



Supplementary Information

Supplementary File 1.

target: myomaker
length: 387
miRNA : gga-miR-133b
length: 21

mfe: -28.5 kcal/mol

```

position 230
target 5' U   CCC   A     A 3'
          GCUGG   AGG GGACCAG
          CGACC   UCC CCUGGUU
miRNA 3' AU   AACU           5'

```

target: myomaker
length: 387
miRNA : gga-miR-1659
length: 23

mfe: -25.1 kcal/mol

```

position 139
target 5' U   A   AG     AUGGGG G     U 3'
          GG GA   CCUUUGU     U UGCCCUG
          CC CU   GGAAGUA     A ACGGGAU
miRNA 3' U   GA           G     5'

```

target: myomaker
length: 387
miRNA : gga-miR-140-3p
length: 21

mfe: -25.3 kcal/mol

```

position 148
target 5' C UU   AU   GGG           C 3'
          U   GU   GG   U UGCCCUGUG
          A   CA   CC   AAUGGGACAC
miRNA 3' C GG           A   G           C 5'

```

target: myomaker
length: 387

miRNA : gga-miR-140-3p
length: 21

mfe: -24.1 kcal/mol

position 179
target 5' C CA C 3'
UCCG UCACCCUGUG
AGGC AGUGGGACAC
miRNA 3' C ACCA A C 5'

target: myomaker
length: 387
miRNA : gga-miR-6543-3p
length: 18

mfe: -21.1 kcal/mol

position 232
target 5' C C A 3'
UGGCC AGGAGG
ACUGG UCCUCC
miRNA 3' UC ACUU G 5'

target: myomaker
length: 387
miRNA : gga-miR-1580
length: 21

mfe: -23.2 kcal/mol

position 113
target 5' U UUUCACUU AAAUCCCCUG U 3'
GCGC UGCU GAGAAGCC
UGCG AUGG CUCUUCGG
miRNA 3' U UCC C 5'

target: myomaker
length: 387
miRNA : gga-miR-6650-5p
length: 24

mfe: -29.1 kcal/mol

position 196
target 5' C UUG CACUUCAC CCCCCUGUCCCC C 3'
GCCC GC CU UCUGCUGGC
CGGG CG GA AGACGACCG

miRNA 3' A UCAA A A 5'

target: myomaker
length: 387
miRNA : gga-miR-1768
length: 22

mfe: -25.8 kcal/mol

position 6
target 5' C A G C 3'
CA CACGG U CUCUGCUC
GU GUGCU A GAGACGAG
miRNA 3' A C GUU G 5'

target: myomaker
length: 387
miRNA : gga-miR-1608
length: 22

mfe: -28.9 kcal/mol

position 174
target 5' G CUCCGCAUCACCCU U CCUU UUCACCUCCCC C 3'
AGAG G GCGC GGCCAC UGUCCC
UCUC C CGCG UCGGUG ACAGGG
miRNA 3' U 5'

target: myomaker
length: 387
miRNA : gga-miR-1789
length: 22

mfe: -25.6 kcal/mol

position 277
target 5' A UU A G 3'
UC UCUGU GU ACACCCA
AG AGGCG CA UGUGGGU
miRNA 3' GUA U UU 5'

target: myomaker
length: 387
miRNA : gga-miR-20a-5p
length: 23

mfe: -20.9 kcal/mol

position 107
target 5' A GA UU C 3'
GCU UGCGCU CACUUUG
UGG ACGUGA GUGAAAU
miRNA 3' GA UAUUC 5'

target: myomaker
length: 387
miRNA : gga-miR-1684a-5p
length: 22

mfe: -23.5 kcal/mol

position 232
target 5' C GCCCAG C U 3'
UG GAGGA CAGAGCU
AC CUCCU GUCUCGA
miRNA 3' CAU AUA UC 5'

target: myomaker
length: 387
miRNA : gga-miR-1722-3p
length: 22

mfe: -28.2 kcal/mol

position 1
target 5' CCAACAC C 3'
GCCCC GGUGCUCUG
CGGGG CUACGAGAU
miRNA 3' GAA AACCA 5'

target: myomaker
length: 388
miRNA : gga-miR-1555-5p
length: 20

mfe: -23.4 kcal/mol

position 58
target 5' C CUUUUCUUCUGUG U C 3'
CAGGG GGA GGUUUGCU
GUCCC CUU CCAAACGA
miRNA 3' CC A C 5'

