Taylor et al., http://www.jcb.org/cgi/content/full/jcb.201403021/DC1

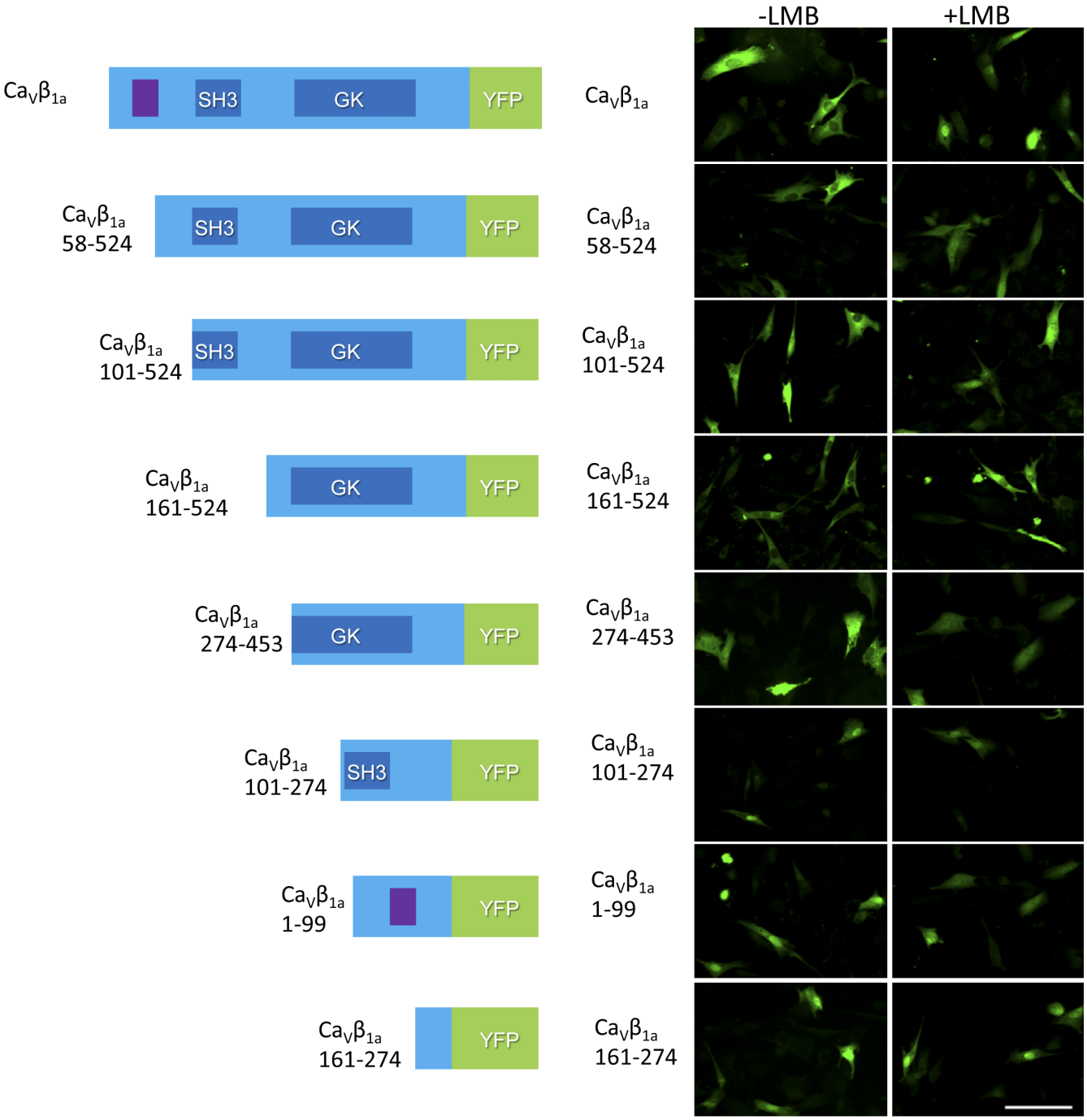

Figure S1. **Representative images of $Ca_V\beta_{1a}$ -YFP mutants with and without LMB treatment.** Bar, 100 µm.

