Expanded View Figures

Figure EV1. Characterization of mouse and human *Myoparr*.

A Schematic diagram of the results of 5′- and 3′-RACE analysis of sense and anti-sense transcripts. The 3′-ends of several sense transcripts overlap with *myogenin* mRNA.

B Coding potential assessment of the indicated RNAs using a coding potential assessment tool (CPAT). Low coding probabilities for anti-sense transcript and sense transcript 1 (Long) and 4 (Short) as well as *lincRNA-p21* are shown.

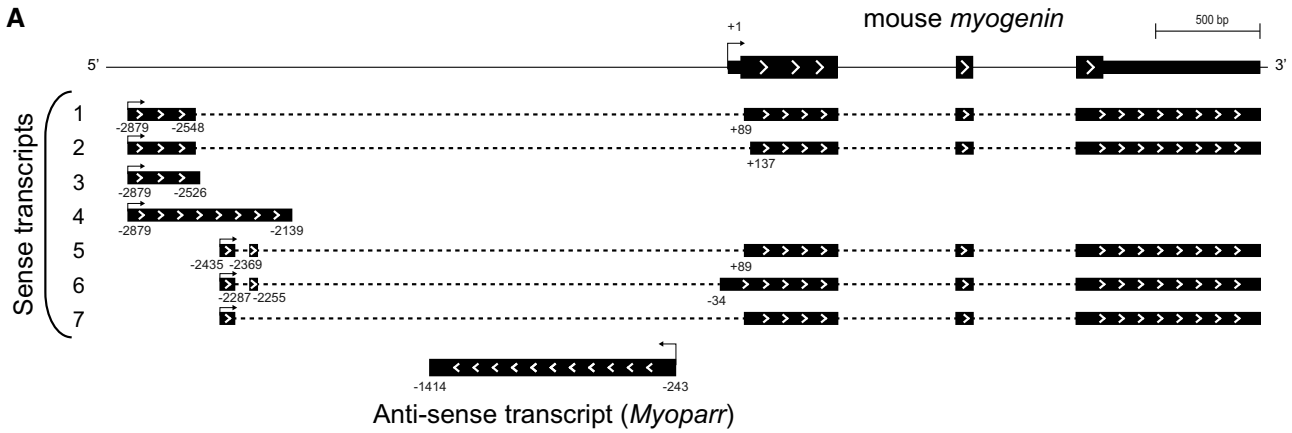
C *In vitro* transcription/translation of pCS2-Anti-Sense, pCS2-Sense (Long), and pCS2-Sense (Short). The pCS2+ vector was used as a negative control. pCS2-EGFP and pCS2-myogenin were used as positive controls.

D The sequence of *Myoparr* cloned from mouse C2C12 cells. The potential RNA nuclear retention signal and putative polyadenylation signal are enclosed in a black and red box, respectively. The LINE-1-like sequence is underlined.

E Schematic representation of the upstream region of human *myogenin* and regions amplified by RT–PCR (top). RT–PCR for novel transcripts in human primary myotubes (bottom). The presence or absence of reverse transcriptase (RT) is indicated by (+) or (−), respectively.

F The primers used for RT–PCR (top). Strand-specific RT–PCR for the novel transcripts in the upstream region of human *myogenin* (bottom).

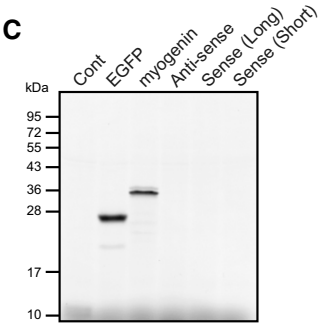
G Schematic diagram of the results of 5′- and 3′-RACE analysis of human *Myoparr*.

