Figure S1.

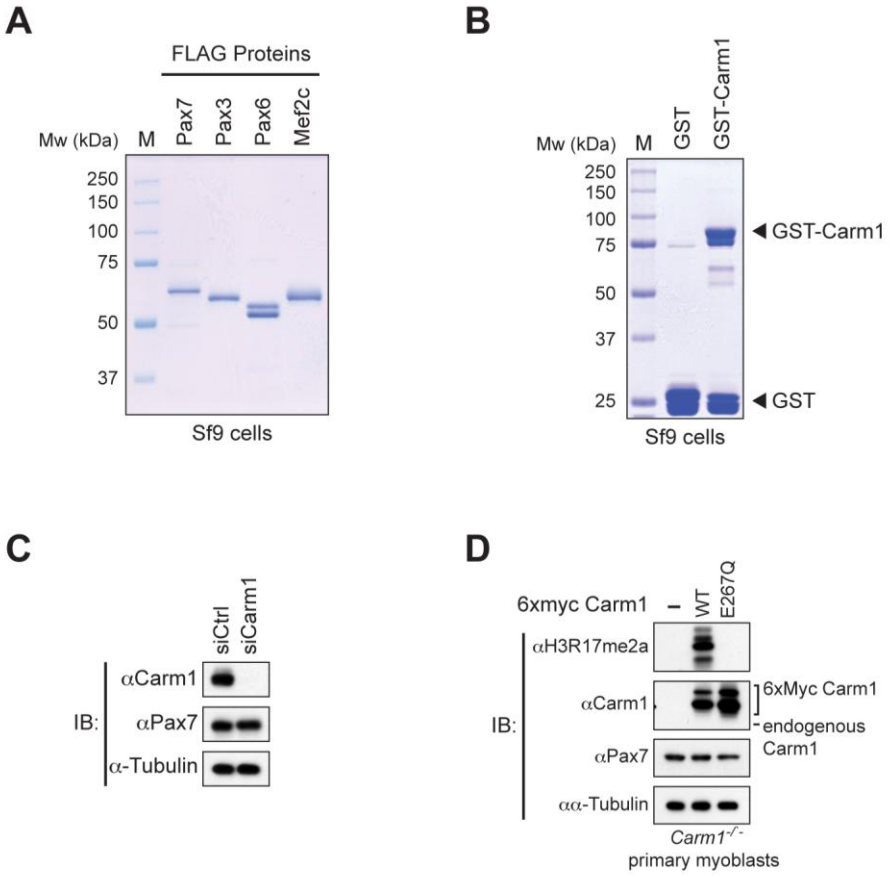

A
FLAG Proteins
Pax7
Pax3
Pax6
Mef2c
Mw (kDa)
M
250
150
100
75
50
37
Sf9 cells
B
GST
GST-Carm1
Mw (kDa)
M
250
150
100
75
50
37
25
GST-Carm1
GST
Sf9 cells
C
siCtrl
siCarm1
IB:
αCarm1
αPax7
α-Tubulin
D
6xmyc Carm1
–
WT
E267Q
IB:
αH3R17me2a
αCarm1
αPax7
αα-Tubulin
6xMyc Carm1
endogenous Carm1
Carm1-/-
primary myoblasts

Figure S2.

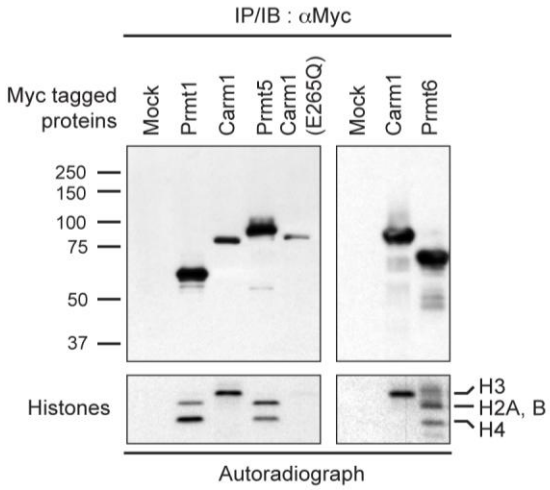

IP/IB : αMyc
Myc tagged proteins
Mock
Prmt1
Carm1
Prmt5
Carm1 (E265Q)
Mock
Carm1
Prmt6
250
150
100
75
50
37
Histones
H3
H2A, B
H4
Autoradiograph

Figure S3.