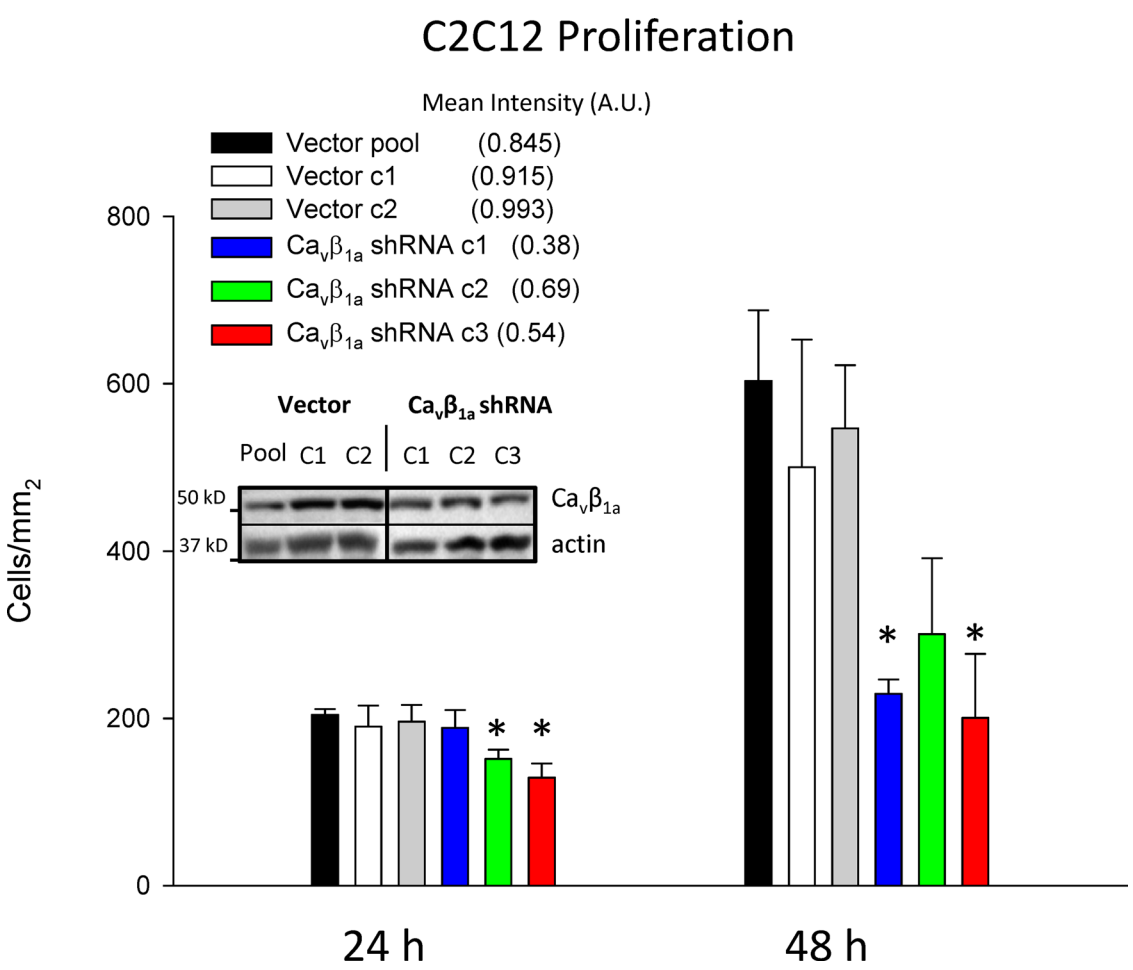

Figure S2. **shRNA-mediated knockdown of $Ca_v\beta_{1a}$ impairs myoblast proliferation.** C2C12 myoblasts were transfected with pLKO.1 (vector) or $Ca_v\beta_{1a}$ sequence-specific shRNA, and selected with puromycin. Individual clones were isolated and expanded (c1–c3), and compared with a vector-transfected pool of cells to control for freezing and extended passaging. $Ca_v\beta_{1a}$ knockdown was evaluated by Western blot and the actin-normalized $Ca_v\beta_{1a}$ intensity is presented in A.U. (average of two experiments). The proliferation rate of each group was evaluated by plating cells at equal densities and quantifying the number of cells per mm^2 using MetaMorph counting software (average of 10 fields per dish, $n = 3$ per group). Data represent mean ± SE (*, $P \leq 0.05$ compared with vector pool). Related to Fig. 4 of the main text.

