

Appendix Supplementary information

Myogenin promoter-associated lncRNA *Myoparr* is essential for myogenic differentiation

Keisuke Hitachi, Masashi Nakatani, Akihiko Takasaki, Yuya Ouchi, Akiyoshi Uezumi, Hiroshi Ageta, Hidehito Inagaki, Hiroki Kurahashi, and Kunihiro Tsuchida

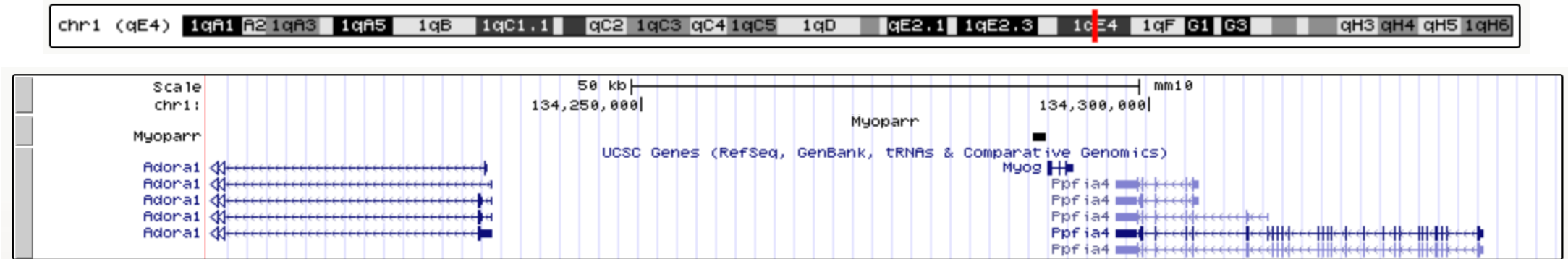
Table of contents

Appendix Figure S1.....	2
Appendix Figure S2.....	3
Appendix Figure S3.....	4
Appendix Figure S4.....	5
Appendix Figure S5.....	6
Appendix Table S1. List of locus information of <i>Myoparr</i>	7
Appendix Table S2. List of siRNA, shRNA, and ASO.....	8
Appendix Table S3. List of primers.....	9
Appendix Table S4. List of ChIRP probes.....	10