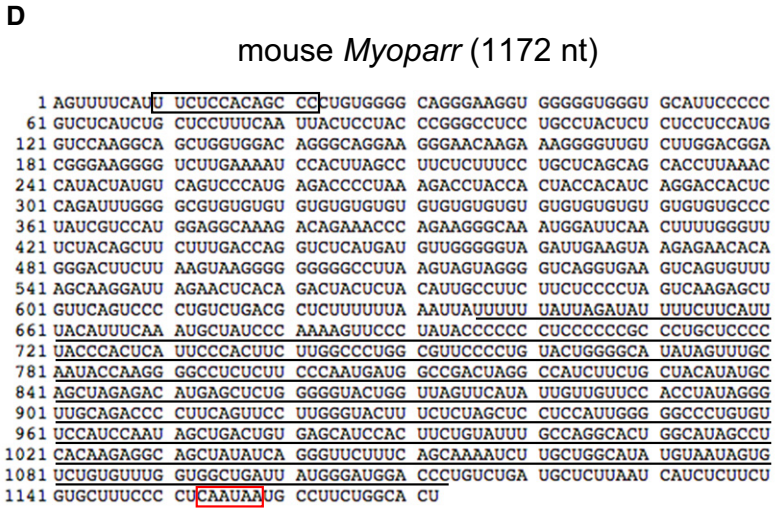


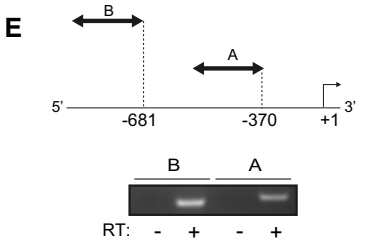
B

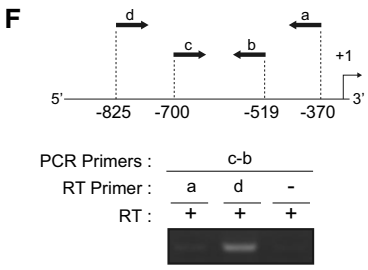
Coding potential assessment by coding potential assessment tool (CPAT)

Sequence Name	RNA size	ORF size	Ficket Score	Hexamer Score	Coding Probability	Coding Label
GAPDH	1228	1002	1.2926	0.541682098	0.998401596	yes
myogenin	1518	675	1.1183	0.441597496	0.965381051	yes
lincRNA-p21	3073	309	0.9806	0.041254541	0.246453936	no
Anti-sense transcript	1172	192	0.7409	-0.117943156	0.047611813	no
Sense transcript (Long)	1762	219	1.0959	-0.136002048	0.102029323	no
Sense transcript (Short)	765	135	0.9152	0.07890528	0.087841224	no









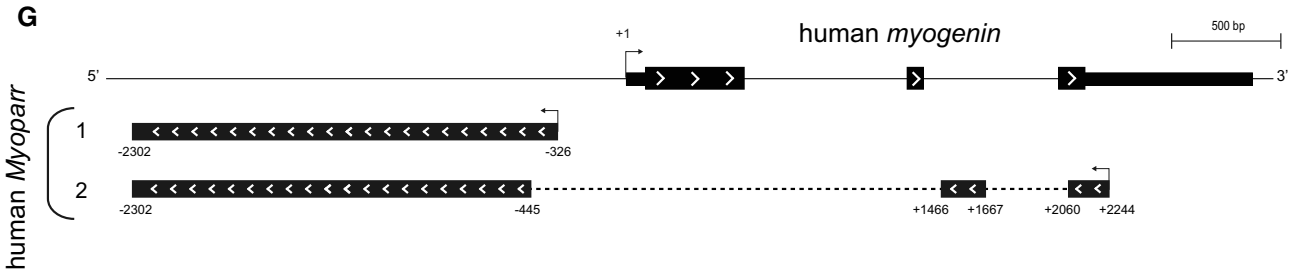


Figure EV1.