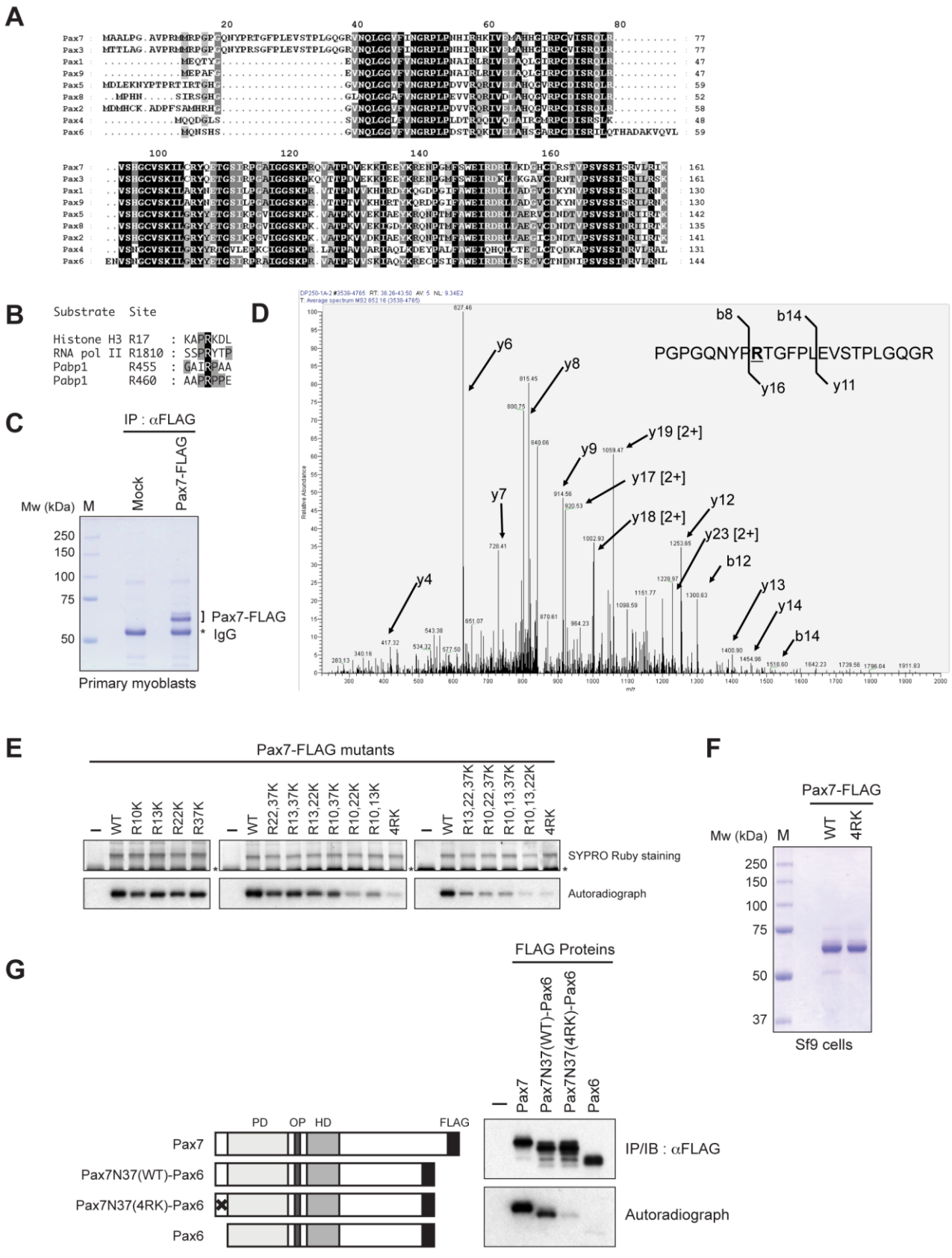

A
Pax7
Pax3
Pax1
Pax9
Pax5
Pax8
Pax2
Pax4
Pax6
B
Substrate Site
Histone H3 R17 : KAPRKDL
RNA pol II R1810 : SSPRYTP
Pabp1 R455 : GAIRPAA
Pabp1 R460 : AAPRPPE
C
IP : αFLAG
Mock
Pax7-FLAG
Mw (kDa) M
250
150
100
75
50
Pax7-FLAG
IgG
Primary myoblasts
D
PGPGQNYPRTGFPLEVSTPLGQGR
b8
b14
y16
y11
y6
y8
y19 [2+]
y9
y17 [2+]
y7
y12
y18 [2+]
y23 [2+]
b12
y4
y13
y14
b14
Relative Abundance
m/z
E
Pax7-FLAG mutants
–
WT
R10K
R13K
R22K
R37K
R22,37K
R13,37K
R13,22K
R10,37K
R10,22K
R10,13K
4RK
R13,22,37K
R10,22,37K
R10,13,37K
R10,13,22K
SYPRO Ruby staining
Autoradiograph
F
Pax7-FLAG
Mw (kDa) M
WT
4RK
250
150
100
75
50
37
Sf9 cells
G
FLAG Proteins
–
Pax7
Pax7N37(WT)-Pax6
Pax7N37(4RK)-Pax6
Pax6
PD
OP
HD
FLAG
Pax7
Pax7N37(WT)-Pax6
Pax7N37(4RK)-Pax6
Pax6
IP/IB : αFLAG
Autoradiograph

Figure S4.

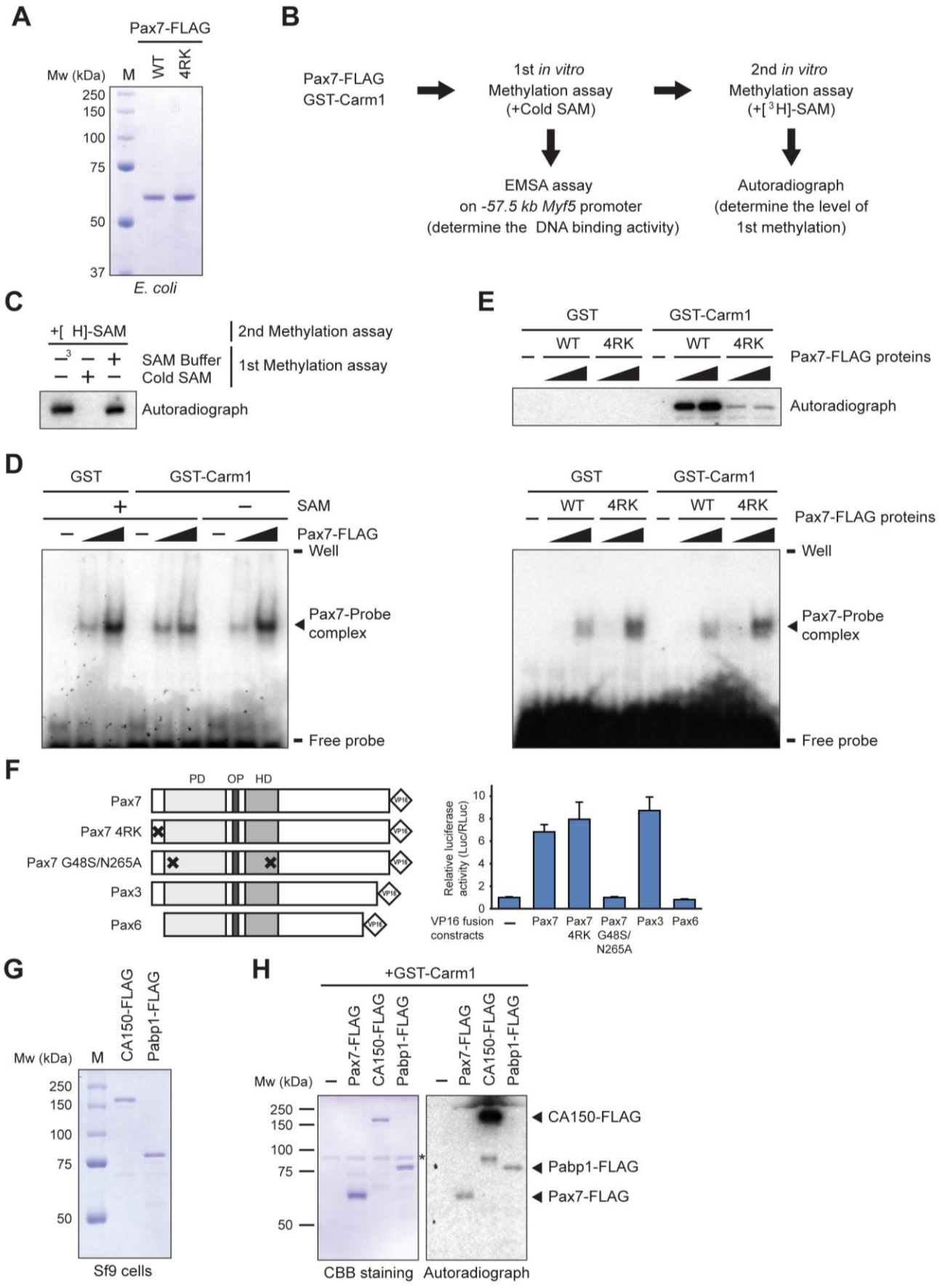

A
Pax7-FLAG
WT
4RK
Mw (kDa)
M
250
150
100
75
50
37
E. coli
B
Pax7-FLAG
GST-Carm1
1st in vitro
Methylation assay
(+Cold SAM)
2nd in vitro
Methylation assay
(+[3H]-SAM)
EMSA assay
on -57.5 kb Myf5 promoter
(determine the DNA binding activity)
Autoradiograph
(determine the level of
1st methylation)
C
+[H]-SAM
3
SAM Buffer
Cold SAM
2nd Methylation assay
1st Methylation assay
Autoradiograph
E
GST
GST-Carm1
WT
4RK
Pax7-FLAG proteins
Autoradiograph
D
GST
GST-Carm1
SAM
Pax7-FLAG
Well
Pax7-Probe
complex
Free probe
F
PD
OP
HD
Pax7
Pax7 4RK
Pax7 G48S/N265A
Pax3
Pax6
VP16
Relative luciferase
activity (Luc/RLuc)
VP16 fusion
constracts
Pax7
Pax7
4RK
Pax7
G48S/
N265A
Pax3
Pax6
G
CA150-FLAG
Pabp1-FLAG
Mw (kDa)
M
Sf9 cells
H
+GST-Carm1
Pax7-FLAG
CA150-FLAG
Pabp1-FLAG
CA150-FLAG
Pabp1-FLAG
Pax7-FLAG
CBB staining
Autoradiograph

Figure S5.