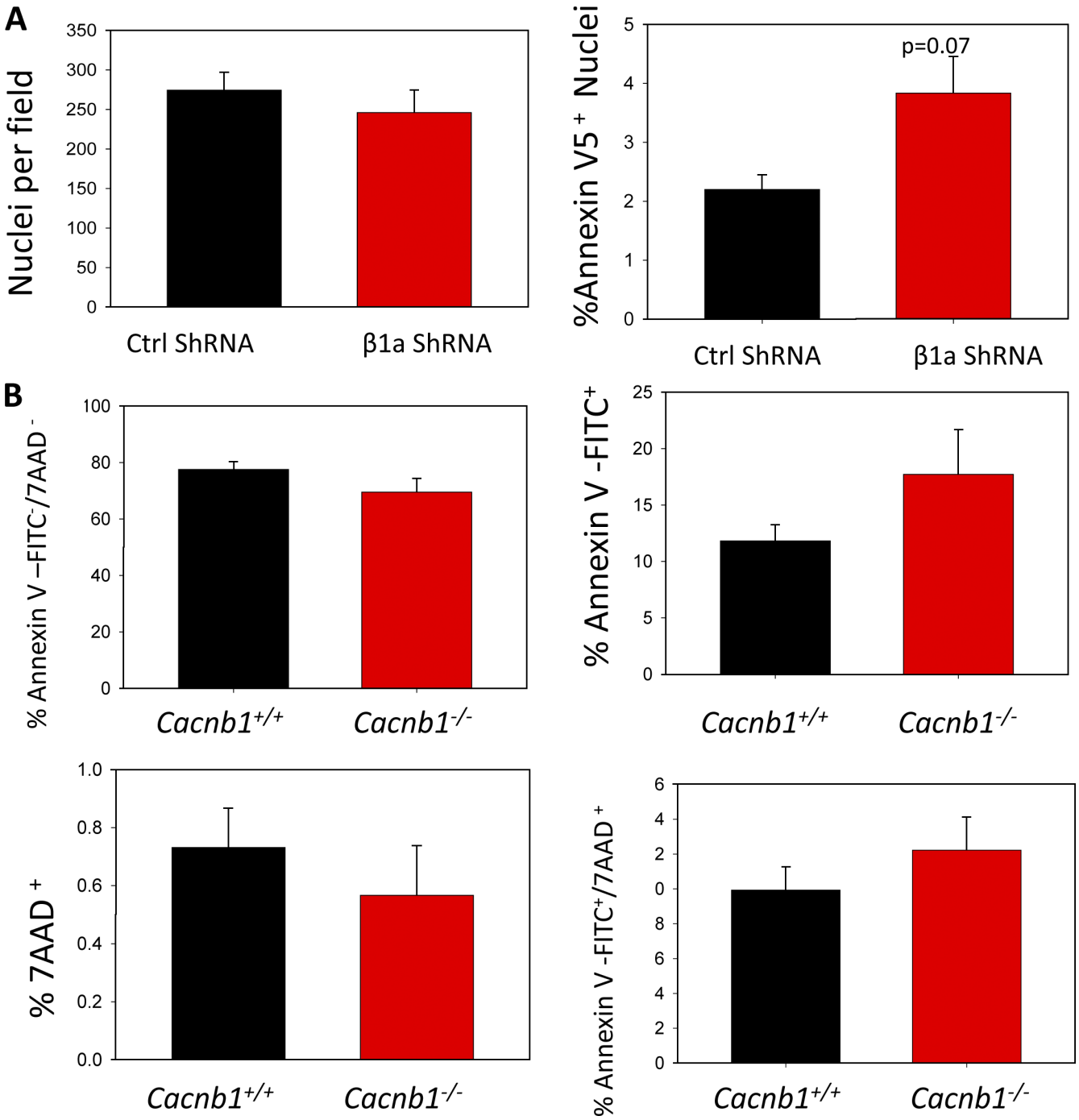

Figure S3. **Analysis of cell death in $Ca_v\beta_{1a}$ knockdown and *Cacnb1*$^{-/-}$ cells.** (A) Control and $Ca_v\beta_{1a}$ shRNA-treated primary myoblasts were stained with AnnexinV-FITC and quantified by fluorescent microscopy (n = 3 each). (B) Cells were isolated from hindlimbs of E12.5 *Cacnb1*$^{+/+}$ (n = 4) and *Cacnb1*$^{-/-}$ (n = 3) mice by enzymatic digestion, preplated for 1 h on plastic, stained for AnnexinV-FITC and 7AAD, and analyzed by flow cytometry. AnnexinV is a marker of apoptosis, and 7AAD is a marker of general cell death.