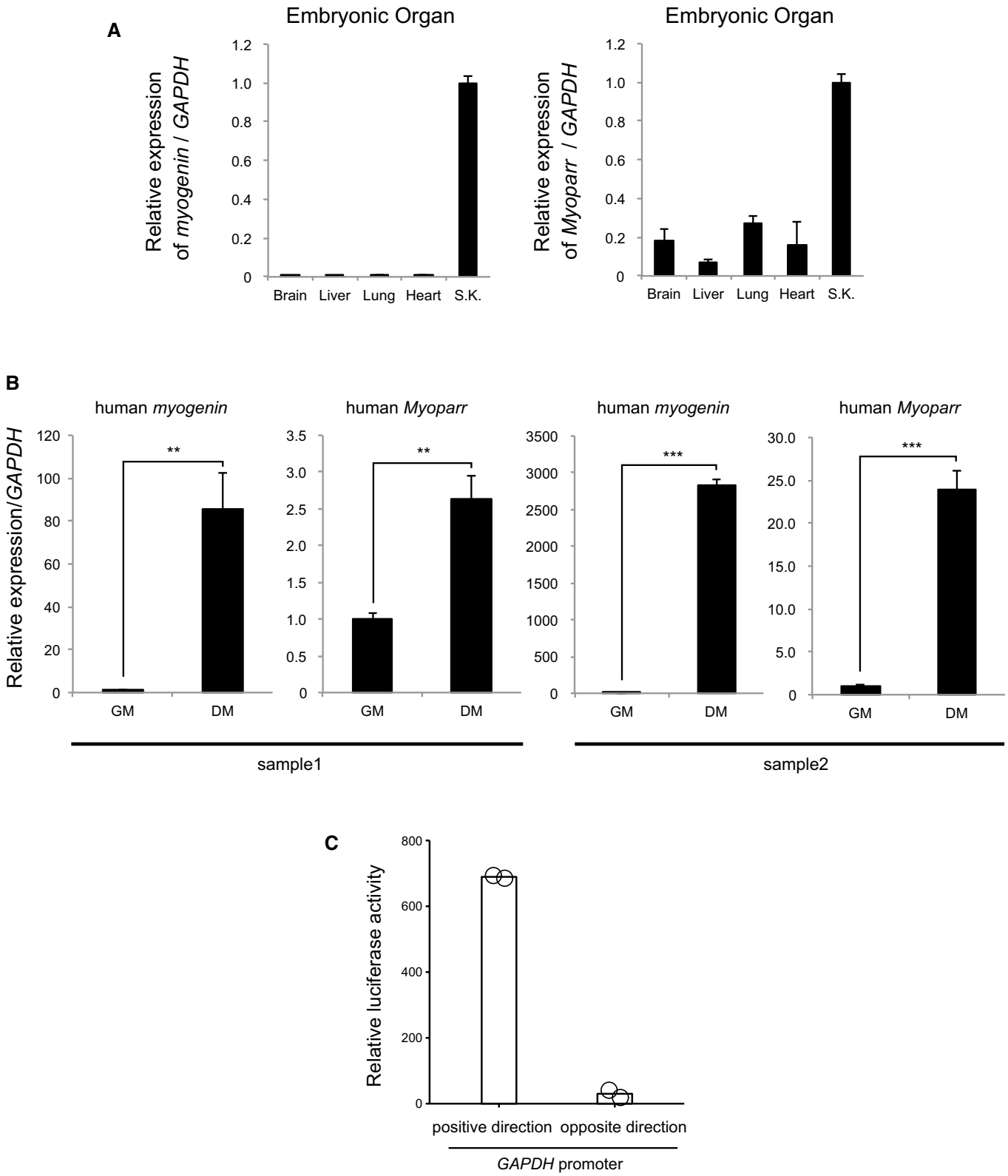

A
Embryonic Organ
Relative expression of myogenin / GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0
Brain Liver Lung Heart S.K.
Embryonic Organ
Relative expression of Myoparr / GAPDH
1.2
1.0
0.8
0.6
0.4
0.2
0
Brain Liver Lung Heart S.K.
B
human myogenin
human Myoparr
human myogenin
human Myoparr
Relative expression/GAPDH
120
100
80
60
40
20
0
**
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
**
3500
3000
2500
2000
1500
1000
500
0
***
30.0
25.0
20.0
15.0
10.0
5.0
0
***
GM DM
GM DM
GM DM
GM DM
sample1
sample2
C
Relative luciferase activity
800
600
400
200
0
positive direction opposite direction
GAPDH promoter

Figure EV2.