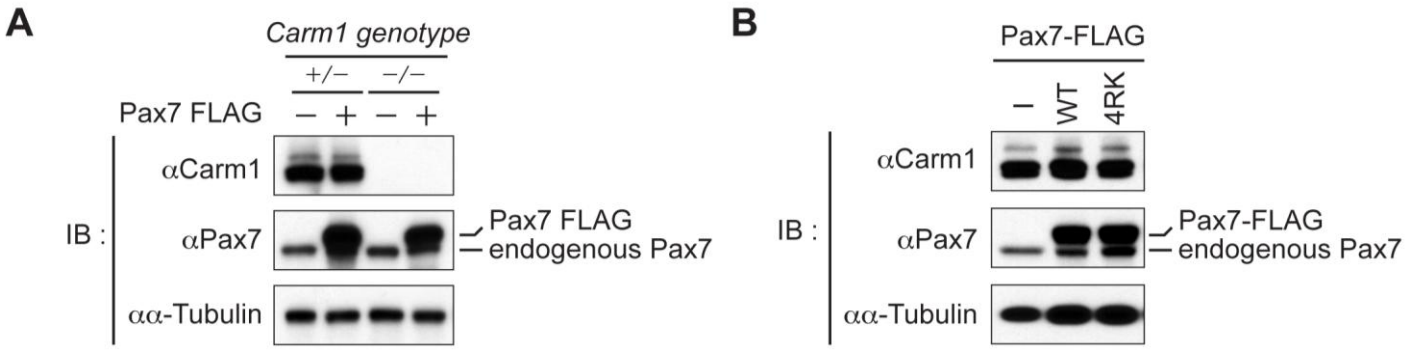
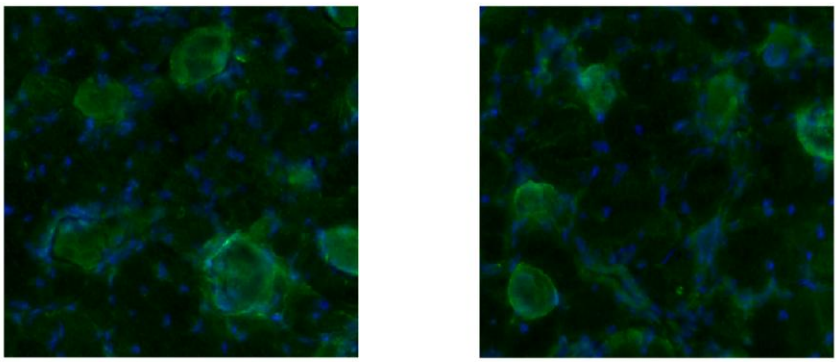
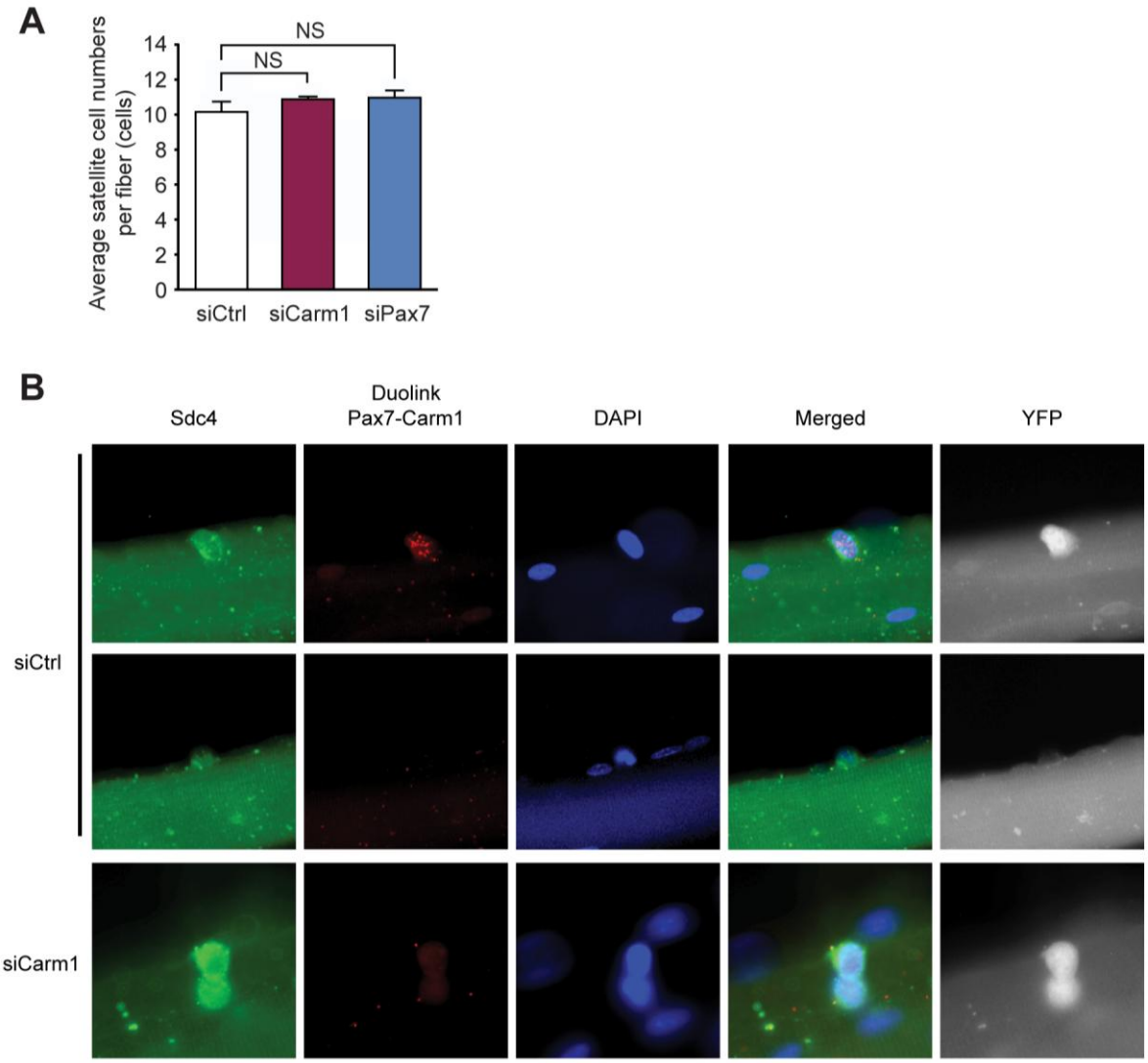

A
Carm1 genotype
+/−
−/−
Pax7 FLAG
− + − +
IB :
αCarm1
αPax7
αα-Tubulin
Pax7 FLAG
endogenous Pax7
B
Pax7-FLAG
−
WT
4RK
IB :
αCarm1
αPax7
αα-Tubulin
Pax7-FLAG
endogenous Pax7

Figure S6.