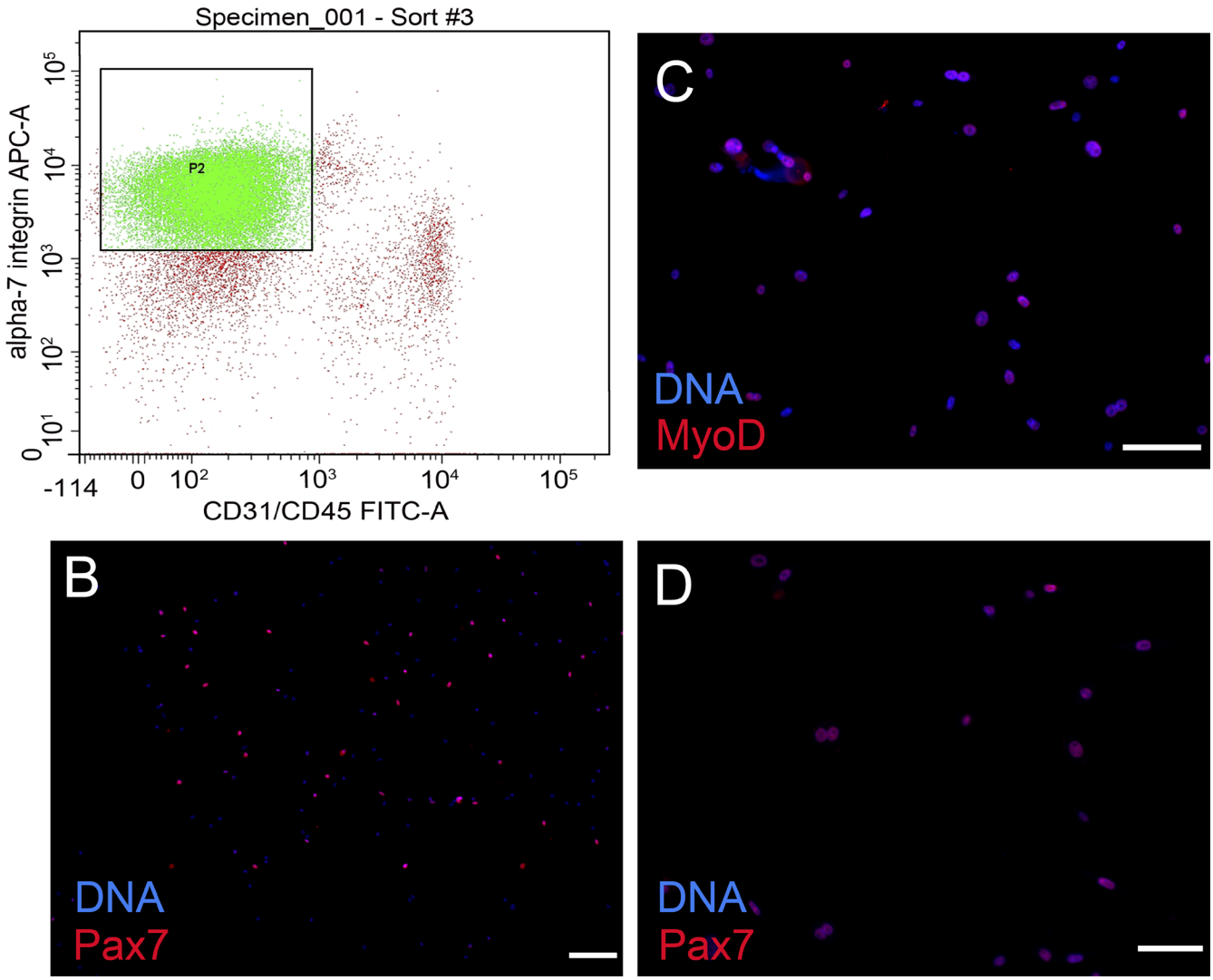

Figure S4. **FACS of muscle precursor cells from E18.5 embryos.** (A) Gating strategy for α7 integrin-APC, CD45-FITC, CD31-FITC–labeled mononucleated cells isolated from skeletal muscle. Gate was set to isolate the α7 integrin-APC$^+$, CD45-FITC$^-$, CD31-FITC$^-$ population. (B) Pax7 staining (red) of sorted cells immediately after FACS. Approximately 50% were Pax7$^+$. (C and D) After several weeks in culture, >95% of cells were myoD (C) or Pax7 (D) positive. Bar, 100 µm. Related to Figs. 1, 4, and 6 of the main text.