Figure EV2. Expression of mouse and human *Myoparr*.

A Quantitative RT–PCR for mouse *myogenin* and *Myoparr* in embryonic organs. $n = 3$, mean ± SD. S.K., skeletal muscle.

B The expression levels of human *myogenin* and *Myoparr* in human primary myoblasts (growth medium, GM) and myotubes (differentiation medium, DM) evaluated by qRT–PCR. $n = 4$, mean ± SD. $^{**}P < 0.01$. $^{***}P < 0.001$ (unpaired two-tailed Welch's *t*-test). Preparations from two independent specimens are shown.

C Relative luciferase activities of the *GAPDH* promoters (positive and opposite directions) in C2C12 cells. Bars indicate the average of two independent experiments, and open circles represent the values of each experiment.

Figure EV3. Regulation of *myogenin* expression by *Myoparr*.

A Quantitative RT–PCR for *Myoparr* expression in differentiating C2C12 cells transfected with control or *Myoparr* anti-sense oligonucleotides (ASOs). Cells were transfected with 50 nM ASOs, and the levels of *Myoparr* expression were quantified by qRT–PCR 48 h after knockdowns. $n = 3$, mean ± SD. $^{*}P < 0.05$ (unpaired two-tailed Student's *t*-test).

B qRT–PCR showing decreased *myogenin* expression in differentiating C2C12 cells transfected with control or *Myoparr* ASOs. $n = 4$, mean ± SD. $^{*}P < 0.05$ (unpaired two-tailed Student's *t*-test).

C Decreased myogenin protein expression in differentiating C2C12 cells 48 h after *Myoparr* knockdown using ASOs. Expression of tubulin served as an internal control.

D ChIP-qPCR detection of Pol II occupancy and histone modification status at the *GAPDH* promoter in *Myoparr*-depleted differentiating C2C12 cells. The data were normalized to input values. $n = 3$, mean ± SD. n.s., not significant. Statistical analyses were performed using an unpaired two-tailed Student's *t*-test (Pol II and H3K4me3). In cases of unequal variances (H3K27ac), an unpaired two-tailed Welch's *t*-test was used.

E A schematic diagram of the CpG sites at the *myogenin* upstream region. Red lines indicate individual CpG sites. The methylation status at the −474/−18 region in *Myoparr*-depleted C2C12 cells was examined.

F The methylation status at the −474/−18 region is shown. C2C12 cells were transfected with 50 nM siRNAs. The methylation status was evaluated 1 and 3 days after differentiation induction. The day 0 sample is from non-transfected cells. The CpG sites are indicated by circles (black and white circles indicate methylated and unmethylated cytosine sites, respectively), and each row represents an individual clone.

G The methylation status of (F) is shown as a methylated/unmethylated ratio.

H, I Decreased MHC expression by *Myoparr* knockdowns using siRNAs (H) or ASOs (I) in C2C12 myotubes. Expression of tubulin served as an internal control.

J–L The expression changes of *MyoD1* (J), *Myf5* (K), and *MRF4* (L) quantified by qRT–PCR either in *Myoparr*- or *myogenin*-depleted differentiating C2C12 cells. $n = 3$, mean ± SD. $^{*}P < 0.05$. $^{**}P < 0.01$ (unpaired two-tailed Student's *t*-test).

M Decreased Pol II occupancy at the *MyoD1* promoter detected by ChIP-qPCR in *Myoparr*-depleted differentiating C2C12 cells. The data were normalized to input values. $n = 3$, mean ± SD. $^{*}P < 0.05$ (unpaired two-tailed Student's *t*-test).