Carm1$^{+/-}$ Carm1$^{-/-}$

Figure S7.


A
Average satellite cell numbers per fiber (cells)
14
12
10
8
6
4
2
0
NS
NS
siCtrl
siCarm1
siPax7
B
Sdc4
Duolink Pax7-Carm1
DAPI
Merged
YFP
siCtrl
siCarm1

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. CBB staining of purified proteins used in *in vitro* binding assay and *in vitro* methylation assay, related to Figure 1.

(A) CBB staining of C-terminally tagged recombinant Pax7, Pax3, Pax6 and Mef2c proteins used in Figure 1B (right). Proteins were expressed in Sf9 cells and purified by anti-FLAG M2 agarose.

(B) CBB staining of recombinant GST and GST tagged Carm1 protein used in Figures 1B, 1C, 2D, 3A, 3B, 4A, 4B, 4C, and 4E and Supplementary Figures S4B, S4D, S4E, S4I, and S9. Proteins were expressed in Sf9 cells and purified by Glutathione sepharose.

(C) Western blot analysis from control (siCtrl) and Carm1 (siCarm1) siRNA transfected primary myoblasts.

(D) Western blot analysis from mock, 6xMyc-Carm1 WT and E267Q mutant over-expressing *Carm1*$^{-/-}$ primary myoblasts.

Figure S2. Histone methyltransferase activity of immuno-purified Prmts from Prmt over-expressing HEK293T cells, related to Figure 2.

6xMyc-Prmts were ectopically expressed in HEK293T cells and purified by anti-Myc antibody. The immunopurified 6xMyc-Prmts were incubated with core histones in the presence of S-adenocyl [^{3}H]-AdoMet. Proteins were resolved by SDS-PAGE. 6xMyc-Prmts were detected by western blot analysis using anti-Myc antibody (top) and histone methyltransferase activity was detected by autoradiography (bottom).

Figure S3. Identification of specific arginines in the N-terminal 37 a.a. region of Pax7 that is the major target of Carm1 methylation, related to Figure 3.

(A) Amino acid sequence alignment of N-terminal paired domains between Pax1-9 in mouse.

(B) Comparison of arginines methylated by Carm1 in known substrates (Histone H3, RNA polII CTD, Pabp1). Conserved arginine residues are highlighted in black and proline glycine methionine rich (PGM) motifs are highlighted in gray.

(C) Purification of Pax7-FLAG ectopically expressed in primary myoblasts by using anti-FLAG M2 agarose. The purified Pax7 protein was resolved by SDS-PAGE and stained by CBB. The band correspond to Pax7 was excised and analyzed by LC-MS/MS spectrometry.

(D) MS/MS spectrum of a di-methylated peptide at arginine 22 in Pax7. Representative MS/MS spectrum of the arginine dimethylated peptide PGPGQNYP**R**(2me)TGFPLEVSTPLGQGR from mouse Pax7 was interpreted. Fragment assignments were determined using Mascot software. 39 fragment ions were assigned using the 76 most intense peaks. For clarity, only selected high-intensity peaks are labeled.

(E) Identification of Carm1 methylation sites in Pax7 by *in vitro* methylation assay. Indicated C-terminal FLAG tagged Pax7 mutants were ectopically expressed in HEK293T cells and purified by anti-FLAG M2 agarose. Methylation of these mutants was analyzed by *in vitro* methylation assay using purified GST-Carm1 with S-adenocyl [^{3}H]-AdoMet and detected by autoradiography (bottom). FLAG tagged proteins were detected by SYPRO Ruby (Sigma) staining (top).

(F) CBB staining of C-terminally tagged recombinant Pax7 WT and 4RK mutant protein used in Figure 3C. Proteins were expressed in Sf9 cells and purified by anti-FLAG M2 agarose.

(G) Arginine residues in N-terminal 37 a.a. portion of Pax7 is the major target for Carm1 methylation. C-terminal FLAG tagged full-length Pax7, Pax6 and N-terminal 37 a.a. Pax7 fused

with Pax6 constructs (left) were ectopically expressed in HEK293T cells and purified by anti-FLAG M2 agarose. Methylation of these proteins were analyzed by *in vitro* methylation assay using purified GST-Carm1 and detected by autoradiography (right bottom). FLAG tagged proteins were detected by western blot analysis using anti-FLAG antibody (right top).

Figure S4. DNA binding activity of methylated or unmethylated Pax family proteins and *in vitro* methylation assay for PGM motif containing proteins, related to Figure 4.

(A) CBB staining of C-terminally tagged recombinant Pax7 WT and 4RK mutant protein used in Figures S4B, S4C, S4D, and S4E. Proteins were expressed in *E. coli* and purified by anti-FLAG M2 agarose.

(B) Schematic presentation of EMSA assay for methylated or unmethylated Pax7 and 4RK mutant purified from *E. coli.*

(C) Detection of Pax7 methylation state of *in vitro* methylated Pax7 WT with non-radioactive S-Adenocyl Methionine (SAM) by Carm1. *In vitro* methylated Pax7 by Carm1 with non-radioactive SAM (1st methylation assay) was used for a 2nd second methylation assay with S-adenocyl [^{3}H]-AdoMet.

(D) DNA binding activity of *in vitro* methylated Pax7 WT or unmethylated Pax7 was detected by EMSA assay using oligonucleotide of *-57.5 kb Myf5* promoter as the probe.

(E) DNA binding activity of *in vitro* methylated Pax7 WT and 4RK mutant or unmethylated Pax7 WT or 4RK mutant was detected by EMSA assay using oligonucleotide of *-57.5 kb Myf5* promoter as the probe (bottom). Methylation of Pax7 WT and 4RK mutant by GST-Carm1 was detected by *in vitro* methylation assay (top).

(F) DNA binding activity of VP16 fused constructs (left) was analyzed with a one-hybrid assay using luciferase reporter containing Pax7/3 binding sequence of -*57.5 kb Myf5* promoter. Relative luciferase activities are indicated in the right panel.

(G) CBB staining of C-terminally tagged recombinant CA150 and Pabp1 proteins used in Figure 3E and Figure S9. Proteins were expressed in Sf9 cells and purified by anti-FLAG M2 agarose.