target: myomaker
length: 388
miRNA : gga-miR-1582

length: 20

mfe: -25.7 kcal/mol

```
position 71
target 5' U      GGA      UUG      C 3'
          CUGUG  UGGU    CUCUUUC
          GACAC  ACCG    GAGAAAG
miRNA  3'      AAG      A        5'
```

target: myomaker

length: 388

miRNA : gga-miR-133b

length: 21

mfe: -28.5 kcal/mol

```
position 230
target 5' U      CCC      A      A 3'
          GCUGG  AGG GGACCAG
          CGACC  UCC CCUGGUU
miRNA  3' AU      AACU      5'
```

target: myomaker

length: 388

miRNA : gga-miR-133c-3p

length: 22

mfe: -28.5 kcal/mol

```
position 230
target 5' U      CCC      A      A 3'
          GCUGG  AGG GGACCAG
          CGACC  UCC CCUGGUU
miRNA  3' CGU      AACU      5'
```

target: myomaker

length: 388

miRNA : gga-miR-1732

length: 21

mfe: -23.8 kcal/mol

```
position 1
target 5'      CC      A      C 3'
```

```
          GCCC   CA CACGGUG
          CGGG   GU GUGCCAC
miRNA 3' GU   CUGUC C       5'
```

target: myomaker

length: 388

miRNA : gga-miR-6665-5p

length: 22

mfe: -22.3 kcal/mol

position 233

```
target 5' U   C   GGACCAGAG   C 3'
          GGC CAGGA   CUUCAGGC
          CCG GUUUU   GAAGUCCG
miRNA 3' C   UA   AG   U 5'
```

target: myomaker

length: 388

miRNA : gga-miR-1594

length: 21

mfe: -29.6 kcal/mol

position 20

```
target 5' U   CGGG   U G   G 3'
          GCUCCA   AUCU U CCCACCA
          UGGGGU   UGGG A GGGUGGU
miRNA 3' U   U G   5'
```

target: myomaker

length: 388

miRNA : gga-miR-6675-3p

length: 22

mfe: -26.0 kcal/mol

position 225

```
target 5' C   U   UG CCCAG   G A   C 3'
          CCUC GC G   GA G CCAGAG
          GGAG UG C   CU C GGUCUC
miRNA 3'   UGA   G A   U 5'
```

target: myomaker

length: 388

miRNA : gga-miR-6675-3p
length: 22

mfe: -22.2 kcal/mol

position 146
target 5' G UGUAUG G UGCCC G G 3'
CCUU G GUG UGU CCAGAGA
GGAG U CAC GCA GGUCUCU
miRNA 3' UG G U 5'

target: myomaker
length: 388
miRNA : gga-miR-1744-5p
length: 22

mfe: -20.5 kcal/mol

position 230
target 5' U GGCCCAG GAC U 3'
GCU GAG CAGAGC
CGA CUC GUCUCG
miRNA 3' U AAA AAGUU U 5'

target: myomaker
length: 388
miRNA : gga-miR-7482-5p
length: 25

mfe: -31.1 kcal/mol

position 227
target 5' C G CCCAG GACCA UUC U 3'
UCU CUGG GAG GAGC AGGCCAG
AGA GACC CUC CUUG UUCGGGUC
miRNA 3' A A C 5'

target: myomaker
length: 388
miRNA : gga-miR-7482-5p
length: 25

mfe: -26.6 kcal/mol

position 31

```
target 5' A      CCCACC  UCCAU  C      G 3'
          UCUUUG  AGU    ACG GCCCAGG
          AGAGAC  UCA    UGU CGGGUCC
miRNA  3'      CAC    CU     U     5'
```

target: myomaker
length: 388
miRNA : gga-miR-6562-5p
length: 22

mfe: -25.3 kcal/mol

```
position 108
target 5' G UG U    U  A  UUGC UAAA      G 3'
          C  A GCGC UUC CU      UUCCCCU
          G  U CGUG AAG GA      AAGGGGA
miRNA  3' G UG      U                      5'
```

