

Appendix

3'UTR shortening alleviates miRNA repression of mRNAs critical for muscle stem cell differentiation

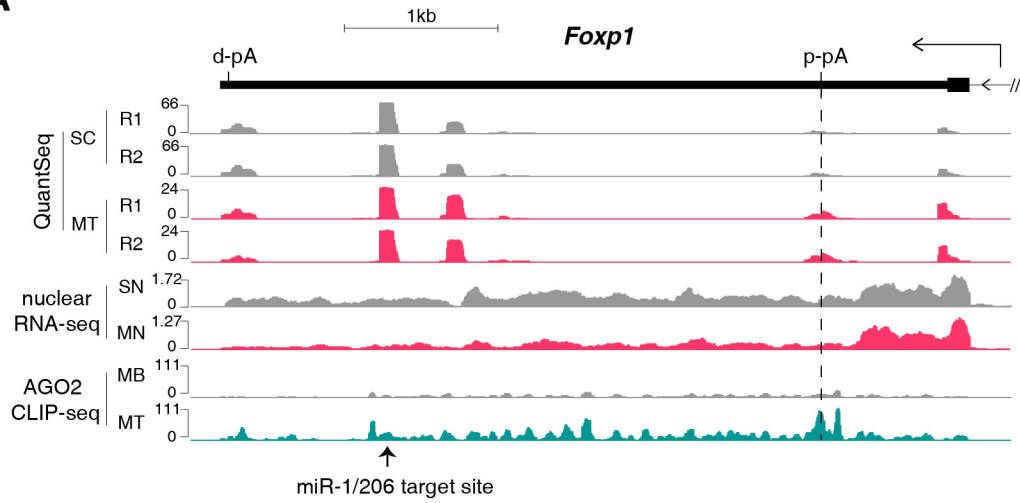
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Appendix Figure S1

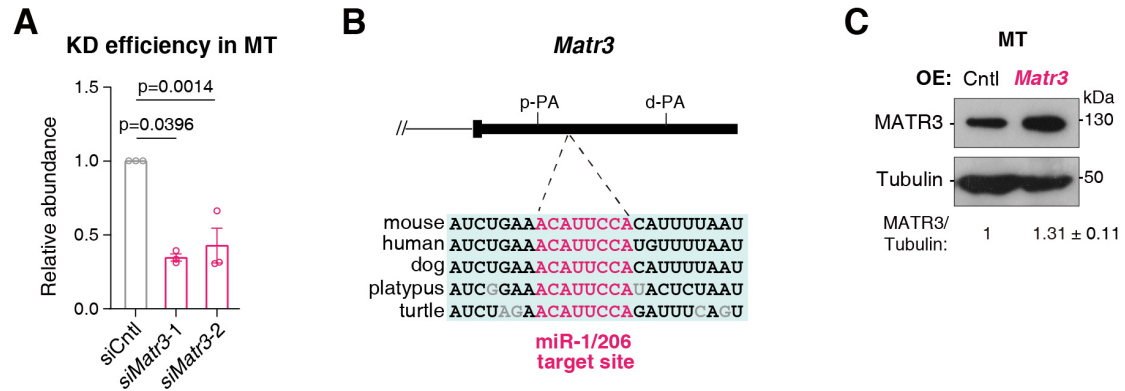
A



Appendix Figure S1. Predicted miR-1/206 target site in *Foxp1* aUTR.

(A) Screenshot of QuantSeq, nuclear RNA-seq, and AGO2 CLIP-seq signals of *Foxp1* aUTR. Predicted miR-1/206 target site is indicated by an arrow.