Appendix Figure S1

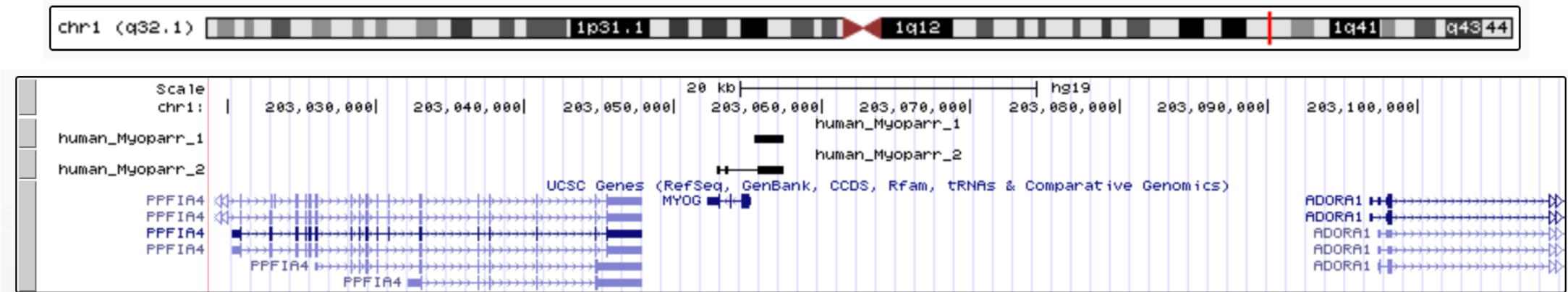
A

Mouse *Myoparr*



B

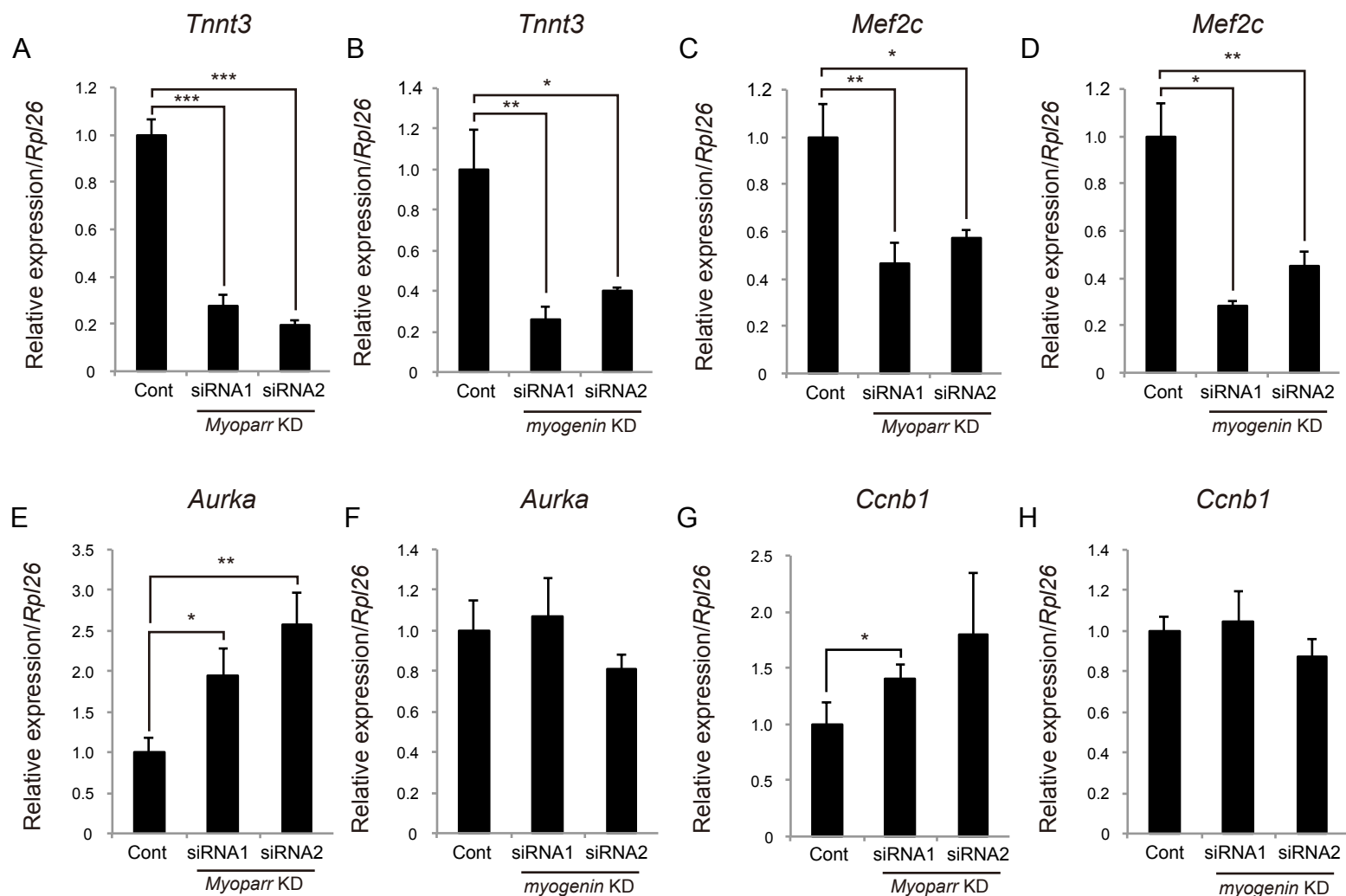
Human *Myoparr*



Appendix Figure S1. Genome Browser screenshot.

