Supplemental Figure Legends

Figure S1: PCR amplification control data. Purified BAC DNA was used as a template to examine amplification between the indicated primer and primers spanning the *Ckm* locus. Interaction frequencies were normalized to the Ct value of the corresponding ligated BAC amplicon.

Figure S2: Interactions between *Des* and *Acta1* promoters but not between *Myog* and either *Des* or *Acta1*. 3C experiments demonstrating inter-chromosomal interactions between the *Des* and *Acta1* promoters and the lack of detectable interactions between the *Myog* and *Acta1* or the *Myog* and *Des* promoters at the onset of C2C12 cell differentiation. The maximum interaction frequency value was normalized to 1 and all other values are shown relative to that value. Values represent the average plus/minus the standard deviation from three independent experiments.

Figure S3: Expression profiles of myogenic genes as a function of time of C2C12 cell differentiation. Quantitative-PCR (qPCR) was performed on RNA isolated from C2C12 cells proliferating in growth media or differentiated for the indicated times. The expression of the housekeeping gene *Eef1α1* is shown as a control. Expression levels in growth media were normalized to 1; all other values are relative to that value. The results represent the average plus/minus the standard deviation from three independent experiments.

Figure S4: siRNA against MyoD inhibited MyoD binding to target gene promoters. C2C12 cells transfected with a control siRNA or siRNA against MyoD were differentiated and harvested for ChIP experiments. MyoD binding to the indicated promoter sequences is shown ChIP with IgG is shown as a control. The binding observed in control siRNAsample was normalized to 1; all other values are relative to that value. The results represent the average plus/minus the standard deviation from three independent experiments.

Figure S5: MyoD, but not Brg1, binds to the *Acta1* promoter at the onset of differentiation. ChIP assays assessing binding of Brg1 and MyoD to the *Acta1* promoter binds to both enhancer and promoter sequences in a Brg1 independent manner at the onset of differentiation. Data in (A-B) represent the average plus/minus the standard deviation from three independent experiments. For each data set, the value of the mock-differentiated, plus tet sample was set at 1.0. M; mock-differentiated, where cells were infected with an empty, instead of a MyoD encoding, retrovirus.