target: myomaker
length: 388
miRNA : gga-miR-99a-3p
length: 21

mfe: -28.7 kcal/mol

```
position 233
target 5' U          ACCA      C 3'
          GGCCCA GGAGG  GAGCUU
          CUGGGU UCUUC  CUCGAA
miRNA  3' U      A      G      C 5'
```

target: myomaker
length: 388
miRNA : gga-miR-20b-5p
length: 23

mfe: -21.9 kcal/mol

```
position 107
target 5' A  GA      UU      C 3'
          GCU  UGCGCU  CACUUUG
          UGG  ACGUGA  GUGAAAC
miRNA  3' GA      UACUC      5'
```

target: myomaker

length: 388

miRNA : gga-miR-7454-5p

length: 23

mfe: -23.1 kcal/mol

position 250

```
target 5' A      GCCCA   GAGCA   U 3'
          GCUUCAG   GUUU   CACAGA
          UGAAGUC   CAGA   GUGUCU
miRNA 3' CG      AAA      U 5'
```

target: myomaker

length: 388

miRNA : gga-miR-6637-3p

length: 23

mfe: -27.8 kcal/mol

position 234

```
target 5' G   CAG  GGAC  A  UUCA      U 3'
          GCC  GA   CAG  GC  GGCCCAGU
          UGG  CU   GUC  CG  UCGGGUCG
miRNA 3'      AAC      UG      5'
```

target: myomaker

length: 388

miRNA : gga-miR-1736-5p

length: 22

mfe: -27.6 kcal/mol

position 12

```
target 5' C      U  CUCCA   UCU   A 3'
          GGUGC CUG   CGGGA  UUGCCC
          UCGUG GAU   GUCCU  AACGGG
miRNA 3'      A      U      A 5'
```

target: myomaker

length: 388

miRNA : gga-miR-7439-3p

length: 23

mfe: -25.8 kcal/mol

position 113
target 5' U UCACUUU AAUUCCC G 3'
GCGCUU GCUA CUGGAGAA
CGUGAG UGAU GACCUCUU
miRNA 3' U UCCU 5'

target: myomaker
length: 388
miRNA : gga-miR-757
length: 21

mfe: -27.7 kcal/mol

position 4
target 5' C ACAC GU U 3'
CCCA G GCUCUGC
GGGU C CGAGACG
miRNA 3' CUUA AGA GU 5'

target: myomaker
length: 388
miRNA : gga-miR-214
length: 21

mfe: -24.7 kcal/mol

position 218
target 5' C CCC G 3'
CCUGUC UCUGCUG
GGACAG GGACGAC
miRNA 3' GAC ACAC A 5'

target: myomaker
length: 388
miRNA : gga-miR-7472-5p
length: 22

mfe: -26.8 kcal/mol

position 245
target 5' A AG GGCCCAG G C 3'
CC AGCUUCA UUU AGCACA
GG UCGAAGU GAG UCGUGU
miRNA 3' A A G A 5'

target: myomaker

length: 388

miRNA : gga-miR-6672-3p

length: 20

mfe: -23.7 kcal/mol

position 145

```
target 5' A C   GUA   GG     U 3'
          G CUUU  UGG  UGUGCCC
          C GAAG  GUC  ACACGGG
miRNA  3' A A           AA     U 5'
```

target: myomaker

length: 388

miRNA : gga-miR-7452-5p

length: 21

mfe: -30.3 kcal/mol

position 151

```
target 5' U           GU     G 3'
          GUAUGG  GGU  GCCCUGU
          CGUGCC  UCG  CGGGACA
miRNA  3'           UAG  GU     5'
```

target: myomaker

length: 388

miRNA : gga-miR-6625-5p

length: 22

mfe: -24.0 kcal/mol

position 196

```
target 5' C   C   GCCA   ACC     U 3'
          GC CUUG  CUUC  UCCCCC
          UG GAAC  GAAG  AGGGGG
miRNA  3'   A           GUCA   U 5'
```

target: myomaker

length: 388

miRNA : gga-miR-1773-5p

length: 23

mfe: -29.9 kcal/mol

position 196
target 5' C CCUU A UCA U 3'
GC GG CC CU CCUCCCC
CG CC GG GA GGAGGGGG
miRNA 3' UU UCU A U 5'

target: myomaker
length: 388
miRNA : gga-miR-1757
length: 21

mfe: -24.9 kcal/mol

position 211
target 5' C CCCC C 3'
ACCU CUG UCC CCUCUG
UGGA GAC AGG GGAGAC
miRNA 3' U U A C U 5'