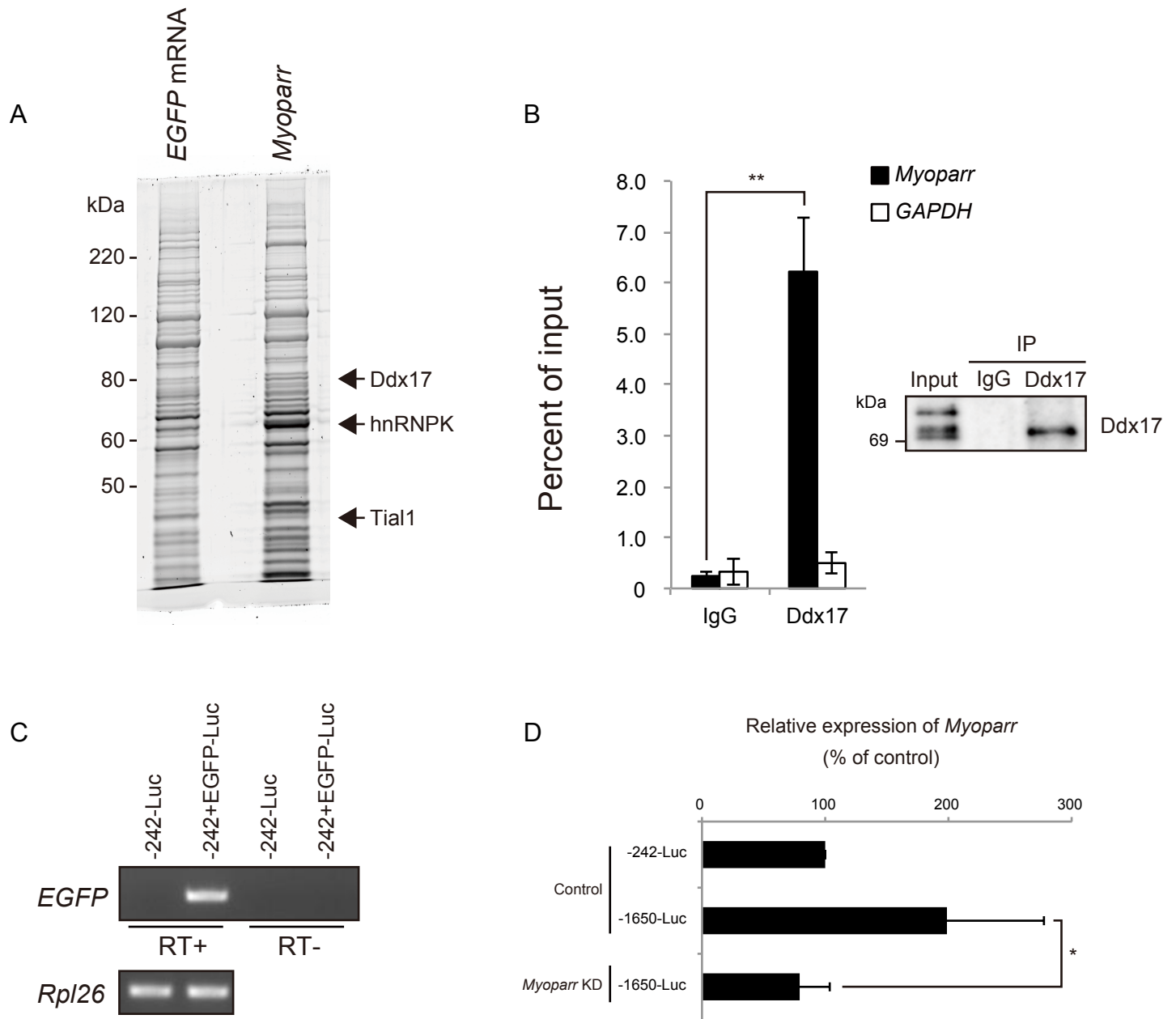
(A-B) UCSC Genome Browser screenshot showing the locations of mouse (A) and human (B) *Myoparr*.