Appendix Figure S2



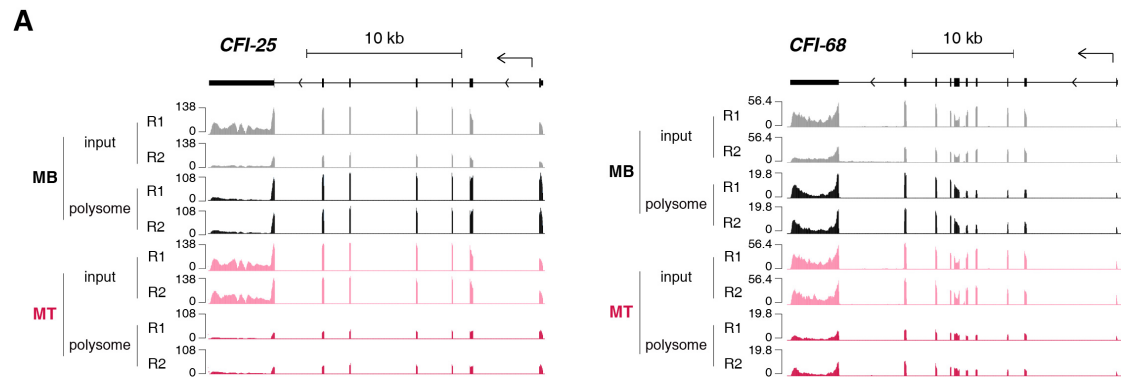
Appendix Figure S2. The APA-miRNA interplay fine-tunes *Matr3* expression for efficient muscle differentiation.

(A) RT-qPCR to detect KD efficiency of *Matr3* in differentiated MTs ($n = 3$). Bars represent RNA abundance normalized to *Gapdh*. Error bars, mean $\pm$ SEM. *P*-values, two-sided unpaired student's *t* test.

(B) The sequences of the conserved miR-1/206 target site in *Matr3* aUTR are labeled in magenta.

(C) Western blots showing *Matr3* protein levels in MTs differentiated from MBs transfected with Ctrl or *Matr3* expression plasmid. Relative protein abundance to Tubulin (mean $\pm$ SEM, $n = 3$) are shown at the bottom.

Appendix Figure S3



Appendix Figure S3. Reduced polysome association of *CFI-25* and *CFI-68* upon differentiation.

(A) Polysome profiling showing the distribution of *CFI-25* and *CFI-68* in MBs and MTs.

Appendix Table S1. siRNAs.

Target gene	Targeting sequence (5' to 3')
Cntl	CGTACGCGGAATACTTCGA
<i>CFI-68</i>	CCTGTTGTAACTCCATGCAAT
<i>Matr3</i> (1#)	GCTCCTCCAAGTAGCAATA
<i>Matr3</i> (2#)	GGACAAGATTGAGGAACTT
<i>Pabpc1</i>	CGTGCTTTGGACACCATGAAT
<i>Pabpn1</i>	TAGAGCGACATCATGGTATTC

Appendix Table S2. Antisense morpholino oligonucleotides (AMOs).

Target gene	AMO sequences (5' to 3')
Control	CCTCTTACCTCAGTTACAATTTATA
<i>Matr3-p</i>	TTGGTTTTGTTCTTTATTGAATGGA
<i>Matr3-tss</i>	TCAGATTAAAATGTGGAATGTTTCA

Appendix Table S3. Antisense oligonucleotides (ASOs).

Target gene	ASO sequences (5' to 3')
Control	CCUUCCCUGAAGGUUCCUCC
<i>Cpd</i>	CGACCCUUUUUAUUUUUCACU
<i>Foxp1</i>	UUAAAAAAUUUGGGAUAAUUAUUUUUAA
<i>Il6st</i>	UAGCUUUUAUUAGACGGUUUU
<i>Neol</i>	GGCCUUUAAUAAUUAUAAUA
<i>RalA</i>	UAAAUUAUAUUUAUUAGUAU
<i>Sec63</i>	CAAAGGAUUUAUUAUCAUUU

ASOs were engineered with full substitution of 2'-O-methoxyethyl oligonucleotides and a phosphorothioate backbone.

Appendix Table S4. Primers.