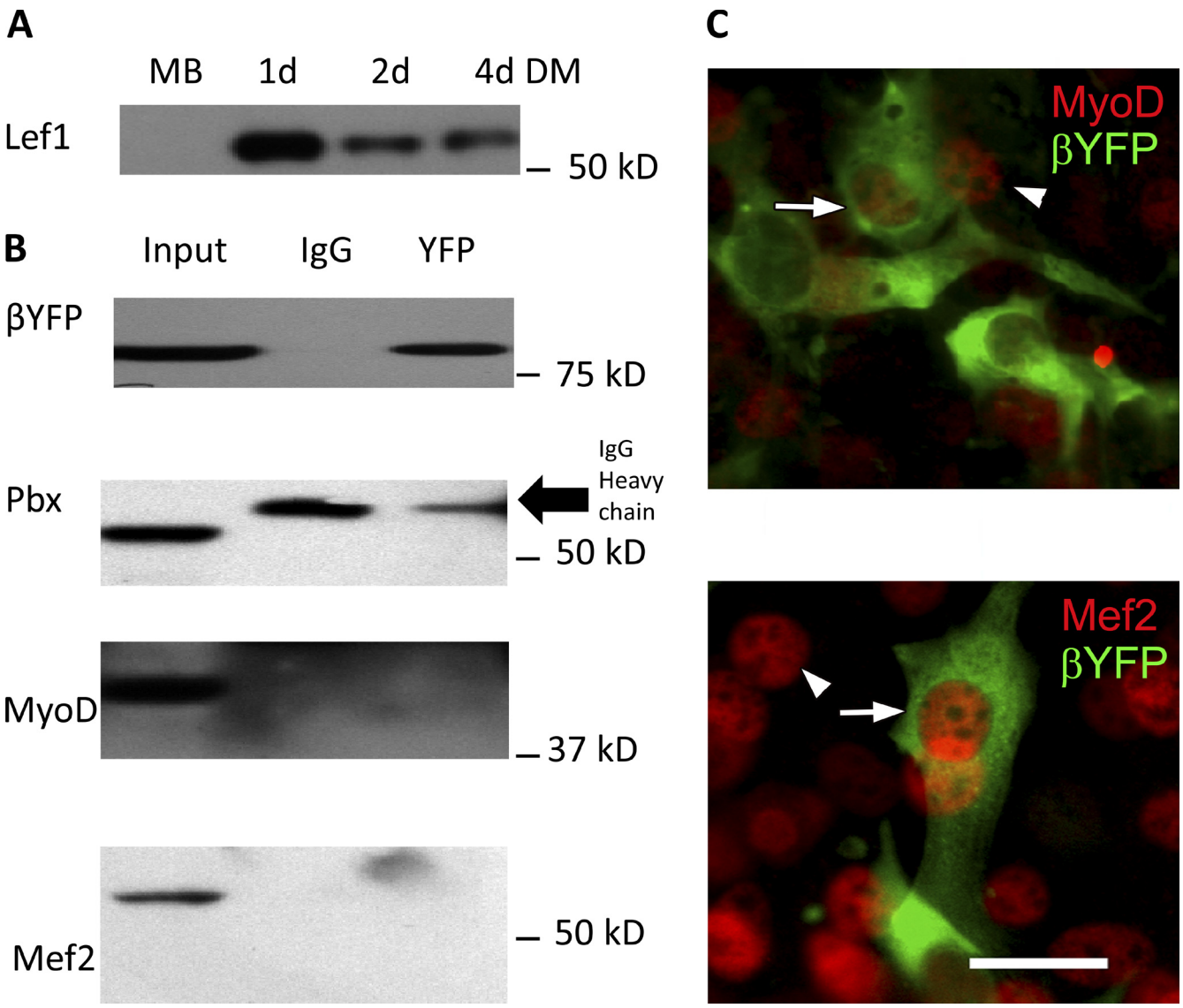

Figure S5. **Screening for $Ca_V\beta_{1a}$-binding partners at the *Myog* promoter.** (A) Western blot analysis of Lef1 protein in proliferating myoblasts (MB) and 1, 2, and 4 d differentiated myotubes. (B) Pbx, MyoD, or Mef2 are all expressed in proliferating myoblasts, but do not coimmunoprecipitate with $Ca_V\beta_{1a}$-YFP. All blots are from the same membrane. $Ca_V\beta_{1a}$-YFP is specifically precipitated by YFP antibody, but not nonspecific control IgG (top). (C) Immunofluorescent images of MyoD (top) and Mef2 (bottom) localization (red) in $Ca_V\beta_{1a}$-YFP cells. Arrow points to $Ca_V\beta_{1a}$-YFP–expressing cell, arrowhead points to untransfected cell.