Table S1: 3C Primer and BAC information

Table S2: qPCR primer list

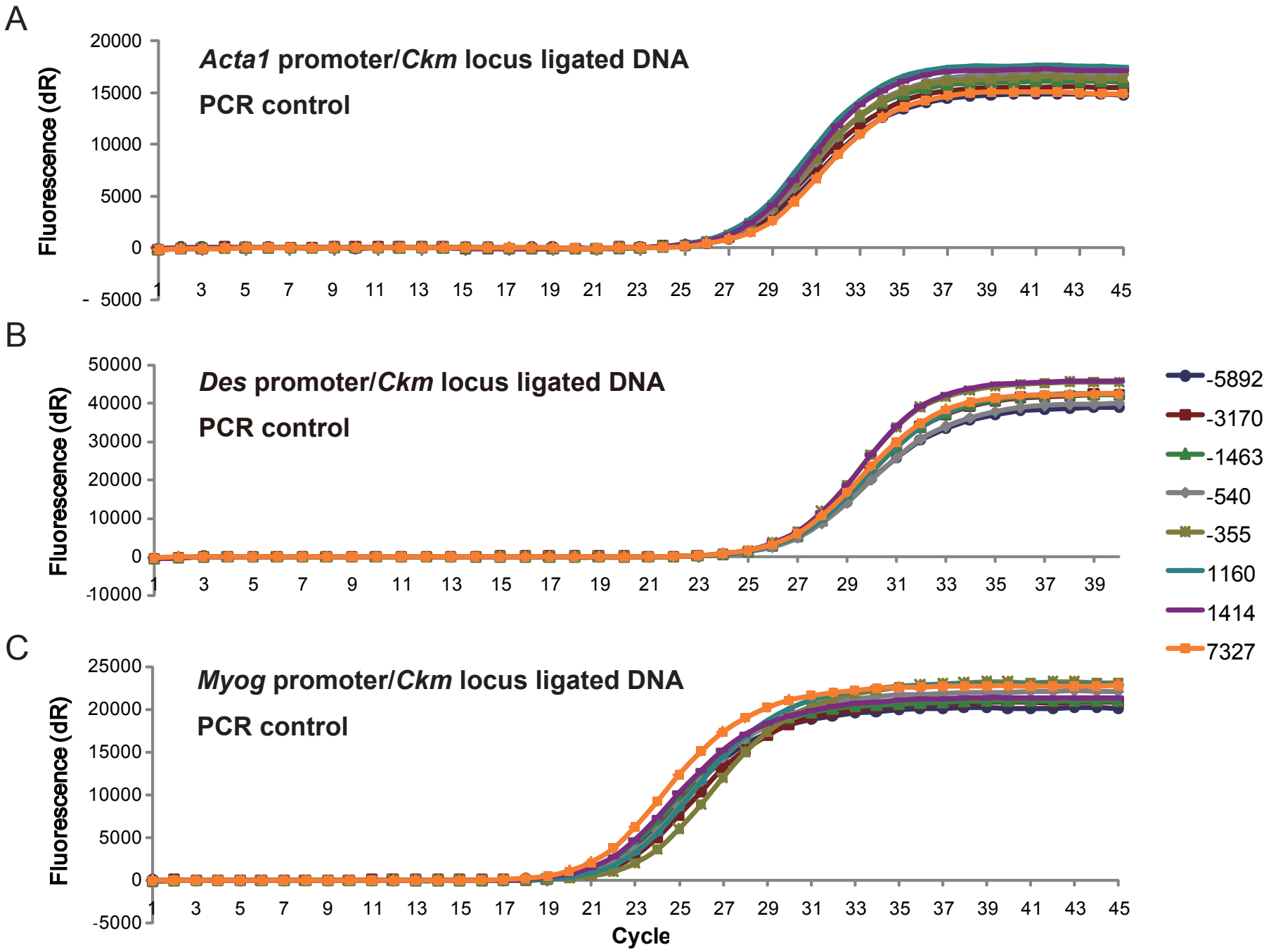
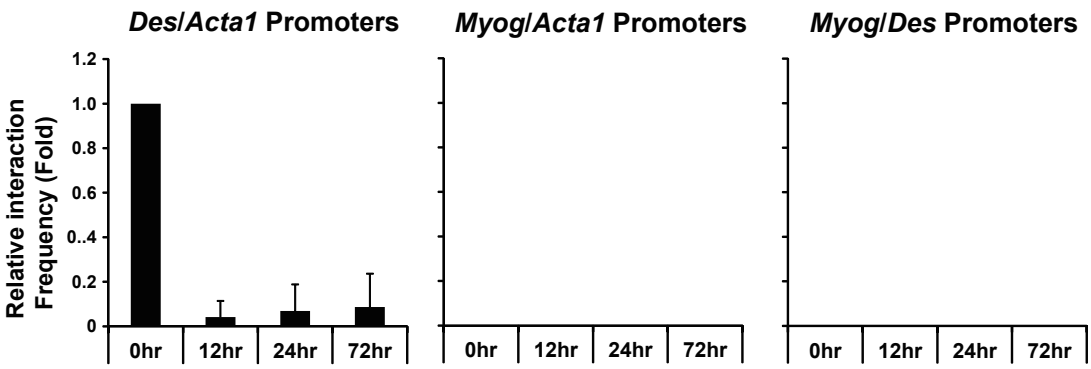

A
Acta1 promoter/Ckm locus ligated DNA
PCR control
Fluorescence (dR)
20000
15000
10000
5000
0
- 5000
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45
B
Des promoter/Ckm locus ligated DNA
PCR control
Fluorescence (dR)
50000
40000
30000
20000
10000
0
-10000
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39
-5892
-3170
-1463
-540
-355
1160
1414
7327
C
Myog promoter/Ckm locus ligated DNA
PCR control
Fluorescence (dR)
25000
20000
15000
10000
5000
0
-5000
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45
Cycle

Figure S1


Des/*Acta1* Promoters
Myog/*Acta1* Promoters
Myog/*Des* Promoters
Relative interaction Frequency (Fold)
1.2
1.0
0.8
0.6
0..4
0.2
0
0hr
12hr
24hr
72hr