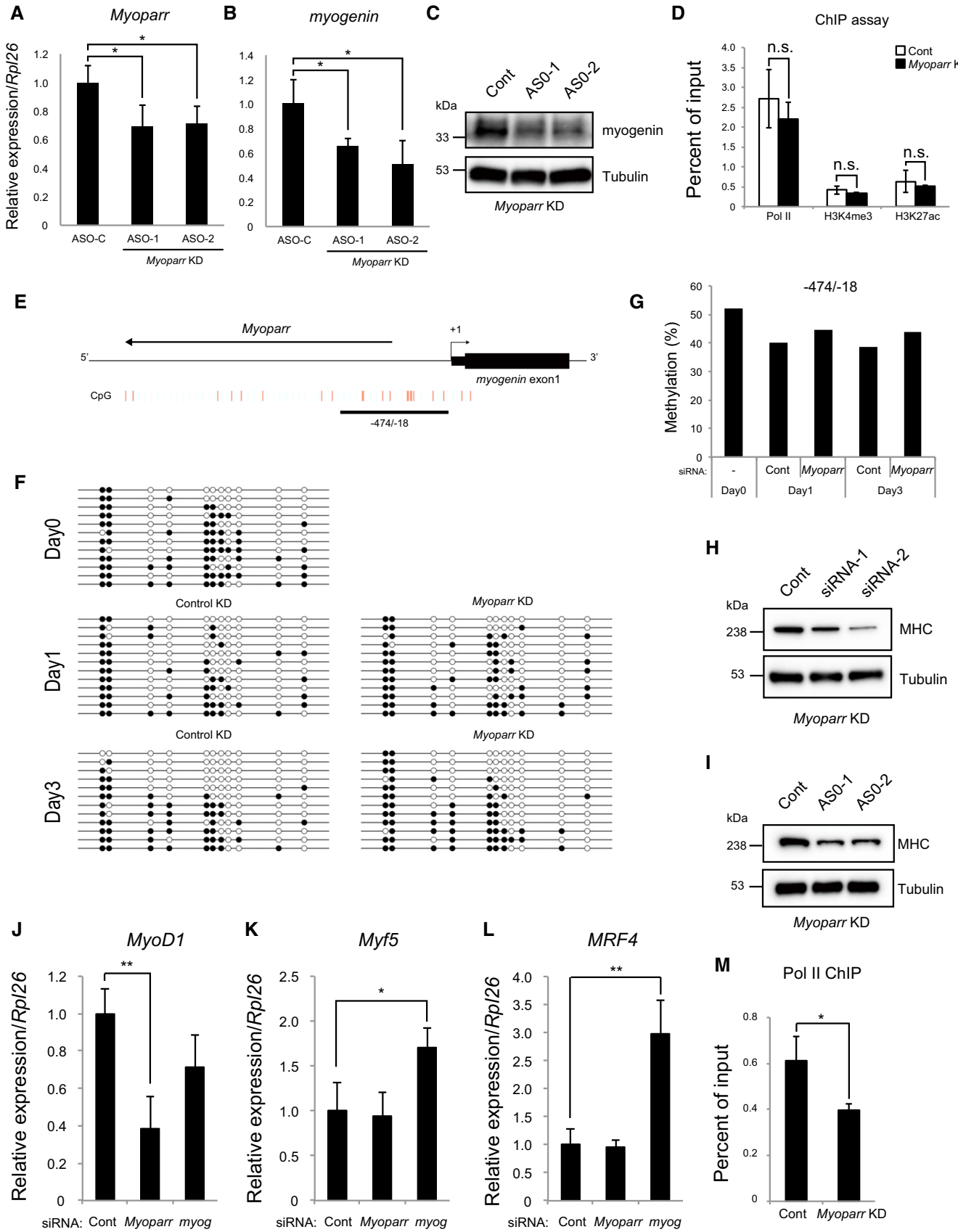

A
Myoparr
Relative expression/Rpl26
ASO-C
ASO-1
ASO-2
Myoparr KD
B
myogenin
C
Cont
ASO-1
ASO-2
kDa
33
53
myogenin
Tubulin
Myoparr KD
D
ChIP assay
Percent of input
Cont
Myoparr KD
n.s.
Pol II
H3K4me3
H3K27ac
E
Myoparr
+1
5'
3'
myogenin exon1
CpG
-474/-18
G
-474/-18
Methylation (%)
siRNA:
-
Cont
Myoparr
Day0
Day1
Day3
F
Day0
Day1
Day3
Control KD
Myoparr KD
H
Cont
siRNA-1
siRNA-2
kDa
238
53
MHC
Tubulin
Myoparr KD
I
Cont
ASO-1
ASO-2
kDa
238
53
MHC
Tubulin
Myoparr KD
J
MyoD1
Relative expression/Rpl26
siRNA: Cont Myoparr myog
K
Myf5
Relative expression/Rpl26
siRNA: Cont Myoparr myog
L
MRF4
Relative expression/Rpl26
siRNA: Cont Myoparr myog
M
Pol II ChIP
Percent of input
Cont
Myoparr KD

Figure EV3.

Figure EV4. *Myoparr* depletion prevents skeletal muscle atrophy.

A Three days after denervation, weights of innervated (−) and denervated (+) tibialis anterior (TA) muscles were measured. $n = 4$, mean ± SEM. $^{**}P < 0.01$ (unpaired two-tailed Student's *t*-test).

B Expression of *myogenin* in innervated and denervated TA muscles detected by qRT–PCR 3 days after denervation. $n = 4$, mean ± SD. $^{***}P < 0.001$ (unpaired two-tailed Welch's *t*-test).