(H)Methylation of purified PGM motif-containing proteins (left) was analyzed by *in vitro* methylation assay using purified GST-Carm1 with S-adenocyl [^{3}H]-AdoMet and detected by autoradiography (right).

Figure S5. Over expression of effector proteins in WT and Carm1 mutant myoblasts, related to Figure 5.

(A) Western blot analysis of transfected effectors in Carm1+/- and Carm1-/- myoblasts.

(B) Western blot analysis of effector proteins in Pax7 WT or 4RK mutant over-expressing primary myoblasts.

Figure S6. TA muscle cross-sections of *Pax7-creERT2:Carm1$^{floxed/+}$:R26R-eYFP* and *Pax7-creERT2:Carm1$^{floxed/floxed}$:R26R-eYFP* mice after Carm1 inactivation at P14 and injury at P21, related to Figure 6.

Immunohistochemistry staining against YFP on frozen sections identifies myofiber that are the progeny of Carm1+/-YFP+ and Carm1-/-YFP+ satellite cells.

Figure S7. Functional role of Carm1 in adult satellite cells, related to Figure 7.

(A) Total satellite cell numbers do not change following knock down. (n=4 mice, >160 fibers, error bars represent s.e.m., *P*-values are indicated in individual analysis, NS, not significant)

(B) Proximity ligation assay performed on satellite cells in single myofiber cultures transfected with siCtrl or siCarm1. Note, Duolink signal is completely abolished in siCarm1 conditions.

SUPPLEMENTAL EXPERIMENTAL PROCEDURE

Materials, antibodies and plasmid constructions

Commercially available antibodies against Pax7 (DSHB), MyoG (DSHB), MyHC (DSHB), Carm1 (Bethyl), Carm1 IHC (Bethyl), Wdr5 (Bethyl), Mll1 N-terminal (Mll1,Bethyl), Ash2l (Bethyl), Rbbp5 (Bethyl), Myc (Rabbit; Bethyl), Histone H3 (Abcam), Histone H3 di-methyl R17 (Abcam), GFP (Chicken; Abcam), CD34 (eBiosciences), Histone H3K4^{me3} (Millipore), GST (Millipore), Rabbit control IgG (Millipore), Mouse control IgG (Millipore), FLAG M2 (Sigma), FLAG (Rabbit; Sigma), and α-Tubulin (Sigma) were purchased from individual vendors. Retrovirus expression vector for C-terminal 3xFLAG tagged Pax7 and Pax3 (pBRIT-Pax7 and Pax3) and cDNA for Wdr5 and ASH2L were previously described (McKinnell et al., 2008). cDNA for Pax6, Carm1, Prmt1, Prmt5 and Prmt6 were obtained from Open Biosystems. cDNA for RBBP5 (a gift from Dr. Kai Ge, National Institute of Health; Addgene plasmid 15550) (Cho et al., 2007) and MLL2/TRX2 (a gift from Dr. Meyersin Matthew, Harvard Medical school; Addgene plasmid 11017) (Liu et al., 1995) were obtained from Addgene. cDNA for MLL1 was a generous gift from Dr. Hess L Jay (University of Pennsylvania) (Hughes et al., 2004). cDNAs for SMN-Tdr and TDRD3-Tdr were generous gift from Dr. Jocelyn Cote (University of Ottawa) (Cote et al., 2005). The full-length cDNAs for CA150 and Pabp1 have been described previously (Cheng et al., 2007; Lee et al., 2002). For generation of all expression vectors, cDNA fragments were PCR amplified using appropriate primers with restriction digestion sites and tags

(C-terminal 3xFLAG tag, N-terminal 6xMyc tag, N-terminal GST tag and N-terminal or C-terminal VP16 transcriptional activation domain sequence), restriction enzyme digested and inserted into the appropriate vectors (pBRIT, pHAN, pcDNA3 (Invitrogen), pFastBac1 (Gibco-BRL) and pET23d (Stratagene)) (McKinnell et al., 2008). Pax7, Pax3 and Pax6 C-terminal 3xFLAG tagged deletion mutants were generated by PCR site-directed mutagenesis and sub-cloned into pBRIT vector. To generate the chimeric Pax6 protein containing the N-terminal 37 amino acids of Pax7, N-terminal sequences from Pax7 WT or 4RK mutant and full length Pax6 were PCR amplified using appropriate primers that have restriction enzyme digestion sites or 5' phosphorylation and were sub-cloned into pBRIT vector. A series of substitution mutants were generated by PCR site-directed mutagenesis. For luciferase reporter plasmid DNA carrying mouse *Myf5 -57.5 kb* enhancer region, two annealed complementary oligonucleotides of the *Myf5* enhancer region and *Myf5* minimal promoter containing restriction enzyme sites were subcloned into pGL4.10 vector (Promega). All PCR amplifications were performed using Phusion DNA polymerase (New England Biolabs.). Detailed strategies for plasmid DNA construction can be requested to the corresponding author (M.A.R.). FLAG tagged Mef2c protein was a kind gift from Dr. F Jeffrey Dilworth (Ottawa Hospital Research Institute) (Rampalli et al., 2007). Mouse Pax7, Carm1 and control siRNA were purchased from Dharmacon and mixture of two effective sequences showed same trend in preliminary experiments was used for siRNA knockdown (Invitrogen). (Pax7; siPax7-1 5'-CCAAGAUUCUGUGCCGAUA-3', siPax7-2 5'-UGACCAAUGUACACCGAUU-3', Carm1; siCarm1-1 5'-GCUACAUGCUCUUCAAUGA-3', siCarm1-2 5'-GGAAGCACCUAUAAUCUCA-3', Control; ON-TARGET*plus* Non-targeting siRNA #1)

Cell culture, transfection and retrovirus production and infection

Primary myoblasts were isolated from 3 week-old BalbC or SV129 mice and cultured in Ham F10 (WISENT Inc.) supplemented with 20 % fetal bovine serum (FBS, Thermo scientific), Fibroblast growth factor basic (Millipore) and penicillin and streptomycin (P/S, WISENT Inc.) (Kuang et al., 2007). HEK293T cells, Phoenix-Eco cells (a gift from Dr. Garry Nolan, Stanford university) and C3H10T1/2 cells were cultured in DMEM (WISENT Inc.) supplemented with 10 % FBS, P/S. Sf9 cells were cultured in Sf900 III medium (Invitrogen) with P/S. Primary myoblasts and C3H10T1/2 cells were transfected with indicated plasmid DNA using Lipofectamine 2000 (Invitrogen) and with indicated siRNA (20 nM) using Lipofectamine RNAiMAX (Invitrogen). HEK293T and Phoenix-Eco cells were transfected with plasmid DNA using GeneJuice (Stratagene) for transient expression and retrovirus production. Retrovirus infection of primary myoblasts was performed according to manufacturer's protocol and puromycin resistant cells were selected by treatment with puromycin (Sigma) or GFP expressing cells sorted by MoFlo cytometer (DakoCytomation).