Appendix Figure S2



Appendix Figure S2. Expression changes of genes regulated by *Myoparr* knockdown and *myogenin* knockdown. (A-H) Quantitative RT-PCR for *Tnnt3* (A-B), *Mef2c* (C-D), *Aurka* (E-F), and *Ccnb1* (G-H) in differentiating C2C12 cells transfected with 50 nM of indicated siRNAs. *Tnnt3* and *Mef2c* are categorized in skeletal muscle contraction and cell differentiation in GO terms. *Aurka* and *Ccnb1* are categorized in cell cycle and cell division in GO terms. n = 3, mean \pm SD. * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Statistical analyses were performed using unpaired two-tailed Student's t-test. In cases of unequal variances, unpaired two-tailed Welch's t-test was used.

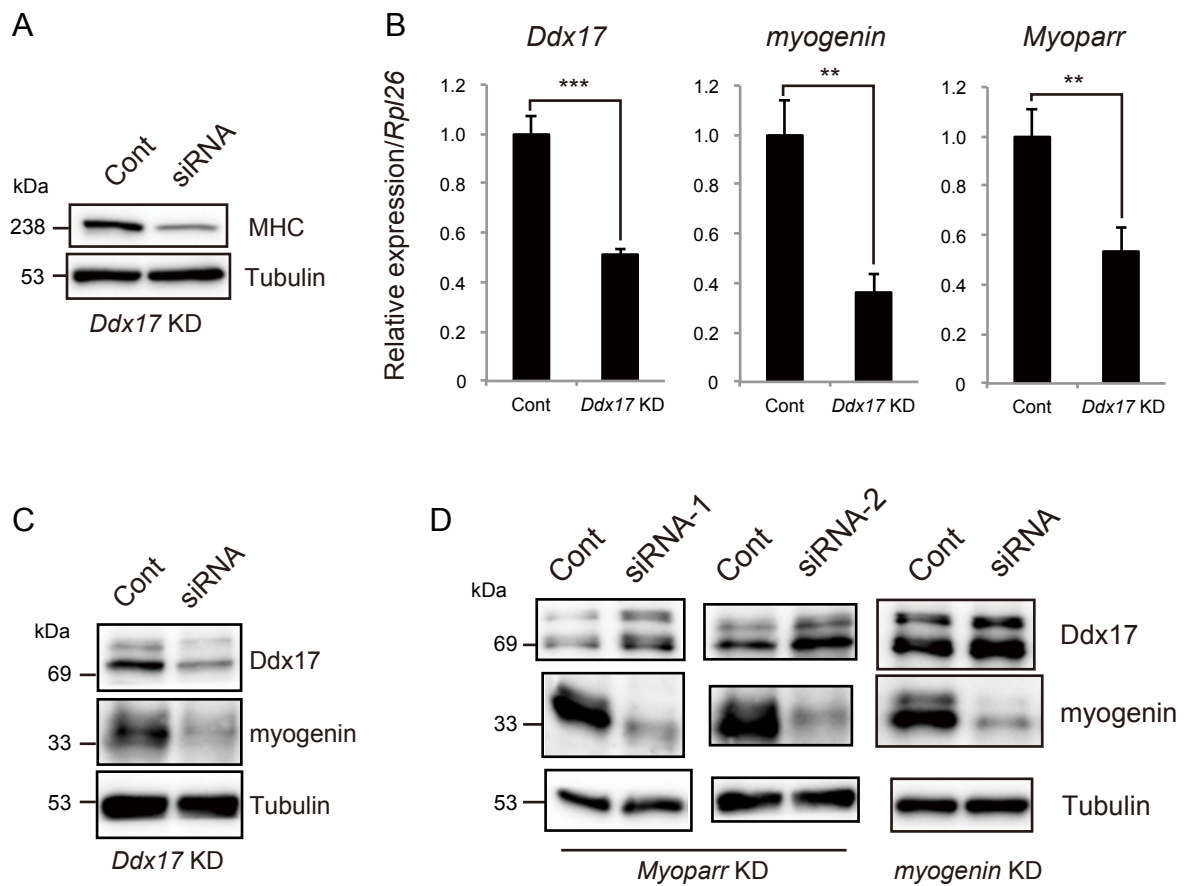
Appendix Figure S3



Appendix Figure S3. Identification of *Myoparr*-interacting proteins.