target: myomaker
length: 388
miRNA : gga-miR-133a-3p
length: 22

mfe: -28.5 kcal/mol

position 230
target 5' U CCC A A 3'
GCUGG AGG GGACCAG
CGACC UCC CCUGGUU
miRNA 3' UGU AACU 5'

target: myomaker
length: 388
miRNA : gga-miR-1704
length: 21

mfe: -26.1 kcal/mol

position 217
target 5' C CCC G 3'
CCCUGUC UCUGCUG
GGGGUAG AGACGAC

mfe: -26.0 kcal/mol

position 60
target 5' A UCUUCUGUGGGAUG GCUCU C 3'
GGGCUUU GUUU UUCCACCAU
UCCGGAA CAAG AAGGUGGUA
miRNA 3' CG 5'

target: myomaker
length: 388
miRNA : gga-miR-6645-5p
length: 22

mfe: -31.9 kcal/mol

position 185
target 5' A CUGUGCGCCCUUG U C 3'
UCACC GCCACU C ACCUCCC
AGUGG UGGUGA G UGGAGGG
miRNA 3' C A U 5'

target: myomaker
length: 388
miRNA : gga-miR-6647-5p
length: 23

mfe: -28.2 kcal/mol

position 230
target 5' U C GGA A U 3'
GCUG G C CA GG CCAGAGC
CGAC C G GU CC GGUCUCG
miRNA 3' U G A U A C 5'

length: 388
miRNA : gga-miR-3524b-3p
length: 21

mfe: -22.5 kcal/mol

position 42
target 5' C UU ACGCG G CUUUUC G 3'
CAG CCAU CCCA GG UUCUGUG
GUC GGUA GGGU UU AAGACAC

miRNA 3' G 5'

target: myomaker
length: 388
miRNA : gga-miR-1790
length: 20

mfe: -30.6 kcal/mol

position 161
target 5' G G UG AGAGAGCUCCGCA U 3'
U CCC UGCC UCACCC
G GGG GCGG AGUGGG
miRNA 3' A G UG G C 5'

target: myomaker
length: 388
miRNA : gga-miR-1680-5p
length: 22

mfe: -28.6 kcal/mol

position 229
target 5' C CUG C 3'
UG GCCCAGGAGGA
AC UGGGUCCUCCU
miRNA 3' CGUCUCA A U 5'

target: myomaker
length: 388
miRNA : gga-miR-7458-3p
length: 22

mfe: -26.9 kcal/mol

position 226
target 5' C GCCCAGGAGGACC GCUUCAGG U 3'
CUCUCUG AGA CCCAGU
GAGACGAC UCU GGGUCA
miRNA 3' ACUA C 5'

target: myomaker
length: 388
miRNA : gga-miR-6669-5p
length: 24

mfe: -28.8 kcal/mol

position 160

```

target 5' U   CCUGU   AGAGA       C       C 3'
              GUGC    GCC     GCUC CG AUCACC
              UACG    CGG     CGAG GC UAGUGG
miRNA  3'   U       AA       G A       5'
    
```

target: myomaker

length: 388

miRNA : gga-miR-1728-3p

length: 22

mfe: -23.1 kcal/mol

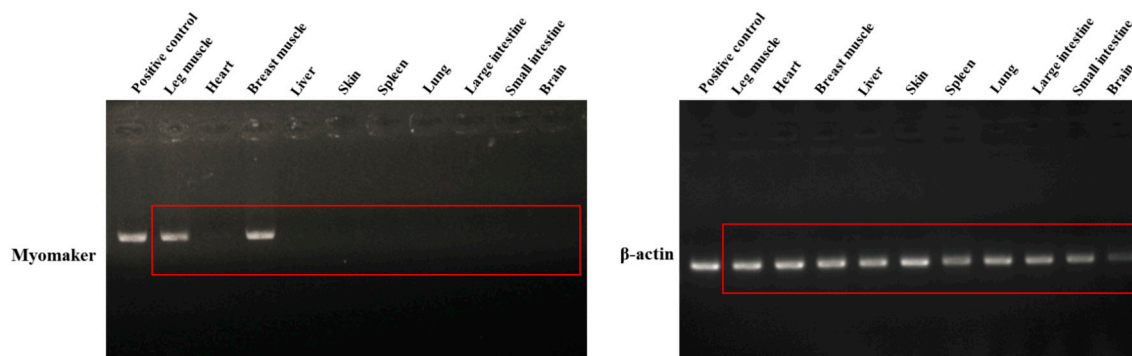
position 105

```

target 5' C A   UGCGCUUUCAC   UAAAUUCCCCUGG   U 3'
              G GCUGA           UUUGC           AGAAGCCU
              C CGACU           AGAUG           UCUUCGGA
miRNA  3' CGA                                     5'
    
```