FACS isolation of quiescent satellite cells

YFP+ satellite cells were sorted from *Pax7-CreERT2:Carm1*$^{+/flox}$*:R26R-eYFP* heterozygous and *Pax7-CreERT2:Carm1*^{floxflox}*:R26R-eYFP* homozygous mice that were Tamoxifen injected at P14. Hindlimb muscles were dissected, minced and digested in Collagenase/Dispase solution. Mononuclear cells were isolated by filtration through a 50um nylon filter (Nynex) and YFP expressing cells were sorted by MoFlo cytometer (DakoCytomation). Cells were spun down, lysed and RNA was extracted using the Picopure RNA isolation kit (Applied Biosystems).

Mouse lines and injury model

Myf5-Cre/ROSA26-YFP mouse was previously described[5]. *Pax7-CreERT2* homozygote mouse and *Carm1*$^{flox/flox}$ mouse were crossed to obtain *Pax7-CreERT2/Carm1*$^{+/flox}$ mouse (Yadav et al.,

2003; Nishijo et al, 2009). *Pax7-CreERT2:Carm1*$^{+/flox}$ and *Carm1*$^{flox/flox}$ mice were obtained from crossing of *Pax7-CreERT2:Carm1*$^{+/flox}$ and *Carm1*$^{flox/flox}$ mouse. To establish the *Pax7-CreERT2:Carm1*$^{+/flox}$ heterozygous (*Carm1*$^{+/flox}$) and *Pax7-Cre:Carm1*$^{flox/flox}$ homozygous (*Carm1*$^{flox/flox}$) knockout primary myoblasts, 4 daily injections of 3 mg Tamoxifen (Sigma) were performed intraperitoneally to 6 week-old mice. Primary myoblasts were isolated one week after the initial tamoxifen administration.

For regeneration studies in juvenile mice, *Pax7-CreERT2:Carm1*$^{+/flox}$ and *Carm1*$^{flox/flox}$ mice were crossed with *R26R-eYFP* lineage reporter mice to generate *Pax7-CreERT2:Carm1*$^{+/flox}$*:R26R-eYFP* heterozygous and *Pax7-CreERT2:Carm1*^{floxflox}*:R26R-eYFP* homozygous mice. *Carm1* was conditionally excised by intraperitoneal Tamoxifen injections (75mg/kg body weight; Sigma) over three consecutive days. Injury was induced by a single 50uL intramuscular injection of 10uM Cardiotoxin (Sigma). All mice were maintained inside a Step-down barrier facility and experiments were performed following the University of Ottawa regulations for animal care and handling.

Western blot analysis

Whole cell extract from the primary myoblasts was prepared by lysing cells in radio immune precipitation buffer containing 300 mM NaCl and protease inhibitor cocktail (Nacalai tesque inc.) on ice for 20 min. The lysate was cleared by centrifugation and mixed with 5x SDS sample buffer. Proteins were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membrane (Millipore). Membranes were immunoblotted with the indicated antibodies. HRP conjugated anti-mouse (Bio-Rad), rabbit (Bio-Rad), or mouse or rabbit light chain specific antibody (Jackson ImmunoResearch) were used for the secondary antibody. Proteins were visualized by Immobilon substrate (Millipore).

Protein expression and purification (*E.coli*, Sf9 cells and HEK293T cells)

C-terminally 3xFLAG-tagged Pax7, Pax3, Pax6, CA150, Pabp1 and GST-Carm1 were expressed using baculovirus system. Recombinant baculoviruses were generated using the Bac-to-Bac system (Gibco-BRL) and infect into Sf9 cells. Infected Sf9 cells were incubated for 2 to 3 days and then collected by centrifugation. Cells were washed with cold calcium- and magnesium-free phosphate-buffered saline (PBS). To purify Pax7, Pax3 and Pax6 FLAG proteins, cell pellets were resuspended in hypotonic buffer (10 mM Tris HCl, pH 7.6, 1 % Nonidet P-40, 2mM $MgCl_2$, 2 mM dithiothreitol; DTT) and incubated on ice for 10min. Nuclei were collected by centrifugation, resuspended in high salt buffer (25mM Tris HCl, pH 7.6, 0.1 % Triton X-100, 2mM $MgCl_2$, 420mM NaCl, 2 mM DTT, protease inhibitor cocktail), and incubated on ice for 30 min. For GST-Carm1, CA150 FLAG and Pabp1 FLAG, cells were resuspended in lysis buffer (25 mM Tris HCl, 1 % Triton X-100, 300 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT). After centrifugation, extracts were pre-cleared by incubated with protein G sepharose (GE healthcare life science) at 4°C for 30 min and then subsequently incubated with anti-FLAG M2-agarose beads (Sigma) or Glutathione Sepharose (GE Healthcare) at 4°C for 3 hr. The resin was washed with wash buffer 1 (25 mM Tris HCl pH 7.6, 0.1 % Triton X-100, 2 mM $MgCl_2$, 300mM NaCl, 2 mM DTT) for three times and then washed twice with wash buffer 2 (25 mM Tris HCl pH 7.6, 0.1 % Triton X-100, 2 mM $MgCl_2$, 100mM NaCl). Proteins that remained bound to the resin were eluted by 500 mM 3xFLAG peptide (Sigma) in wash buffer 2 for FLAG tagged proteins twice or by glutathione elution buffer (50 mM Glutathione (Sigma), 50 mM Tris HCl, pH 9.6, 100 mM NaCl, 1 mM EDTA and 1 mM DTT) for GST-tagged proteins. Eluted fractions were dialyzed with dialysis buffer (10 mM Tris HCl pH 7.6, 100mM NaCl, 2 mM DTT, 0.5 mM PMSF, Sigma) for FLAG tagged proteins or dialysis buffer with 20 % glycerol for GST-fusion

proteins. The purity of the eluted fractions was analyzed by SDS-PAGE followed by CBB staining. For *E. coli* expression, expression vectors were transformed into BL21-Codon-Plus(*DE3*)-RIL (Stratagene). Overnight culture of *E. coli* was diluted in LB medium by 1/20 dilution and precultured for 1.5 hr. Protein expression was induced by the presence of 1 mM IPTG for 3-5 hr. After centrifugation, cells were washed with cold PBS. To purify proteins, cells were resuspended in sonication buffer (1 % Triton X-100, 1 mM EDTA, 1 mM DTT in PBS) and sonicated on ice until the suspension became clear. After clearance by centrifugation, the soluble fraction was pre-cleared by protein G sepharose and subsequently incubated with anti-FLAG M2-agarose beads (Sigma). Beads were washed with wash buffer 3 (20 mM Tris HCl, pH7.6, 500 mM NaCl, 1 % Triton X-100, 2 mM DTT) and FLAG tagged protein was eluted by 500 mM FLAG peptide in dialysis buffer. The eluted protein was dialyzed with dialysis buffer. 6xMyc tagged Prmts and 3xFLAG tagged Pax7 mutants for methyltransferase assays were expressed in HEK293T cells. Cells were lysed in lysis buffer (20 mM Tris HCl, pH8.0, 300 mM NaCl, 1 % Triton X-100, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT) and centrifuged to clear the lysate. The cell lysate was precleared by protein G sepharose and purified with anti-Myc agarose beads (Nacalai) or anti-FLAG M2-agarose beads (Sigma). Beads were washed 3 times with lysis buffer and then twice with methyltransferase reaction buffer. The protein bound beads were directly used for methyltransferase assay.

