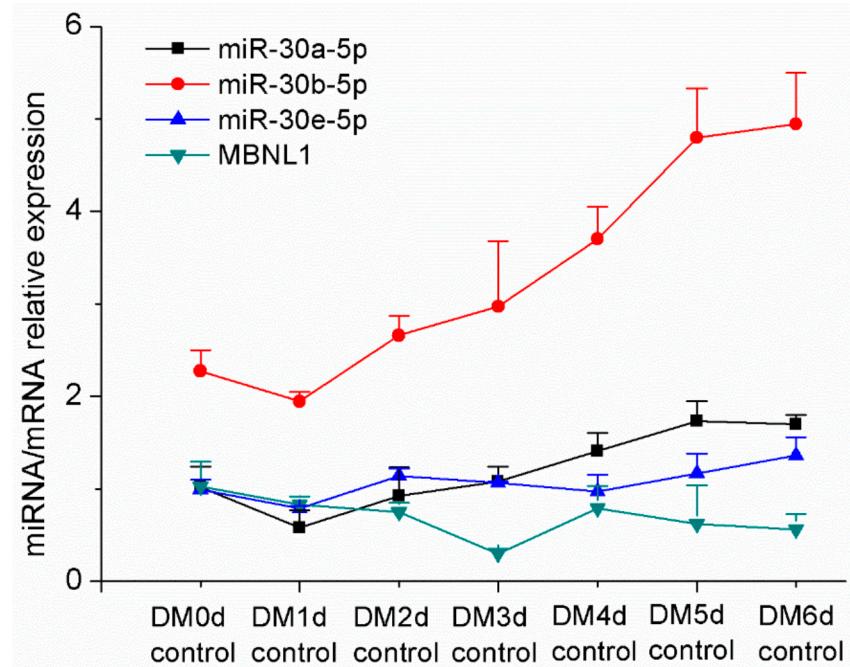
**Table S3.** Primers for constructs of the mutant 3' UTR.

Name of Primers	Sequence of Primers (5'-3')	Amplicon Size
Mut-MBNL1-3' UTR-F1	GCTCTAGATCAGCCACAAGACATCCACA	336 bp
Mut-MBNL1-3' UTR-R1	GACCTTTGTTATTGATTG <u>CTTGAAGAAATA</u>	
Mut-MBNL1-3' UTR-F2	TATTCTTCAA <u>ACAAATCAAATAACAAAGGT</u>	147 bp
Mut-MBNL1-3' UTR-R2	GCTCTAGATCAGATCCCTCCCTCACAC	
Mut-MBNL2-3' UTR-F	GCTCTAGAGGGTTGTA <u>ACTGACTACAGCAT</u>	187 bp
Mut-MBNL2-3' UTR-R	GCTCTAGAAGTATT <u>AAAAAGAAAACACATT</u>	
Mut-MBNL3-3' UTR-F1	GCTCTAGATTCAACCCGCCTAGATAGAT	185 bp
Mut-MBNL3-3' UTR-R1	GAGCAAAAG <u>TTTGATACATGTGGATTCT</u>	
Mut-MBNL3-3' UTR-F2	AGAACCCACATGT <u>ACAAAAC</u> TTTGCTC	210 bp
Mut-MBNL3-3' UTR-R2	AAATACTGTGGAATAACCCT	

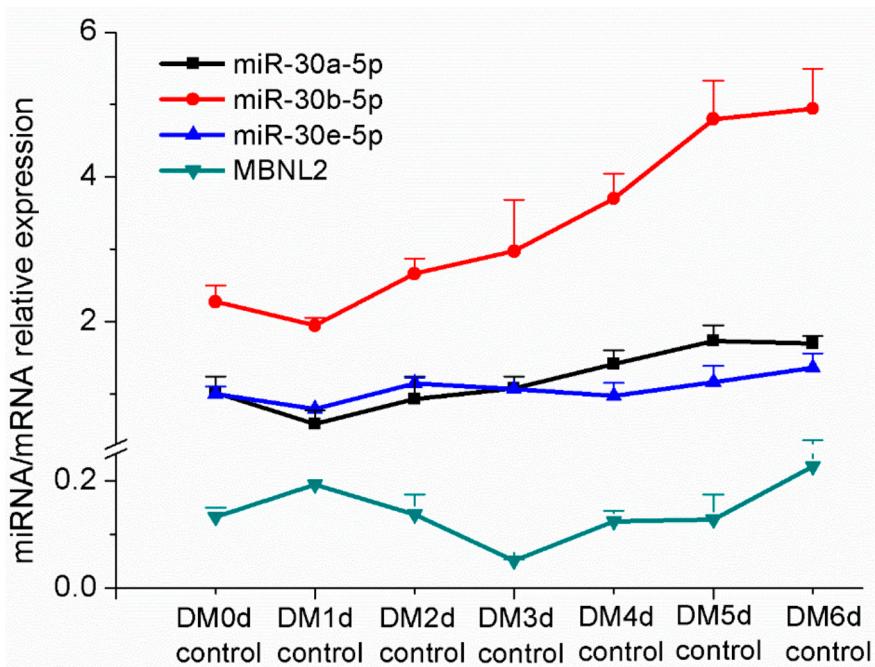
The red font represents the sequence sites recognized by restriction enzyme *Xba*I; the underline font represents the mutant sites in the target sequences recognized by miR-30-5p.