Table S1. **List of primers used in this study**

Primer name	Forward (5′→3′), or sequence (shRNA), or product ID (TaqMan probes)	Reverse (5′→3′)	Experiment
$Ca_V\beta_{1a}$	GTCCAGAAGAGCGGCATGTC	GAAGGGGATGCGCTTGCCGT	RT-PCR
β-Actin	TGAGCTGCGTTTTACACCCTTTCT	ACTCAGGGCATGGACGCGAC	RT-PCR
$Ca_V\beta_{1a}$-58-524	GCTAGAATTCCATGTCCGACGGGAGCACCT	GCTAGTCGACATGGCATGTTCCTGC	$Ca_V\beta_{1a}$-YFP truncations
$Ca_V\beta_{1a}$-101-524	GCTAGAATTCCATGGTGGCTTTTGCTGTTCGGACAAAT	GCTAGTCGACATGGCATGTTCCTGC	$Ca_V\beta_{1a}$-YFP truncations
$Ca_V\beta_{1a}$-161-524	GCTAGAATTCCATGAGGCCCATCATCCTGGTG	GCTAGTCGACTGGTTTGGTCTTGGCTTT	$Ca_V\beta_{1a}$-YFP truncations
$Ca_V\beta_{1a}$-101-274	GCTAGAATTCCATGCCCGTCAAACTGGACAGC	GCTAGTCGACGGAAGGCACCACGTC	$Ca_V\beta_{1a}$-YFP truncations
$Ca_V\beta_{1a}$-1-99	GCTAGAATTCCATGGTCCAGAAGAGCGGCATGTCC	GCTAGGATCCTTTGGTCTTGGCTTTCTCGAG	$Ca_V\beta_{1a}$-YFP truncations
$Ca_V\beta_{1a}$-161-274	GCTAGAATTCC ATG GTGGCTTTTGCTGTTCGGACAAAT	GCTAGTCGACGGAAGGCACCACGTC	$Ca_V\beta_{1a}$-YFP truncations
69048 (Open Biosystems)	CCGGCCAGTGGTAATGAAATGACTACTCGAGTAGTCATTTCATTACCACTGGTTTTTG		shRNA
69049 (Open Biosystems)	CCGGCCCAGCAAACACATCATCATTCTCGAGAATGATGATGTGTTTGCTGGGTTTTTG		shRNA
69050 (Open Biosystems)	CCGGCGAGGGAAGTCTCAATCCAAACTCGAGTTTGGATTGAGACTTCCCTCGTTTTTG		shRNA
69051 (Open Biosystems)	CCGGCCTCGGATACAACATCCAACACTCGAGTGTTGGATGTTGTATCCGAGGTTTTTG		shRNA
69052 (Open Biosystems)	CCGGGCTCAGGAGAAATCTCAGCTTCTCGAGAAGCTGAGATTTCTCCTGAGCTTTTTG		shRNA