***In vitro* methyltransferase assay**

Purified methyltransferase was mixed with core histone (Upstate) or the purified substrate in reaction buffer (50 mM Tris HCl, pH8.0, 100 mM NaCl and 0.5 mM EDTA) and incubated for 1 hr in the presence of radioactive S-Adenosyl-L-[*methyl*-^{3}H] Methionine (S-adenocyl [^{3}H]-AdoMet; Perkin Elmer) or non-radioactive S-Adenosyl-Methionine (SAM; New England

biolabs). Proteins were resolved by SDS-PAGE. The gel was fixed, soaked with Amplify (GE Healthcare) and dried. Protein methylation was detected by autoradiography.

***In vivo* labeling of primary myoblasts**

In vivo labeling of primary myoblasts was performed as previously described with some modification (Liu et al., 1995). Mock or Pax7-FLAG over- expressing primary myoblasts were washed with PBS and methionine free DMEM (Sigma) and cultured in methionine free DMEM supplemented with 20 % dialyzed fetal bovine serum, sodium pyruvate (Invitrogen), L-glutamine (Invitrogen), b-FGF and protein synthesis inhibitors (40 μg/ml cyclohezamide, Sigma, 40 μg/ml chloramphenicol, Sigma) for 30 min to inhibit protein synthesis. The cells were incubated for an additional 3 hr in the presence of L-[*methyl*-^{3}H] Methionine (10 μCi/ml, Perkin Elmer). Cells were washed with PBS and lysed in lysis buffer (20 mM Tris HCl, pH8.0, 300 mM NaCl, 1 % Triton X-100, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, protease inhibitor cocktail). The lysate was cleared by centrifugation and precleared with protein G sepharose for 30 min. Pre-cleared lysate was incubated with anti-FLAG M2 agarose for 3 hr. The protein bound beads were washed 5 times with lysis buffer and resuspended in SDS-sample buffer. Proteins were resolved by SDS-PAGE and analyzed by western blot analysis or autoradiography.

Co-immunoprecipitation

Primary myoblasts were lysed with Lysis buffer (20 mM Tris HCl, pH7.6, 150 mM NaCl, 0.5 % Triton X-100, 2 mM $MgCl_2$, 1 mM EDTA, 1mM DTT, protease inhibitor cocktail) and incubated on ice for 20 min. The lysate was cleared by centrifugation and incubated with anti-Carm1 antibody for 3 hr. Protein G sepharose was added into the cell lysate and then incubated for 2 hr. Beads were washed 5 times with lysis buffer and resuspended in SDS-sample buffer. The proteins were resolved by SDS-PAGE and subjected to western blot analysis.

***In vitro* binding assay**

Purified proteins were immobilized with glutathione sepharose (GE healthcare) for GST fusion proteins or anti-FLAG M2 agarose (Sigma) for FLAG tagged proteins and mixed with either indicated purified proteins, *in vitro* transcribed/translated proteins (from TNT *in vitro* transcription/translation system (Promega)) or 10 mg of nuclear extract prepared from primary myoblasts using nuclear extraction method with some modification (Dignam et al., 1983). The proteins were incubated in binding buffer (20 mM Tris HCl pH7.6, 100 mM NaCl, 10 % glycerol, 0.05 % tween 20, 0.1 mM EDTA, 1mM $MgCl_2$, 1 mM DTT, 10 μg of bovine serum albumin, Pierce) at 4°C for 3 hr. The protein bound beads were washed 4 times with binding buffer and then subsequently eluted in SDS-sample buffer. Eluted proteins were resolved by SDS-PAGE and subjected to western blot analysis.

Electrophoresis gel mobility shift assay

An oligonucleotide encoding the Pax7/Pax3 binding element *-57.5 kb* upstream of the *Myf5* promoter (5'-AATCATAAAGGCATGACTAATTGCATGG-3') was radiolabeled with ^{32}P-γATP (Perkin Elmer) by polynucleotide kinase (New England Biolabs.) and then annealed with complementary oligonucleotide. The annealed double stranded DNA was gel filtrated by MicroSpin G-25 column (GE Healthcare) and then used as the probe. Purified protein was incubated with radio-labeled probe in a binding buffer (20 mM Tris HCl, pH7.6, 75 mM NaCl, 4 mM $MgCl_2$, 2 mM EDTA, 1 mM DTT, 10 % Glycerol, 2.5 μg/ml bovine serum albumin) containing poly-dIdC (Sigma) and reticulocyte lysate (Promega) for 20 min at room temperature. DNA-protein complexes were separated on 5 % polyacrylamide gel in 0.5xTBE buffer at 50 V/C and detected by autoradiography.

Luciferase reporter assay

The indicated plasmids were transfected into primary myoblasts or C3H10T1/2 cells with the firefly and renilla luciferase reporters using Lipofectamine 2000 (Invitrogen). Cells were cultured for 24 hr (reporter assay) or 48 hr (three-hybrid assay). Firefly or renilla reporter activation was measured by LUMIstar OPTIMA (BMG Labtech) using Dual-Luciferase Reporter Assay System (Promega).

RNA extraction and RT-qPCR analysis

Total *RNA* was isolated using RNeasy Mini kit (Qiagen) or Nucleospin RNA II kit (Macherey-nagel) according to the manufacture's instructions. 1st-strand cDNA was synthesized using superscript III (Invitrogen) or iScript (Bio-Rad) reagents according to the manufacture's instructions. Quantitative reverse transcriptase-PCR (RT-qPCR) was accomplished with iQ-Syber (Bio-Rad) using Mx3000P Real Time PCR System (Stratagene) with oligonucleotide primer sets. Sequences are listed as follows; *Gapdh* (5'-TGTCCGTCGTGGATCTGAC-3', 5'-GGTCCTCAGTGTAGCCCAAG-3'), *Pax7* (5'-GACGACGAGGAAGGAGACAA-3', 5'-ACATCTGAGCCCTCATCCAG-3'), *Carm1* (5'-TCCTCATCCAGTTTGCCACAC-3', 5'-ATTCCTCTGTCCGCTCACTGA-3'), *Myf5* (5'-ACCTCCAACTGCTCTGACGG-3', 5'-CTGCAGCACACATGCATTTGATAC-3'),

Mass spectrometry analysis

Protein complex purification was described previously (McKinnell et al., 2008). For the identification of *in vivo* Pax7 methylation sites, approximately $1x10^9$ cells of Pax7-FLAG over-expressing primary myoblasts were lysed in lysis buffer (20 mM Tris HCl, pH8.0, 300 mM NaCl, 1 % Triton X-100, 2 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, protease inhibitor cocktail) and immunoprecipitated by anti-FLAG M2 agarose. Protein was resolved by SDS-PAGE and stained with CBB. Selected gel bands were excised and analyzed at the OHRI Proteomics Core