**Table S4.** Primers for real-time PCR of genes.

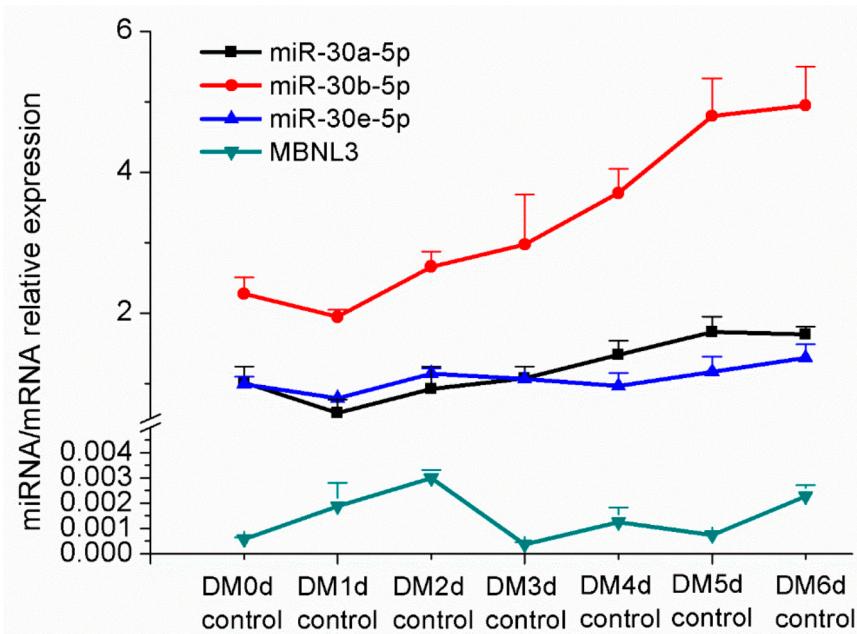
Name of Primers	Sequence of Primers (5'-3')	Amplicon Size
MBNL1-F	CAACAAACATCTGCCACAA	109 bp
MBNL1-R	TACATCTGGGTAAACATACTTG	
MBNL2-F	CGTAACCGTTGTATGGATTACAT	76 bp
MBNL2-R	GTGTGCAGGAGGGTAAA	
MBNL3-F	TGATAAACTGTGACCATCTGC	79 bp
MBNL3-R	AGGAGGGATGAAA <u>ACTTGC</u>	
MyoG-F	GTCCCAACCCAGGAGATCATT	70 bp
MyoG-R	GACGTAAGGGAGTGCAGATTGTG	
MHC-F	CAATAAACTGCCGGCAAAGAC	75 bp
MHC-R	CTTGCTCACTCCTCGCTTCA	
MyoD-F	GGAAGGGAAGAGCAGAAG	82 bp
MyoD-R	AAGGACTACAACAACAACAC	
Trim55-F	AGTGAGTGGTAAGGAGTC	97 bp
Trim55-R	CCAGATGTAGTAGAGAATAAGAA	
INSR-F	TGGAGGAGTCTTCATTCA	100 bp
INSR-R	CTACTGTCCTCGGCACCAT	
GAPDH-F	AACTTGGGATTGTGGAAGG	222 bp
GAPDH-R	ACACATTGGGGTAGGAACA	



**Figure S1.** The expression of these miR-30-5p and MBNL1 in non-transfected C2C12 cells.



**Figure S2.** The expression of these miR-30-5p and MBNL2 in non-transfected C2C12 cells.



**Figure S3.** The expression of these miR-30-5p and MBNL3 in non-transfected C2C12 cells.