SHC002 (Sigma-Aldrich)	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT		shRNA
Casp2	TGCAGCCCGGGAAAAGCTGG	AGGCGAGAAGTAGCGCGGGA	ChIP-PCR
Dlx2	GCGGGCAACGTCACCTAGCA	GTCCTGTTGCGGGCCATCCC	ChIP-PCR
Cbx5#1	ACGGATCCTTCCCTGCGCCT	ACGATGTTCCGCCTGCTGCC	ChIP-PCR
Cbx5#2	GGCAGCAGGCGGAACATCGT	ACGGCATCCGTTTGGGCGTT	ChIP-PCR
Pax3#1	GGCGGACGAGTTTGGTGCGA	GCACTCGGTGTCACGACGGG	ChIP-PCR
Pax3#2	CCCGTCGTGACACCGAGTGC	GCCTGGGGACCGTCAGGGAT	ChIP-PCR
Tbx5	GACGCTCGCTTGCTCCGTGT	AGAGGTCGGGTGGGTCTGCG	ChIP-PCR
Tnnt3	CCCCACCAGCCACACACGAC	GGCCATGAGCAGACCTCGCC	ChIP-PCR/ChIP-qPCR
Tnnt3	GCCTGCTTGGGCACACCCTC	GGCTGGGGGCTCCTTGTGGA	ChIP-PCR
Wnt3	GCCCAAGCCGACCCCTTCAC	GCCCGCGGCCTAAGGTAAG	ChIP-PCR
BDNF I	TGATCATCACTCACGACCACG	CAGCCTCTCTGAGCCAGTTACG	ChIP-PCR
Cacnb1	GAGAGACATGACAGACTCAGCTCGGAGA	ACACCCCCTGCCAGTGGTAAGAGC	ChIP-PCR/ChIP-qPCR
Myog (Applied Biosystems)	Mm00446194_m1		qPCR
GAPDH (Applied Biosystems)	Mm03302249_g1		qPCR
Myog 5′	CAGCAGGGAGGGTTTAAATGG	CCATCAGGTCGGAAAAGGCTT	ChIP-qPCR
Myog 3′	AGCAGCTTTCTCACACCCTC	GACCCTTGGCACTGCTATGT	ChIP-qPCR
ChIP-chip motif sequence 1 (NC E-box)	GAGGCCAGCCTGGTCTACAAAGTGAGTTCCAGGACAGCCAG		GSMA
ChIP-chip motif sequence 2 (O-box)	TGGTGGCGCATGCCTTTAATCCCAGCACTCGGGAG		GSMA
Myog promoter	CGTCTTGATGTGCAGCAACAGCTTAGA		GSMA

Underlined letters represent DNA consensus motifs of ChIP-seq–identified NC E-box, O-box, and NC E-box within Myog promoter, respectively..