C Quantitative RT–PCR showing increased *Myoparr* expression in denervated TA muscles 3 days after denervation. $n = 4$, mean ± SD. $^{***}P < 0.001$ (unpaired two-tailed Student's *t*-test).

D Evaluation of the inhibitory effect of *Myoparr* expression by *Myoparr* shRNAs in NIH3T3 cells. $n = 3$, mean ± SD. $^{**}P < 0.01$. $^{***}P < 0.001$ (unpaired two-tailed Welch's *t*-test). The results were normalized to *Rpl26* expression. Data are shown as percent of the control.

E *In vivo* inhibitory effect of *Myoparr* shRNA against the expression of *Myoparr* and *myogenin* in innervated TA muscles. $n = 4$, mean ± SD. $^{*}P < 0.05$ (unpaired two-tailed Student's *t*-test).

F, G Distributions of single myofiber areas in innervated (F) and denervated (G) TA muscles in the presence of control or *Myoparr* shRNA. All EmGFP-positive myofibers (innervated control shRNA; $n = 3{,}326$, innervated *Myoparr* shRNA; $n = 3{,}035$, denervated control shRNA; $n = 3{,}141$, denervated *Myoparr* shRNA; $n = 5{,}417$) were counted. The percentage of myofibers with indicated areas per total fibers were plotted. A Mann–Whitney nonparametric test was used for comparisons between each group (innervated control shRNA *vs.* innervated *Myoparr* shRNA, $P < 0.001$; denervated control shRNA *vs.* denervated *Myoparr* shRNA, $P < 0.001$). Data are shown as mean ± SEM.