(A) Nuclear extracts prepared from C2C12 myotubes were immunoprecipitated with BrU labeled *Myoparr* or *EGFP* RNA. Isolated proteins were resolved by SDS-PAGE, and stained with SYPRO Ruby Protein Gel Stain. The protein bands specifically detected in *Myoparr* sample were retrieved and analyzed by mass spectrometry. (B) RNA immunoprecipitation assays showing intracellular interaction between endogenous *Myoparr* and Ddx17. Nuclear lysates of C2C12 myotubes were immunoprecipitated with an anti-Ddx17 antibody or a control rabbit IgG. After washing, the presence of *Myoparr* was detected by qRT-PCR (left). *GAPDH* was used as a negative control. The data were normalized to input values. $n = 3$, mean \pm SD. $**p < 0.01$ (unpaired two-tailed Welch's t-test). Specific immunoprecipitation (IP) of Ddx17 by an anti-Ddx17 antibody was confirmed by western blot (right). (C) RT-PCR for *EGFP* in C2C12 cells transfected with the -242-Luc or -242-EGFP-Luc construct. The presence or absence of reverse transcriptase (RT) is indicated by (+) or (-), respectively. Expression of *Rpl26* served as an internal control. (D) Relative expression of *Myoparr* in Figure 4H was quantified by qRT-PCR. The results were normalized to *Rpl26* expression. Data are shown as percent of the control (-242-Luc) and are the mean \pm SD. $n = 3$, $*p < 0.05$ (unpaired two-tailed Welch's t-test).