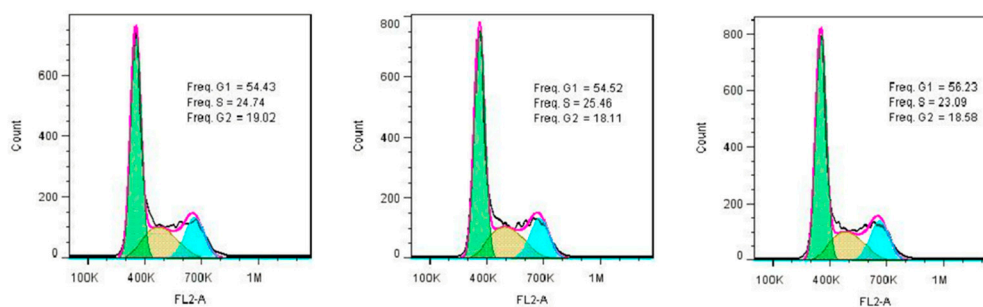
Supplementary File 2.

A. The used in the main figures of full-gels are shown. All cropped gels have been run under the same experimental condition.

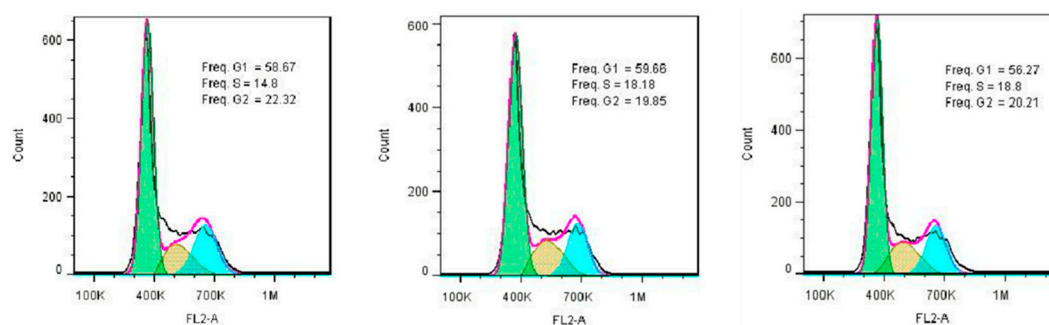


B. The histograms of cell cycle analyzed by flow cytometry.

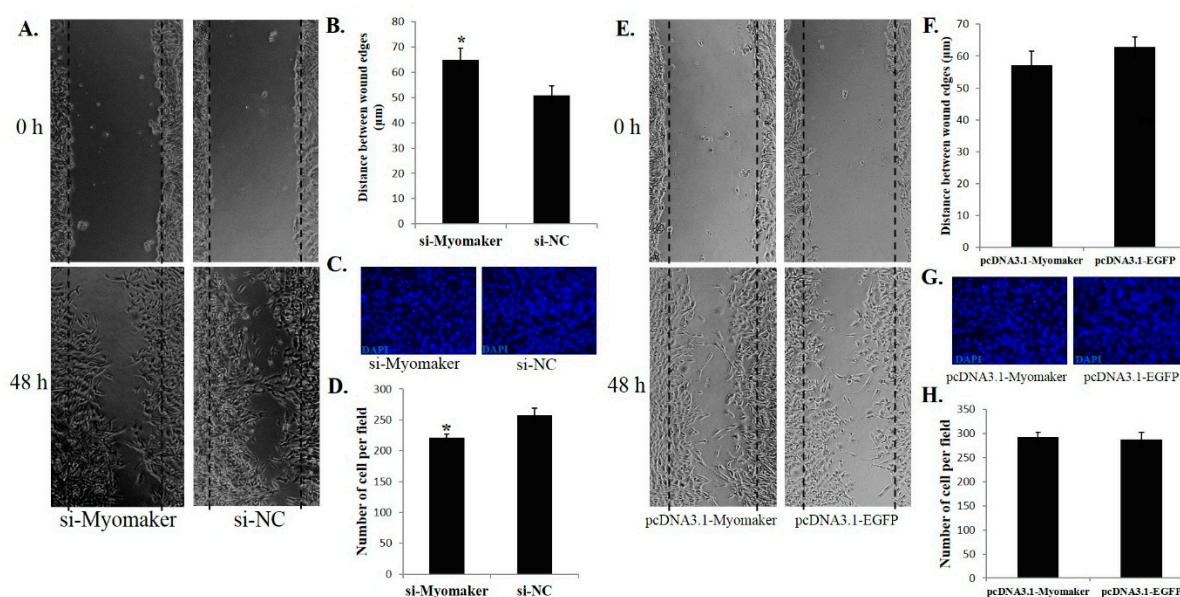
miR-140-3p



NC



Supplementary File 3.



A. Representative classic scratch assay results for myoblasts transfected with si-Myomaker or si-NC. **B.** The statistical results of the distance between wound edges in the classic scratch assay for myoblasts transfected with si-Myomaker or si-NC. **C.** Transwell migration assay using myoblasts transfected with si-Myomaker or si-NC. **D.** The statistical results for the cell number in the Transwell migration assay for myoblasts transfected with si-Myomaker or si-NC. **E.** Representative classic scratch assay results for myoblasts transfected with pcDNA3.1-Myomaker or pcDNA3.1-EGFP. **F.** The statistical results of the distance between wound edges for the classic scratch assay for myoblasts transfected with pcDNA3.1-Myomaker or pcDNA3.1-EGFP. **G.** Transwell migration assay using myoblasts transfected with pcDNA3.1-Myomaker or pcDNA3.1-EGFP. **H.** The statistical results of the cell number for the Transwell migration assay for myoblasts transfected with pcDNA3.1-Myomaker or pcDNA3.1-EGFP.

Methods