FigureS2

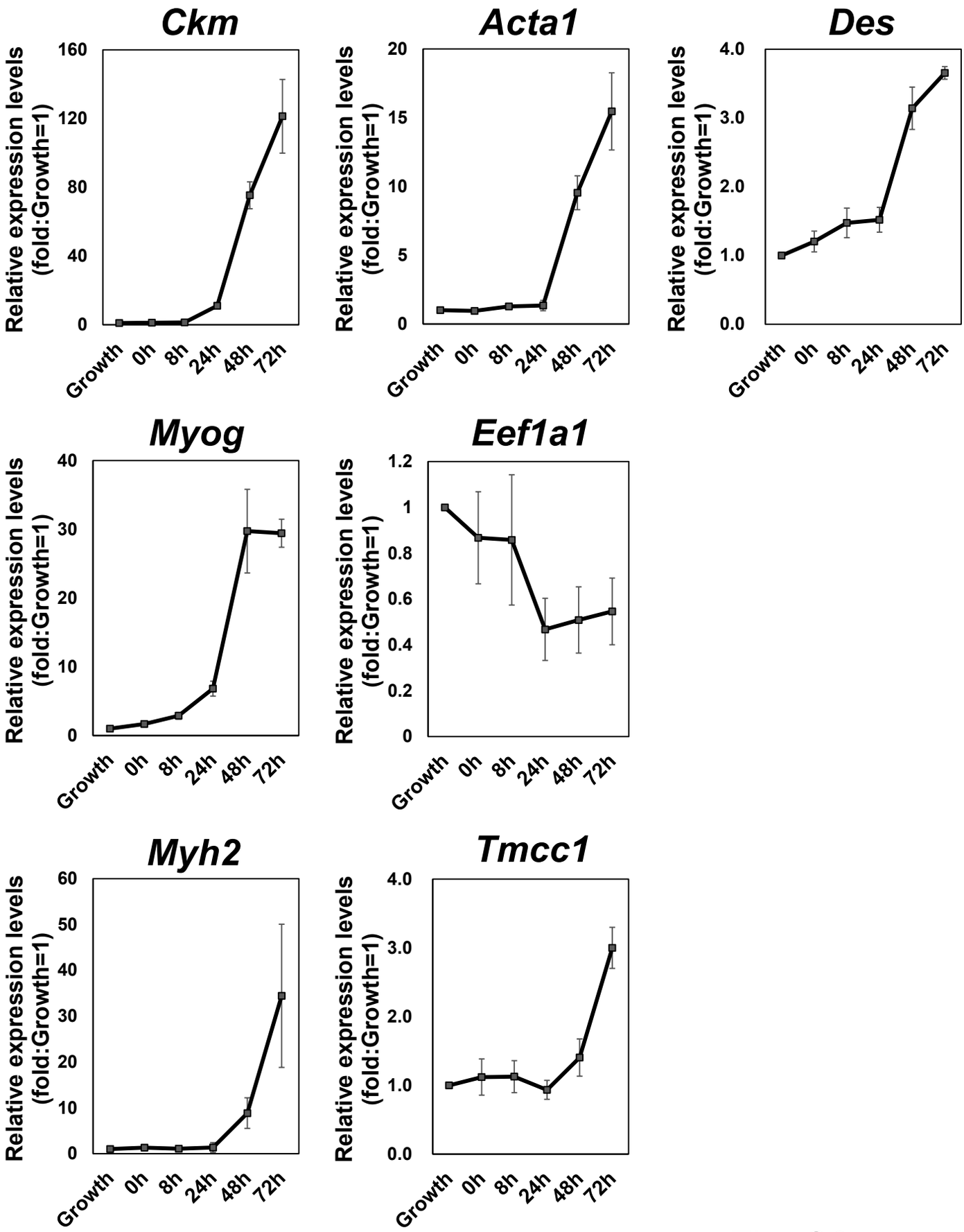

Ckm
Acta1
Des
Myog
Eef1a1
Myh2
Tmcc1
Relative expression levels (fold:Growth=1)
Growth
0h
8h
24h
48h
72h