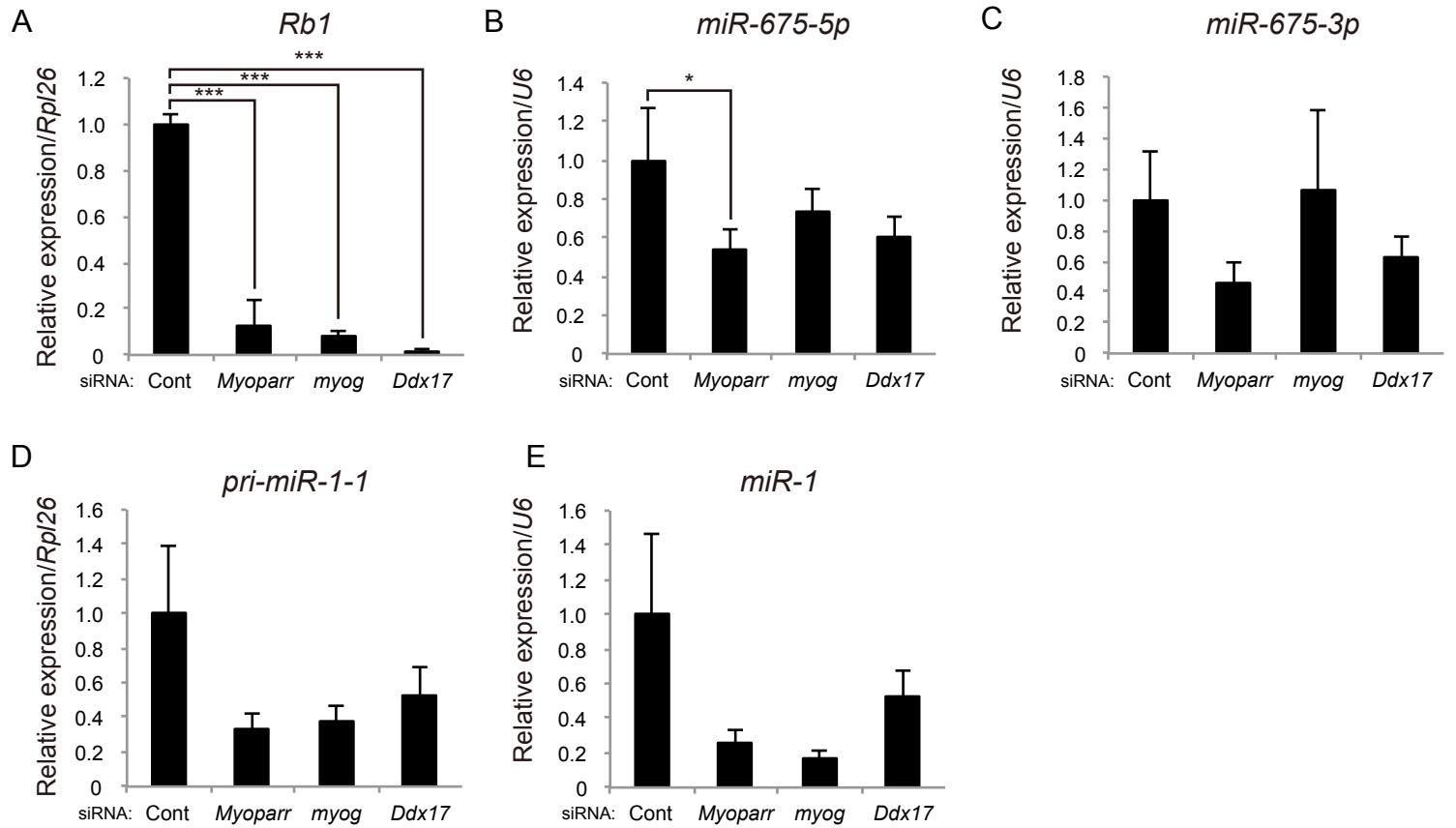
Appendix Figure S4



Appendix Figure S4. Expression changes of genes and proteins in *Ddx17*-deficient C2C12 cells.

(A) Western blot for MHC in *Ddx17*-depleted differentiating C2C12 cells. Tubulin expression served as an internal control. (B) Relative expression of *Ddx17*, *myogenin*, and *Myoparr* quantified by qRT-PCR in *Ddx17*-depleted C2C12 cells. $n = 3$, mean \pm SD. $**p < 0.01$. $***p < 0.001$ (unpaired two-tailed Student's t-test). (C) Decreased expression of myogenin protein by *Ddx17* knockdown shown by western blot. (D) Western blots for *Ddx17* and myogenin protein in C2C12 cells transfected with *Myoparr* or *myogenin* siRNAs.

Appendix Figure S5



Appendix Figure S5. Expression changes of genes regulated by *Myoparr*, *Ddx17*, and *myogenin* knockdown. (A-E) Quantitative RT-PCR for *Rb1* (A), *miR-675-5p* (B), *miR-675-3p* (C), *pri-miR-1-1* (D), and *miR-1* (E) in differentiating C2C12 cells transfected with 50 nM of indicated siRNAs. n = 3, mean \pm SD. * p < 0.05. *** p < 0.001 (unpaired two-tailed Student's t-test).

Appendix Table S1. List of locus information of *Myoparr*.

Locus information (mouse) mm10	
<i>Myoparr</i> (from skeletal muscle of C57BL/6J)	chr1: 134288595-134289760
<i>myogenin</i> -associated sense long non-coding RNA isoform-1	chr1: 134287131-134292548
<i>myogenin</i> -associated sense long non-coding RNA isoform-2	chr1: 134287131-134292548
<i>myogenin</i> -associated sense long non-coding RNA isoform-3	chr1: 134287131-134287485
<i>myogenin</i> -associated sense long non-coding RNA isoform-4	chr1: 134287131-134287873
<i>myogenin</i> -associated sense long non-coding RNA isoform-5	chr1: 134287575-134292550
<i>myogenin</i> -associated sense long non-coding RNA isoform-6	chr1: 134287575-134292550
<i>myogenin</i> -associated sense long non-coding RNA isoform-7	chr1: 134287575-134292550
Locus information (human) hg19	
<i>Myoparr</i> isoform-1 (from human myoblasts)	chr1: 203055492-203057468
<i>Myoparr</i> isoform-2 (from human myoblasts)	chr1: 203052923-203057468

Appendix Table S2. List of siRNA, shRNA, and ASO.