Primer Name	Primer Sequences
Isoform-specific (RT)	CAAGCAGAAGACGGCATACGAGATTTT TTTTTTTTTTTTTTTTTTTTTTTVN
<i>CFI-25</i> Forward	CATTTCTAGTCTTCCTCAGCTGCTG
<i>CFI-25</i> Reverse	TGTAAGCTGTGCTCATAGAGACGG
<i>CFI-68</i> Forward	CTGACGCTAGTGCTGGTGAT
<i>CFI-68</i> Reverse	TTGATCGTTCACGTCTTCTGGAT
<i>Cpd-S</i> Forward	TCAGTGTGCTTGGCTGGTT
<i>Cpd-L</i> Forward	ACATGTCATGGTGTCTGC
<i>Cpd-L</i> Reverse	GTTATCAACAAGTTGCTAGACAG
<i>Foxp1-S</i> Forward	GCACAGCGCTGCATGTTGATA
<i>Foxp1-L</i> Forward	AGCACCCACCATTGTGAGTA
<i>Foxp1-L</i> Reverse	TGGTCCTGGTCACCTGATTAT
<i>Gapdh</i> Forward	ACCCAGAAGACTGTGGATGG
<i>Gapdh</i> Reverse	ACACATTGGGGGTAGGAACA
<i>Il6st-S</i> Forward	GGAGCACCTCCCTTTGCTCT
<i>Il6st-L</i> Forward	TGTGGTCATCAGCTCGGC
<i>Il6st-L</i> Reverse	CAGTCTCACCAGACAGTGGT
Luc-S Forward	GAGCTCGCTAGCCTCGAG
Luc-L Forward	GAGCTCGCTAGCCTCGAG
Luc-L Reverse	TCAAATGAAAAGTCTGCAAGGC
<i>Matr3-S</i> Forward	ATACGGTTAAGTTAATGAAAATGTT
<i>Matr3-L</i> Forward	CAGCAGCCTTCCTCATTATCA
<i>Matr3-L</i> Reverse	AAATGTGGAATGTTTCAGATG
<i>Matr3</i> Forward	GACTCTTACCGAAGGGTTATC

<i>Matr3</i> Reverse	CCATTCTGGGTAGATTTC
<i>Myh1</i> Forward	CTCTTCCCGCTTTGGTAAGTT
<i>Myh1</i> Reverse	CAGGAGCATTTCGATTAGATCCG
<i>Myh3</i> Forward	ATGCCACCTTCGCTACAACA
<i>Myh3</i> Reverse	G TTCAGCACTCGGTATCTCTGT
<i>Mck</i> Forward	CACCTCCACAGCACAGACAG
<i>Mck</i> Reverse	ACCTTGGCCATGTGATTGTT
<i>Neol-S</i> Forward	CTGAGACATTGCATCCTCTGC
<i>Neol-L</i> Forward	ACCCATGACGCTTCCCCA
<i>Neol-L</i> Reverse	GTCTTCTGCGTGTGGCTGAA
<i>RalA-S</i> Forward	GAGGGAAATACGAGCCAGAAAGA
<i>RalA-L</i> Forward	AGATTATGAGCGTGAGTACCAA
<i>RalA-L</i> Reverse	CAGACCACATTAGCTCTCCTC
<i>Sec63-S</i> Forward	GGTGGTGCGTTTATCTCATCTG
<i>Sec63-L</i> Forward	GCTCTCAGTAAAAGTCAAGGC
<i>Sec63-L</i> Reverse	GTGAGAATGCTAAGTTATGGCC
<i>Pabpc1</i> Forward	GCCACTGGTGTTCCAAGTGTCTA
<i>Pabpc1</i> Reverse	TTAAGATATTTTTCTTCGGTGAAGCA
<i>Pabpn1</i> Forward	CTACAGTGGTTTTAACAGCAGGCC
<i>Pabpn1</i> Reverse	TTCCATCAAGGTCATCTTCTGTTTT
RS	CAAGCAGAAGACGGCATAACGAGA
<i>Matr3</i> ^{mpA/mpA} Forward (genotyping)	TGTGTCCTGAGCCAGTTTAGTTTTGT
<i>Matr3</i> ^{mpA/mpA} Reverse (genotyping)	AGCTACAGGATTCAAGTGACAGGCA

Appendix Table S5. Candidate myomiR target sequences.

myomiRs	targeting sequence (5' to 3')
mmu-miR-1	ACATTCC
mmu-miR-133	GGACCAA
mmu-miR-206	ACATTCC
mmu-miR-208	AAAAGCT
mmu-miR-486	GTACAGG
mmu-miR-499	AGTCTTA