miR-24 and miR-122 Negatively Regulate the Transforming Growth Factor-β/Smad Signaling Pathway in Skeletal Muscle Fibrosis

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A: After culturing for 0, 4, 8, 12, and 24 h with TGF-β stimulation (10ng/mL), the level of miR-345 in C2C12 cells was quantified by using RT-qPCR.

B: After culturing for 24h with TGF-β stimulation (0, 5, 10, 15ng/ml) for 12h, the level of miR-345 in C2C12 cells was quantified by using RT-qPCR.

Data are expressed as mean ± SD.
A: Quantification of Figure 3B. B: Quantification of Figure 3C. C: Quantification of Figure 3D.

D: Quantification of Figure 3F. E: Quantification of Figure 3H. Data are expressed as mean ± SD, *: p<0.05, **: p<0.01, ***: p<0.001. NC: negative control.
A: Quantification of Figure 4D. B: Quantification of Figure 4E. C: Quantification of Figure 4F.

D: Quantification of Figure 4K. E: Quantification of Figure 4L. F: Quantification of Figure 4M.

Data are expressed as mean ± SD, *: p<0.05, **: p<0.01, ***: p<0.001. NC: negative control.
### Table S1 Primers used for cloning

| Name                  | Sequence (5’-3’)                              | Product length (bp) |
|-----------------------|-----------------------------------------------|---------------------|
| miR-24 promoter F     | CGGGGTACCTTTTCTAGTCGGCAGTC                   |                     |
| miR-24 promoter R     | CCGCTCGAGACATACCAACAATCCCCT                 | 505                 |
| miR-122 promoter F    | CGGGGTACCGTGAGCCCTCCTTTGTTAT                |                     |
| miR-122 promoter R    | CGCGTCAGTTGGTGTCAGGGTAGTC                   | 471                 |
| smad2 3’UTR F         | TGCTCTAGATGAAAGTGGTGAGGGTT                  |                     |
| smad2 3’UTR R         | AAGGAAAAAGCGGCGCGCTCACATTGGCTGGGTAAA        | 510                 |
| tgfbr2 CDS F          | TGCTCTAGACTGCGCCTGCTGCATATCGT              |                     |
| tgfbr2 CDS R          | AAGGAAAAAGCGGCGCGCAGATTCATCCTGGATTCTAGAACTTCCG | 1358               |

F: forward, R: reverse.

### Table S2 The sequence of miRNA and siRNA

| Name                  | Sense sequence (5’-3’)            | Antisense sequence (5’-3’)             |
|-----------------------|-----------------------------------|----------------------------------------|
| NC mimics             | UUCUCCGAACGUGUCACGUTT             | ACGUGACACGUUCGGAGAATT                   |
| miR-24 mimics         | UGGCUCAGUUCACGAGAACAG             | GUUCCUGUAGAACUGAGCCAUU                 |
| miR-122 mimics        | UGGAGUGUGACAAUUGUUUG              | AACCAUUGUACUCACUCAAU                   |
| NC inhibitor          | CAGUACUUUUUGUGUAACAA             |                                       |
| miR-24 inhibitor      | GUUCCUGUAGAACUGAGCCAUU           |                                       |
| miR-122 inhibitor     | AACCAUUGUACUCACUCAAU             |                                       |
| siSmad4-914           | GGAUGAGUACGUUCACGACGc            | GUCGUGAACGUACUCACUCc                   |
| Agomir NC             | UCACAACCUCUAGAAAGUGUA            |                                       |
| Agomir-24             | UGGCUCAGUUCACGAGCAACAG           |                                       |
| Agomir-122            | UGGAGUGUGACAAUUGUUGUUG           |                                       |
| Name       | Sequence (5'-3') | Product length (bp) |
|------------|-----------------|---------------------|
| 18S-F      | ACCGCAGCTAGGAATAATGGA | 157               |
| 18S-R      | CAAATGCTTTTCGCTCTTGTC |                  |
| TGF-β1-F   | TGACGTCACTGGAGTTGTACGG |                  |
| TGF-β1-R   | GTTTCATGTCACTGGATGGTGTC | 170              |
| Tgfbr2-F   | CCGCTGCAATATCGTCTTG   |                  |
| Tgfbr2-R   | AGTGGATGGATGGTCTATTACA | 131              |
| Smad2-F    | AAGCCATACACACTCGAATTG |                  |
| Smad2-R    | CACTGATCTACCCTATTGCTGT | 100              |
| Smad3-F    | CACGCAGAAGCTGAACACC |                  |
| Smad3-R    | GGCAGTAGATAACGTAAGGA | 101               |
| Smad4-F    | CGGCCGCGTGGCAGGGAAACA |                  |
| Smad4-R    | CTGCAAGAGCTCGTGAGGTGAAT | 215             |
| Col1a1-F   | GCTCCTCTTAGGGCGCACT |                  |
| Col1a1-R   | CCACGTCTCACCCTTTGG | 103               |
| α-SMA-F    | GTCCCCAGACATCGGGAGTAA |                  |
| α-SMA-R    | TCGGATACCTCAGCGAAGGA | 102               |
| Vimentin-F | CGGCTGCAGAGAATTTG |                  |
| Vimentin-R | CCACTTTCCGGATTCAAGTC | 124              |
| U6         | AACTCCAGCTGGCGCAAAATTCGTGAAGC | 120          |
| uniprimer  | CTCAAAGTGTCTCGGAAGTCCGGCA | 120         |
| miR-24-3p  | AACTCCAGCTGGCGTCAGCGTCA | 120        |
| miR-122-5p | AACTCCAGCTGGGATGTCGACATG | 120        |
| miR-345-5p | AACTCCAGCTGGCTCAGCAGTGAAT | 120     |
| mir-122-promoter-SBE-F | GGCTCTTCCCTATGCTCC | 90            |
| Promoter-SBE-F/R       | Sequence                          | Length |
|------------------------|-----------------------------------|--------|
| mir-122-promoter-SBE-R | ATGGCGTTTGATGGTTTG                |        |
| mir-24-promoter-SBE1-F | TGTTGCTAATCTTAACCCAT              |        |
| mir-24-promoter-SBE1-R | GCTTACTCCAGCCCACTA                | 79     |
| mir-24-promoter-SBE2-F | ACCGCCACAGTCAAGCAG                |        |
| mir-24-promoter-SBE2-R | CCACATACCAACAATCCC                | 80     |

F: forward, R: reverse.