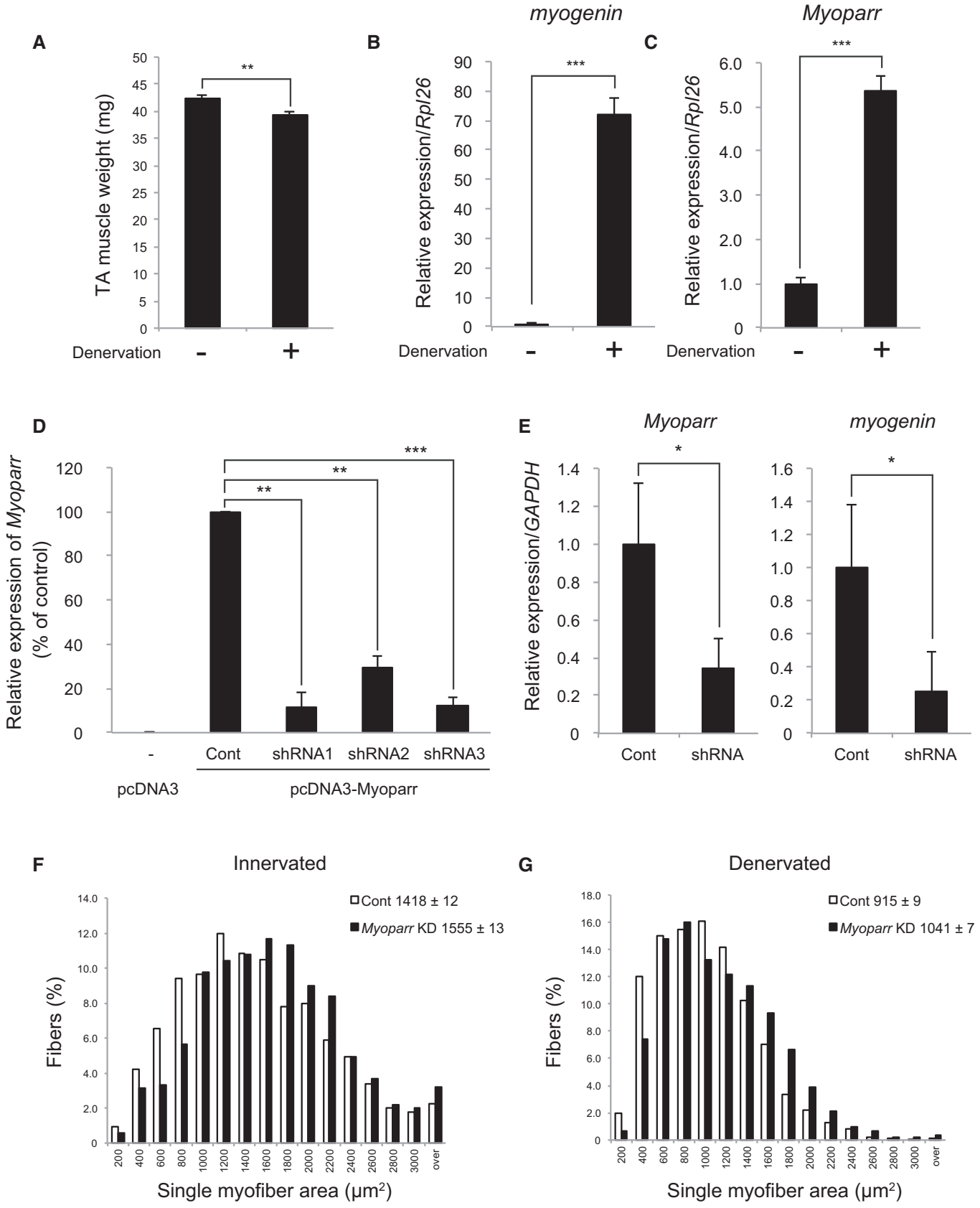

A
TA muscle weight (mg)
Denervation
B
myogenin
Relative expression/Rpl26
C
Myoparr
D
Relative expression of Myoparr (% of control)
Cont
shRNA1
shRNA2
shRNA3
pcDNA3
pcDNA3-Myoparr
E
Myoparr
myogenin
Relative expression/GAPDH
Cont
shRNA
F
Innervated
Cont 1418 ± 12
Myoparr KD 1555 ± 13
Fibers (%)
Single myofiber area (μm²)
G
Denervated
Cont 915 ± 9
Myoparr KD 1041 ± 7

Figure EV4.