Fig. S3

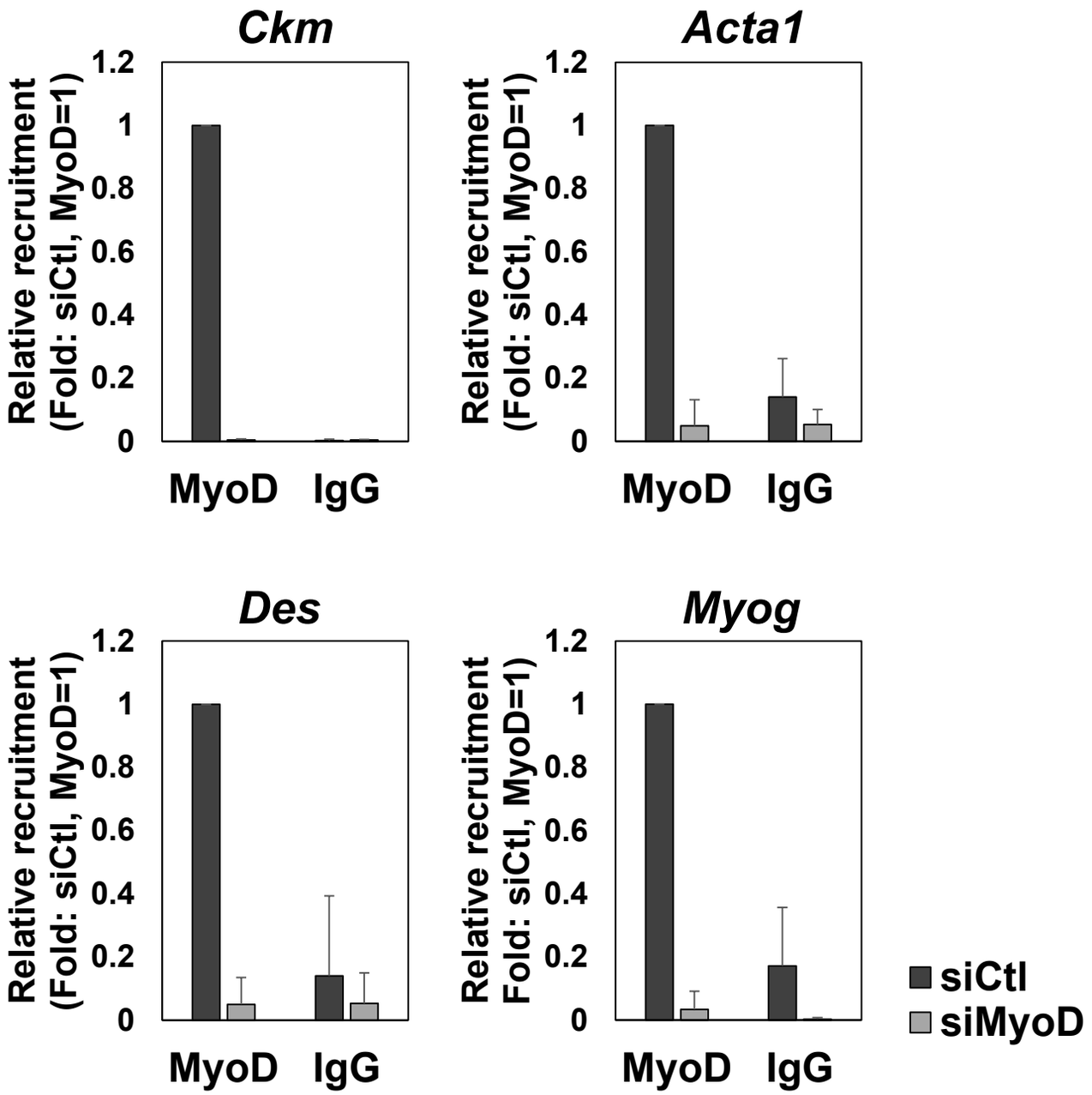

Ckm
Acta1
Des
Myog
Relative recruitment
(Fold: siCtl, MyoD=1)
1.2
1
0.8
0.6
0.4
0.2
0
MyoD
IgG
siCtl
siMyoD