siRNA		
Target name	Target Sequence	
Control 1	Stealth RNAi siRNA negative control, Med GC (Thermo Fisher Scientific)	
Control 2	Stealth RNAi siRNA negative control, Med GC Duplex #3 (Thermo Fisher Scientific)	
Myoparr siRNA 1	CCCTAAAGACCTACCACTACCACAT	
Myoparr siRNA 2	GATGGACCCTGTCTGATGCTCTTAA	
myogenin siRNA 1	GGGAGAAGCGCAGGCTCAAGAAAGT	MSS275910 (Thermo Fisher Scientific)
myogenin siRNA 2	CAGACGCCACCAATCTGCACTCCCT	MSS275912 (Thermo Fisher Scientific)
Ddx17 siRNA	CACCAACAAGGGCACTGCCTATACT	
ASO		
Target name	Target Sequence	
Control	Antisense LNA GapmeR, negative control A, in vitro standard (Takara)	
Myoparr ASO1	CCATACTATGTCAGTC	
Myoparr ASO2	TCTCTTCTGTGCTTTC	
shRNA		
Target name	Target Sequence	
Myoparr shRNA 1	TCTCATCTGCTCCTTTCAATT	
Myoparr shRNA 2	GACCCTGTCTGATGCTCTTAA	
Myoparr shRNA 3	TACCACATCAGGACCACTCCA	

Appendix Table S3. List of primers.