Classic scratch assay: A ~400-µm scratch was made using a sterile pipette tip on a fully confluent cell monolayer 12 h after transfection with siRNA oligos or pcDNA3.1-Myomaker. Then the cells were washed and cultured in growth media. Images were taken using a phase contrast microscope (Nikon). The distance between wound edges were measured using NIS-Elements BR analysis software (Nikon).

Transwell migration assay: A total of 5×10^4 cells in 250 µL sera-free media were seeded in an upper chamber of a noncoated Transwell insert (24-well insert; pore size, 8 µm; BD Biosciences). Media supplemented with serum was used as the chemoattractant in the lower chamber. After 24-h

incubation, cells in the upper chamber were removed with a cotton swab and cells which migrated through the pores were fixed and stained with DAPI (Beyontime). Images of migrated cells were taken with a fluorescence microscope (Nikon) and the numbers of migrated cells were quantified with ImageJ (National Institutes of Health).

Supplementary File 4.

Oligonucleotides:

For sex determination the following primers were used for PCR:

CHD1 s: 5'-GTTACTGATTCGTCTACGAGA

as: 5'-ATTGAAATGATCCAGTGCTTG

For ChIP-qPCR analysis the following primers were used:

Myomaker promoter s: 5'-GTGCATCACAGCCAGCATG

as: 5'-GGTGGCAGGAGGAGTTT

GAPDH s: 5'-ATGGCATCCAAGGAGTGA

as: 5'-GGGAGACAGAAGGGAACAG

For cloning the following primers were used for PCR:

Myomaker CDS cloning for pcDNA3.1: s: 5'-CCGCTCGAGGCCACCATGGGCTCGCTGGTGG

as: 5'-CGGGGCCCGGTATGGAAGTGGTGGG

Myomaker CDS cloning for pcDNA3.1-EGFP:

s: 5'-CCCAAGCTTGCCACCATGGGCTCGCTGGTGG

as: 5'-GGGGTACCGACACAGCAGCACAGCGTGG

MYOG CDS cloning: s: 5'-CCGCTCGAGGCCACCATGGAGCTCTTTGAG

as: 5'-CGGGGCCCACTTGGAAACAGCCACATTG

MYOD CDS cloning: s: 5'-GGAATTCGCCACCATGGACTTACTGGGCCCCATGGAA

as: 5'-CCGCTCGAGGGCTGAACGGAGCAATT

Myomaker 3'UTR Fragment:

s: 5'-CCGCTCGAGTTGCCCACCAGTTCCAT

as: 5'-ACGCGTCGACTTGACGTTTGCCAAGT

Myomaker 0.6kb promoter s: 5'-CGGGGTACCAGTGATCTGCTCCTATTCCTCC

as: 5'-TCCCCCGGGCGATGCTGATGGTGGG

Myomaker 1.3kb promoter s: 5'-CGGGGTACCCCTGTGAAGGGATGCTCG

as: 5'-TCCCCCGGGCAAAGTGCTGAGGGTGGG

Myomaker 2.0kb promoter s: 5'-CGGGGTACCTAGCCCTGTGGAATGTGAAT

as: 5'-TCCCCCGGGCTGTTGGAGATGGAGGAGAA

For quantitative real time RT-PCR, the following primers were used:

Myomaker-qPCR: s: 5'-TGGGTGTCCCTGATGGC

as: 5'-CCCGATGGGTCCCTGAGTAG

MYOD-qPCR: s: 5'-GCTACTACACGGAATCACCAAAT

as: 5'-CTGGGCTCCACTGTCCTCA

MYOG-qPCR: s: 5'-CGGAGGCTGAAGAAGGTGAA

as: 5'-CGGTCCTCTGCCTGGTCAT

MyHC-qPCR: s: 5'-CTCCTCACGCTTTGGTAA

as: 5'-TGATAGTCGTATGGGTTGGT

β -actin-qPCR: s: 5'-ATCTTCTTGGGTATGGAGTC

as: 5'-GCCAGGGTACATTGTGG

For mutagenesis the following primers were used for PCR:

myomaker-3'UTR-mut1:

s: 5'-GGAGAAGCCTTTGTATGGGGTGTGCTTCACACCAGAGAGCTCCGCATCACCCCTGTG

as: 5'-CACAGGGTGTATGCGGAGCTCTCTGGTGTGAAGCACACCCCATACAAAGGCTTCTCC

myomaker-3'UTR-mut2:

s: 5'-CCTGTGCCAGAGAGCTCCGCATCACTTCACACGCCCTTGGCCACTTCACCTCCCC

as: 5'-GGGGGAGGTGAAGTGGCCAAGGGCGTGTGAAGTGTGCGGAGCTCTCTGGCACAGG

Myomaker 0.6kb promoter-mut1:

s: 5'-GTGGCTCAGCTGTGCCACTGCCTGTGAAGATTCCCTGCCTGCAGCTCTCCGGGC

as: 5'-GCCCGGAGAGCTGCAGGCAGGGAATCTTCACAGGCAGTGGCACAGCTGAGCCAC

Myomaker 0.6kb promoter-mut2:

s: 5'-AAGAAGGAATGGAGCATATGGGTGGCTACTTGTGGCCACTGCCTGCAGCTGTTCCCTGC

as: 5'-GCAGGGAACAGCTGCAGGCAGTGGCAACAAGTAGCCACCCATATGCTCCATTCTCTTCTT

For RACE and gene specific PCR the following primers were used:

5' RACE RT-adaptor: 5' -GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTTTTT

5' outer primer: 5' -AAGCAGTGGTATCAACGCAGAGTACGCGGGGGGGGGG

5' inner primer: 5'-AAGCAGTGGTATCAACGCAGAGT

5' Myomaker outer primer: 5'- TGTCTGGATACAGCCCTTTC

5' Myomaker inner primer: 5'- TGAAACATAGCACCGATAAGC

3'RACE Oligo(dT)-anchor primer:

5'-GCTGTCAACGATACGCTACGTAACGGCATGACAGTGTTTTTTTTTTTTTTTTTTT

3' adaptor outer primer: 5'- GCTGTCAACGATACGCTACGTAACG

3' adaptor inner primer: 5'- CGCTACGTAACGGCATGACAGTG

3' Myomaker outer primer: 5'- TGGCTTATCGGTGCTATGTT

3' Myomaker inner primer: 5'- CCCACCAGTTCATACGC