Table S2. **List of antibodies used in this study**

Name	Supplier	Clone	Antigen	Host	Dilution	Application(s)
Primary antibodies						
$Ca_V\beta_{1a}$	Santa Cruz Biotechnology, Inc.	H50	Human $Ca_V\beta_{1a}$ (internal region)	Rabbit	1:500	WB, ICC
$Ca_V\beta_{1a}$	GeneTex	C1C3	Human $Ca_V\beta_{1a}$ (internal region)	Rabbit	1:500	WB
Actin	EMD Millipore	C4	Chicken gizzard actin	Mouse	1:500,000	WB
$Ca_V1.1$	Developmental Studies Hybridoma Bank (DSHB)	IIID5E1	Rabbit $DHPR_{\alpha 1s}$ subunit	Mouse	1:10,000	WB
Troponin T	DSHB	RV-C2	Rabbit troponin T	Mouse	1:500	WB
Normal mouse IgG	Santa Cruz Biotechnology, Inc.	SC-2025	–	Mouse	1:500	WB, ICC
Normal rabbit IgG	Santa Cruz Biotechnology, Inc.	SC-2027	–	Rabbit	1:500	WB, ICC, ChIP-chip
α -Tubulin	Sigma-Aldrich	DM1A	Chicken embryo tubulin	Mouse	1:1,000	WB
GAPDH	Imgenex	1D4	Pig GAPDH	Mouse	1:1,000	WB
HP1	Santa Cruz Biotechnology, Inc.	FL-191	Human HP1α	Rabbit	1:500	WB
GFP	Invitrogen	3E6	Recombinant GFP	Mouse	1:50	IP, ChIP
α7 integrin-APC	AbLab	R2F2	α7 integrin	Rat	1:50	FACS
CD31-FITC	eBioscience	390	Mouse CD31	Rat	1:50	FACS
CD45-FITC	eBioscience	30-F11	Mouse CD45	Rat	1:50	FACS
Ki67	Novus Biologicals	SP6	Human Ki67, synthetic C terminus peptide	Rabbit	1:500	ICC/IHC
Pax7	DSHB	PAX7	Chicken Pax7	Mouse	1:100	ICC/IHC
Myogenin	DSHB	F5D	Rat myogenin	Mouse	1:100	ICC/IHC
Secondary antibodies						
Sheep anti–mouse HRP	Santa Cruz Biotechnology, Inc.	NA931VS	Mouse IgG	Sheep	1:25,000	WB
Donkey anti–rabbit HRP	GE Healthcare	NA9340V	Rabbit IgG	Donkey	1:25,000	WB
Alexa Fluor 488	Invitrogen		IgG heavy chains and other Ig light chains	Goat	1:1,000	ICC/IHC
Alexa Fluor 568	Invitrogen		IgG heavy chains and other Ig light chains	Goat	1:1,000	ICC/IHC

Table S3. **ChIP buffers**

Buffer name	Buffer recipe
Pre-IP dilution buffer (store at RT)	10 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 7.5), 10 mM NaCl, 3 mM $MgCl_2$, 1 mM $CaCl_2$, 4% IGEPAL, and 1 mM PMSF (add fresh)
IP dilution buffer (store at RT without protease inhibitors)	20 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 8), 2 mM EDTA, 1% Triton X-100, 150 mM NaCl, and protease inhibitor stock (add fresh)
Protease inhibitor stock	Prepare a 25× stock by dissolving 1 protease inhibitor tablet in 2 mL of nuclease-free water (store at −20°C)
ChIP wash 1 (store at RT)	20 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 8), 2 mM EDTA, 1% Triton X-100, 150 mM NaCl, and 1 mM PMSF (add fresh)
ChIP wash 2 (store at RT)	20 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 8), 2 mM EDTA, 1% Triton X-100, 0.1% SDS, 500 mM NaCl, and 1 mM PMSF (add fresh)
ChIP wash 3 (store at RT)	10 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 8), 1 mM EDTA, 0.25 M LiCl, 0.5% IGEPAL, and 0.5% deoxycholate (sodium salt)
Elution buffer	25 mM Tris-HCl (made from stock 1 M Tris-HCl, pH 7.5), 10 mM EDTA, and 0.5% SDS (store at room temperature)

Tables S4, S5, and S6 are available online as Microsoft Excel files.