Fig. S4

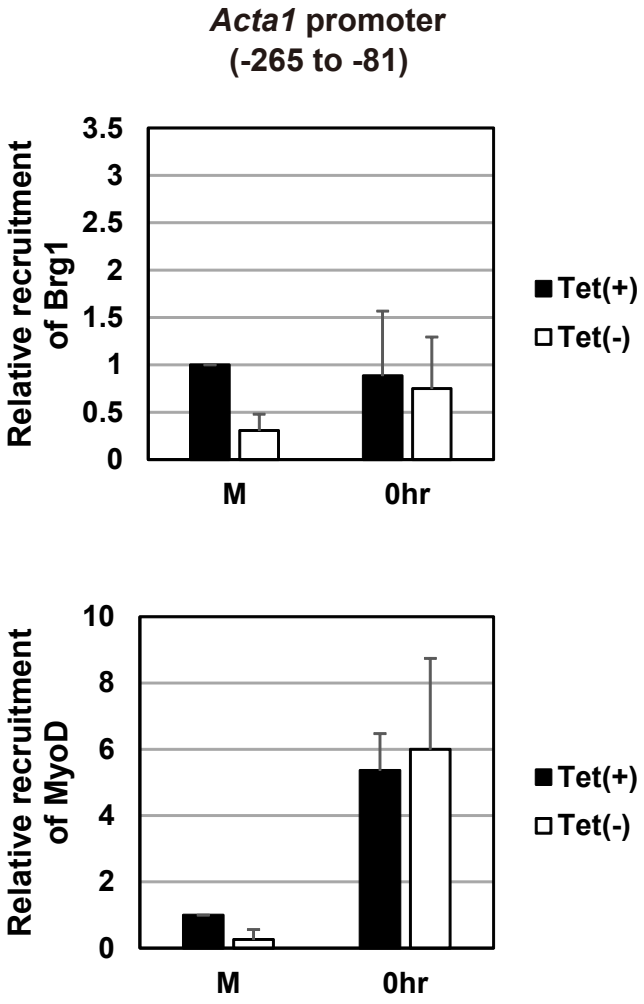

Acta1 promoter
(-265 to -81)
Relative recruitment of Brg1
3.5
3
2.5
2
1.5
1
0.5
0
M
0hr
Tet(+)
Tet(-)
Relative recruitment of MyoD
10
8
6
4
2
0
M
0hr
Tet(+)
Tet(-)

Fig. S5

3C primers

myog

-4918	TGCAATGTCACAGAGGTCTAAGCTCAAGTC
-4479	TCCGCTCCGAAGCCCATGAGACTCCAGAGT
-465	GGGTCTCATGGGACTGACATAGTATGGTTT
3365	CGTACTGCTTGTCTGGTTTCCTGGAGATTA
5589	TGTTCTTTACTGGCTTGCCTCCCCTGGCTT
7190	CAAAGCTAGCCCCAGAGTCCCTAGAAGGAG
rev	TTTTCATTTCTCCACAGCCCCTGTGGGGCA

ckm

-5892	TGCCCTCCTTTTCTGAGGCAGGGTCTCACT
-3170	TCTGACCCTCAGTGGCTCCCATGAACTCCT
-1463	AGGCAGAAGAGGAAACTTCCACAGTGCATC
-540	CATGTCTGAGGCCAGCCTGGACTACATAGG
- 355	GGAGGGTGCTGGCTACAATCAAGGCTGTGG
1160	CCCCATGGTCAGTGCTTCAGGGATCTAGTC
1414	AATGTGCACACCTGTGCACATACATGAGCC
7327	CAGATGGAGAGAATCTGCCTGGCATGCATA
rev	TGAGAGTAGATGAGCTTTCAGCTCGTTGCC

tmcc1

-8019	TACAGCACCTCCTTTGTCCCCACTCCTATC
-3204	AAAACAGCGCTAAAAGGAGCTCATGGGTTG
-473	CACGTATCCTCAGCCCCTTTCTCTGCTTTT
120	CCAGTCCTACTCACCGGTGGAGAGACGACG
3901	AAGTTGAATGGCAACACAATGGCAATGCTC
8412	GCTGTGGCCGTTTACTACTGAGTTTGTCCT
15180	GCAGGAGGTGTGTTAACTCCTTGAGCACCA
104484	TGAGGGTCTGATGAGCTATTGCATTGAACA
rev	CGTTCTCGTTCTAGCTGTCGTTCCCTCGCG

des

rev	AGCCCCTTAGCTGTCTGCACTATTTGTAGC

acta1

rev	CTGTGACCAAACAGGCGACATGCTTTGAGG

myh2

rev	TATATGGCTAGCTATGGAAACAATGCGACT

eef1a1

rev	CGGCCAGAATCTATCCCTACTCTTCCTTAC

BAC clones

Desmin	RP24-335G4
MCK	RP23-457C1
Tmcc1	RP24-188A4
Myh2	RP24-230G5
Acta 1	RP24-271N5
Myog	RP24-343C14

Table S1

Table S2. Quantitative RT-PCR primer list

Acta1:
5’-TTGTGCACCGCAAATGCTTCTAGG-3’
5’-ATGTACACGTCAAAAACAGGCGCC-3’

Ckm:
5’-AAGTCCAATCATTGGGCTCTGTCC-3’
5’-ACGGACTTTTATTTAAGGCAGGGC-3’

Eef1a1:
5’-CTCTGACTACCCTCCACTTGGTCG-3’
5’-ATTAAGACTGGGGTGGCAGGTGTT-3’

Des:
5’-TTTCTCCACTCACAGGCTCTGACC-3’
5’-GAGCTGGGTTCTCTCTTAAGAGCC-3’

Myog:
5’-AAAGCCATCACTTCTGTAGCAGGG-3’
5’-TCTCTGGACTCCATCTTTCTCTCC-3’

Myh2:
5’-AAGTGACTGTGAAAACAGAAGCA-3’
5’-GCAGCCATTTGTAAGGGTTGAC-3’

Tmcc1:
5’-GATCTTAAGCTGCTAATTGTCGAG-3’
5’--CAGGTTTCTAGCTGAGGTCTTTAT3’