Facility (Ottawa Hospital Research Institute). Gel bands were in-gel digested as described by Shevchenko (Shevchenko et al., 2006). Liquid chromatography–tandem mass spectrometry (LC-MS/MS) was performed on an LTQ Orbitrap XL hybrid mass spectrometer (Thermo Scientific, USA) with Agilent 1100 HPLC. Peptides were loaded onto a capillary trap column (Michrom, USA) and eluted over a 60 minute gradient of 3-40 % acetonitrile with 0.1 % formic acid at 300 nanolitres per minute through a 10-cm analytical column (New Objective Picofrit, self-packed with Zorbax C18) and sprayed into the LTQ Orbitrap XL using a nanospray source (Thermo Scientific, USA). Mass spectra were acquired using a data-dependent method. MS scans were acquired using the Orbitrap module while MS^2 scans were acquired in the ion trap module. MS^2 spectra were matched against human or mouse sequences in the SwissProt (version 57.15) database using MASCOT 2.3.01 software (Matrix Science, UK) with MS tolerance of ±5 ppm, MS/MS tolerance of 0.6 Da and semi-trypsin enzyme specificity. Carbamidomethylation of cysteine was searched as a fixed modification. Oxidation of methionine, Gln conversion to pyro-Glu (N-term Gln), phosphorylation (Ser or Thr), acetylation (Protein N-term), methyl (Arg), dimethyl (Arg), and acetyl (Lys) were allowed as variable modifications. The same data was also searched in Error-Tolerant mode with oxidation of methionine and carbamidomethylation allowed as variable modifications. In Error-Tolerant mode, the Mascot software performs a second-pass analysis of the data in which it attempts to determine whether any observed spectra can be matched to a previously identified protein by assuming the presence of any protein modification. MS tolerance was set at ±5 ppm.

Chromatin immunoprecipitation (ChIP)

For immunoprecipitation of histones, cultured primary myoblasts were washed with PBS and cross-linked with 1 % formaldehyde (Sigma) for 10 min at room temperature. Glycine was added

to the fixing solution and incubated for 10 min. For immunoprecipitation of indicated proteins, cultured primary myoblasts were washed with PBS and fixed in PBS containing 5 mM Dimethyl 3,3'-dithiobispropionimidate·2HCl (Thermo scientific) for 30 min at 4°C. An equal volume of quenching buffer (100 mM Tris HCl, pH8.0 and 150 mM NaCl) was added to the fixing solution. Cells were washed with PBS and cross-linked with 1 % formaldehyde for 10 min at room temperature. Glycine was added to fixing solution and incubated for 10 min. Cells were lysed in cell lysis buffer (5 mM PIPES, pH8.1, 85 mM KCl, 0.5 % Nonidet P-40) with protease inhibitor cocktail and incubated on ice for 10 min. Lysed cells were cleared by centrifugation and resuspended in Buffer A (50 mM Hepes KOH, pH7.6, 500 mM NaCl, 1 % Triton X-100, 0.1 % Sodium deoxycholate, 1 mM EDTA, protease inhibitor cocktail) containing 0.1 % SDS. The cell lysate was sonicated by Bioruptor (Diagenode), and the sheared DNA was centrifuged. The chromatin was diluted with Buffer A to 300 μg/ml and pre-cleared with a mixture of protein A and G sepharose and salmon sperm DNA (Millipore). Chromatin for histone immunoprecipitation (100 μg) or other protein immunoprecipitation (300 μg) was incubated with 5 μg of antibody at 4°C overnight and mixed with a mixture of protein A and G agarose and salmon sperm DNA. Immunoprecipitated chromatin was washed 20 times with Buffer A by inversion, twice with Buffer B (50 mM Hepes KOH, pH 7.6, 300 mM LiCl, 1 mM EDTA, 0.5 % Noniodet P-40, 0.7 % Sodium deoxycholate) for 5 min at room temperature and twice with TE buffer by rotation for 5 min at room temperature. Washed beads were suspended in Elution buffer (50 mM $NaHCO_3$, 1 % SDS, 200 mM NaCl, 10 mM EDTA, 10 mM DTT, 3 μg/ml sheared salmon sperm DNA) and incubated at 65°C for 5.5 hr. After RNaseA and Proteinase K treatment, de-cross-linked DNA was recovered by phenol/chloroform extraction and analyzed by quantitative PCR using CFR384 (Bio-rad) with SsoFast™EvaGreen Supermix (Bio-rad). PCR

primers for ChIP-qPCR are listed as following; *IgH* (5'-CTGGACAGAGTGTTTCAAAAC-3', 5'-TGGCAGGAAGCAGGTCATGT-3'), *Myf5 -57.5 kb* (5'-ATACAGACATGCAGGCTTCAC-3', 5'-CTCCGTATGTTTGTTGGAAAGG-3'), *Myf5 -0.7 kb* (5'-ACACGGCTCTTAAAGCAATGG-3', 5'- AACTGCTCTGACGGCATGGTA-3'), *Myf5 -1.5 kb* (5'-ACACGGCTCTTAAAGCAATGG-3', 5'-AACTGCTCTGACGGCATGGTA-3').

Myofiber experiment and immunohistochemistry

Single muscle fibers were isolated from the EDL muscle of *Myf5-Cre/RosaYFP* mice (SV129) as described previously (Kuang et al., 2007; Le Grand et al., 2009). Isolated myofibers were cultured in 12 wells and transfected with indicated siRNA (20 nM) using Lipofectamine RNAiMAX reagent at 4 and 24 hr post-dissection. Transfected culture media was replaced with fresh media at 4hr post-transfection. Transfected myofibers were fixed after 42 hr in culture, and permeabilized with permeabilization buffer (25 mM Tris HCl, pH7.6, 150 mM NaCl, 0.25% Triton X-100, 62.5 mM Glycine). After blocking, myofibers were incubated with indicated primary antibodies (See above; CD34 (Ram34; eBioSciences); Sdc4 (Dr. Bradley Olwin; University of Colorado)). Alexa488, 568, and 647 conjugated antibodies against mouse IgG_1, rabbit IgG and chicken IgG were used for secondary antibody (Invitrogen). Nuclei were counterstained with 4',6-diamino-2-phenylindole, DAPI.

Duolink (OLink) was used according to manufacturer's protocol. In brief, myofibers were incubated with primary antibodies against Sdc4 (chicken), Pax7 (mouse), Carm1 (rabbit). Anti-mouse and Anti-rabbit secondary antibodies conjugated to oligonucleotides were added, ligated and amplified with a Cy3 conjugated DNA probe. Alexa-488 anti-chicken and Alexa-647 conjugated anti-GFP (Invitrogen) was added during the final amplification step. Nuclei were counter stained with a DAPI containing mounting medium (OLink).

Statistical analysis

Statistical evaluation was performed using the Student's *t*-test. Data are presented as mean±s.e.m. and $P<0.05$ was considered as statistically significant. Individual *P*-values are indicated in each figure.

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