Primers for PCR analysis in mouse		
Target name	Forward	Reverse
A, -493/-302	GACATAGTATGGTTTAAAGGTGCTGC	CGTCTCATCTGCTCCTTTCAATTAC
B, -711/-469	CTCTTACTTCAATCTACCCCAACA	GCAGCACCTTAAACCATACTATGTC
C, -927/-702	GGGAACCTTTGGGATAGCAITTGAA	GAAGTAAGAGAACACAGGGACTTCT
D, -1444/-1268	CCCTTCTACAGAAAGGAAAGAGTCA	AGCTATATCAGGGTCTTTTCAGCAA
Target name	Sequence	
a	CGTCTCATCTGCTCCTTTCAATTAC	
b	GCAGCACCTTAAACCATACTATGTC	
c	CTCTTACTTCAATCTACCCCAACA	
d	GGGAACCTTTGGGATAGCAITTGAA	
Primers for PCR analysis in human		
Target name	Forward	Reverse
A, -538/-370	TGCCCATGAATGCCAGAAT	GGGAACAAGGAAGGGTAGGC
B, -825/-681	GGGAGGAGACAGGGAGCTTA	TTGGTTTCTTGGCAGGGGT
Target name	Sequence	
a	GGGAACAAGGAAGGGTAGGC	
b	ATTCTGGGCATTCATGGCA	
c	ACCCCTGCCAAGAAAACCAA	
d	GGGAGGAGACAGGGAGCTTA	
Primers for real-time PCR analysis		
Target name	Forward	Reverse
GAPDH	CCTGGAGAAACCTGCCAAGTATG	AGAGTGGGAGTTGCTGTTGAAGTC
myogenin	CAGTACATTGAGCGCTACAG	GGACCGAACTCCAGTGCAT
Neat1	TTGGGACAGTGGACGTGTGG	TCAAGTGCCAGCAGACAGCA
Myoparr (1)	GTGCCCTATCGTCCATGGAG	CACTGACTTCACCTGACCCC
Myoparr (2)	GGCAGGAAGGGAACAAGAA	CCCCAAATCTGGAGTGGTCC
Sense RNA	CCACCCTGAGATCCACACAC	GGGACGAGGATCACTTGCAA
SRA1	TCCACCTCCTCAAGTAAGGCT	GACCTCAGTCACATGGTCAACC
Malat1	CATGGCGGAATTGCTGGTA	CGTGCCAACAGCATAAGCAGTA
Myod1	CCACTCCGGGACATAGACTTG	AAAAGCGCAGGTCTGGTGAG
Myf5	CCTGTCTGGTCCCGAAAGAAC	GACGTGATCCGATCCACAATG
MRF4	ATCAGCTACATTGAGCGTCTACA	CCTGGAATGATCCGAAACACTTG
EGFP	GAACGGCATCAAGGTGAACT	GAACTCCAGCAGGACCATGT
Ddx17	GATCGGGATCGTGACAGGGA	AGTCAGTCTTGCTACTTCTGGAT
Rpl26	GGTCTATGCCATTCCGAAGG	TCGTTCGATGTAGATGACGTACT
pri-miR-133b	CTGGTCAAACGGAACCAAGT	TGATGGCAAACCAGCATTA
pri-miR-206	TTCTTCTGCGTGACAAGTG	GCCAAAACCCACACTTCTCT
H19	CTGCTCCAAGGTGAAGCTGA	TAGAGGCTTGGCTCCAGGAT
Pola1	AGCTCGGCGAGAAAAGAAATC	CCACATAGCTATCCCATCGTC
Rb1	TGCACTTTATCGCAGCAGTT	GTTCACACGTCCGTTCTAAATTG
pri-miR-1-1	CCTACCTGCTTGGGACACAT	CAGTCTGGCGAGAGAGTTCC
Aurka	CTGGATGCTGCAAACGGATAG	CGAAGGGAACAGTGGTCTTAACA
Tnnt3	GGAACGCCAGAACAGATTGG	TGGAGGACAGAGCCTTTTCTT
Mef2c	TGCTGGTCTCACCTGGTAAC	ATCCTTTGATTCACTGATGGCAT
Ccnb1	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
human Myoparr	ACCCCTGCCAAGAAAACCAA	ATTCTGGGCATTCATGGCA
human myogenin	GGGGAACCTACCTGCCTGTC	AGGCGCTCGATGTACTGGAT
human GAPDH	ACCCACTCTCCACCTTGA	TTGCTGTAGCCAAATTCGTTG
Target name	Sequence	
miR-133b	miScript Primer Assay (QIAGEN, MS00007301)	
miR-206	TGGAATGTAAGGAAGTGTGG	
miR-675-5p	GCGGAAAGGGCCACAGT	
miR-675-3p	CTGTATGCCCTAACCGCTCAGT	
miR-1	miScript Primer Assay (QIAGEN, MS00011004)	
U6	miScript Primer Assay (QIAGEN, MS00014000)	
qPCR primer for ChIP and ChIRP		
Target name	Forward	Reverse
myogenin promoter	GAATCACATGTAATCCACTGGAAA	ACCCAGAGATAAATATAGCCAACG
myogenin ORF	GAGGAAGTCTGTGTCGGTGG	ATCTCCACTTTAGGCAGCCG
Myod1 promoter	AACAGATATTCTCTGAGCCATTCC	GAGAAGAGTGGAGAAATGATGACA
miR-133b promoter	ACGAAGCGATGAGGATCCAC	TGTCCTTGTCTAAATCCCCCT
miR-206 promoter	CCAGGCGTATTGTACTTCC	TGCCAGTGTCCATTCATCTC
H19 promoter	ACCACCGTTCTATGAAGGGC	TCCAAAGACAGCCTCACCAC
GAPDH promoter	AGTCCGTATTATAGGAACCCGGATGGTGG	ATGAGAGAGGCCAGCTACTCGCGCTTTA
myogenin 3'UTR	GAGACATGAGTGCCTGACC	CAGAGGCTTTGGAACCGGAT

Appendix Table S4. List of ChIRP probes.

Biotin-TEG probes	
Odd1	ACAGGGGCTGTGGAGAAATG
Even1	GACCATGGAGGAGAGAGTAG
Odd2	AAGTGGATTTTCAAGACCCC
Even2	TGGTAGTGGTAGGTCTTTAG
Odd3	ACGATAGGGCACACACACAC
Even3	ACATCATGAGACCTGGTCAA
Odd4	AACACTGACTTCACCTGACC
Even4	GTCAGACAGGGGACTGAACA
Odd5	GGGGGTATAGGGAAC TTTG
Even5	CCCTGGTATTGCAA ACTAT
Odd6	ACCCTATAGGTGGAACAACA
Even6	AGAAGTGGATGCTCACAGTC
Odd7	CACTATTACATATGCCAGCA
Even7	CCAGAAGGCATTATTGAGGG