

Table S1

	Age	Cell number (10^7)	Muscle weight (g)	Number	$10^7/g$ (A)	CD31(-) CD45(-)% (B)	SM/C-2,6(+) Sca-1(-)% (C)	Myogenic cell number ($10^7/g$)
CTX-0d	9wks	0.08	0.540	n=1	0.148	65.0	18.1	0.017
	13wks	0.217	1.270	n=2	0.171	65.0	18.0	0.020
	9wks	0.51	1.003	n=2	0.508	69.0	14.0	0.050
	11wks	0.1	0.59	n=1	0.169			
	11wks	0.24	1.194	n=2	0.201	50.0	22.8	0.023
CTX-2d	8wks	2.15	0.625	n=1	3.440			
	10wks	3.52	1.387	n=2	2.538	20.6	7.4	0.039
	8wks	3.55	1.000	n=2	3.550			
	10wks	4.65	1.311	n=2	3.547	25.4	18.0	0.162
	8wks	2.62	0.907	n=2	2.889	31.7	14.0	0.128
	8wks	3.8	1.097	n=2	3.464	27.0	13.0	0.122
	11wks	1.62	0.640	n=1	2.531	32.6	10.8	0.089
CTX-3d	9wks	2.8	0.576	n=1	4.861	50.1	29.1	0.709
	11wks	4.54	0.581	n=1	7.814	35.0	31.8	0.869
	8wks	3.49	0.489	n=1	7.137	34.4	18.5	0.453
	11wks	3.1	0.550	n=1	5.636	29.0	43.0	0.704
	8wks	3.55	0.662	n=1	5.363	35.7	19.7	0.378
CTX-5d	13wks	2.91	0.525	n=1	5.543			
	9wks	3.38	0.604	n=1	5.596	38.8	19.4	0.421
	11wks	3.2	0.621	n=1	5.153	62.6	12.0	0.387
	9wks	2.55	0.606	n=1	4.208	47.5	20.3	0.405
CTX-7d	9wks	1.44	0.450	n=1	3.200	53.8	17.0	0.293
	12wks	2.98	1.038	n=2	2.871	50.0	19.0	0.273
	9wks	4.62	0.824	n=2	5.607	44.2	11.4	0.283
	10wks	2.05	0.545	n=1	3.761	50.0	8.4	0.158
	10wks	4.4	1.186	n=2	3.710	41.4	15.1	0.232
CTX-14d	13wks	1.83	1.003	n=2	1.825	62.0	9.9	0.112
	10wks	1.12	0.884	n=2	1.267	62.0	10.5	0.082
	10wks	0.54	0.517	n=1	1.044	64.0	7.7	0.051
	10wks	1.18	1.043	n=2	1.131	62.8	9.3	0.066

Myogenic cell number: (A) x (B)/100 x (C)/100.

Table S2. Primary antibodies

Antibodies	Clone	Ig type	Conjugate	Supplier	Application & Dilution
anti-CD31	390	Rat IgG2a, κ	FITC	BD PharMingen	FACS: x400
anti-CD31	390	Rat IgG2a, κ	PE	BD PharMingen	FACS: x400
anti-CD45	30-F11	Rat IgG2b, κ	FITC	BD PharMingen	FACS: x800
anti-CD45	30-F11	Rat IgG2b, κ	PE	BD PharMingen	FACS: x1600
anti-Sca-1	D7	Rat IgG2a, κ	PE	BD PharMingen	FACS: x400
anti-Satellite cells	SM/C-2.6	Rat IgG2a	biotin	Ref) Fukada et al. ECR 296, 245-255.	FACS: x200
anti-M-cadherin	polyclonal	Rabbit IgG	Unconjugated	Gift from S.Takeda	IHC: x1000
anti-eMyHC	F1.652	Mouse IgG	Unconjugated	DSHB	IHC: x2
anti-laminin α2	4H8-2	Rat IgG1	Unconjugated	Alexis	IHC: x200
anti-Pax7	PAX7	Mouse IgG1, κ	Unconjugated	DSHB	IHC: x2 (All experiments)
anti-Pax7		Rabbit IgG	Unconjugated	Gift from N. Hashimoto	IHC: x5000 (Fig. 7B, 7C)
anti-MyoD	polyclonal	Rabbit IgG	Unconjugated	Santa Cruz	IHC: x200 (Fig. 1B)
anti-MyoD	5.8A	Mouse IgG1, κ	Unconjugated	BD PharMingen	ICC: x200 (Fig. S1)
anti-myogenin	F5D	Mouse IgG1, κ	Unconjugated	DSHB	IHC: x30
anti-GFP	polyclonal	Rabbit IgG	Unconjugated	Chemicon International	IHC: x800
anti-YFP	polyclonal	Rabbit	Unconjugated	MBL	IHC: x2000
anti-doublecortin	polyclonal	Goat	Unconjugated	Santa Cruz	IHC: x200
anti-Ki67	polyclonal	Rabbit IgG	Unconjugated	Ylem	IHC: x2
anti-Ki67	polyclonal	Rabbit IgG	Unconjugated	Abcam	IHC: x100
anti-dystrophin	polyclonal	Rabbit IgG	Unconjugated	Abcam	IHC: x800

Table S3. Primers
Genotyping primers

Gene		Sequence	Size (bp)
Pax7-CreERT2	Pax7-CE Fwd	ACTAGGCTCCACTCTGTCCTTC	WT:724 Mut:231
	Pax7-CE Rev	GCAGATGTAGGGACATTCCAGTG	
DCX	Dcx-ex2-1	AAATATGAGAGGGTCACGGATG	WT:313
	Dcx-ex2-2	CTTCCAGTTCATCCATGCTTC	
	lacZ F9	CGAAAACCCGAAACTGTGGAG	Mut:499
	lacZ B12	ATTCATTCCCCAGCGACCAG	
Myf5-Cre	Common-Fwd	CGTAGACGCCTGAAGAAGGTCAACCA	WT:603 Mut:400
	Myf5 Rev	CACATTAGAAAACCTGCCAACACC	
	MUT Rev	ACGAAGTTATTAGGTCCCTCGAC	

PCR primers

Gene		Sequence	Size (bp)
Dcx	Fwd	AAATATGAGAGGGTCACGGATG	741
	Rev	TCATCTTGAGCATAGCGG	
Dclk 1	Fwd	AGCTGGTGGAAAGGTGAAAGCTATG	582
	Rev	CATTAACTGAGCTGGTGGAGGC	
Dclk 2	Fwd	GGAAGGTGAAAGTTACGTGTGTC	457
	Rev	TTTCAGGACACGGCATT CGC	
Hprt	Fwd	CTTGCTGACCTGCTGGATTACAT	361
	Rev	GTCAAGGGCATATCCAACAACAAA	

Real-time PCR primers

Gene		Sequence	Size (bp)
Myf5	Fwd	TGAAGGATGGACATGACGGACG	134
	Rev	TTGTGTGCTCCGAAGGCTGCTA	
Myf5 (Standard)	Fwd	GTGTCTCCCTCTGCTGAATC	591
	Rev	CTGCTGTTCTTCGGGACC	
Pax7	Fwd	GTCTGGTTCAGTAACCGGCGTG	52
	Rev	GGTTAGCTCCTGCCTGCTTA	
Pax7 (Standard)	Fwd	CTGGATGAGGGCTCAGATGT	268
	Rev	AGAAGGTGGTTGAAGGCGG	
DCX	Fwd	CTCAACAAGAAAACAGCCC	133
	Rev	AGAAATCATGGAGACAGGTG	
DCX (Standard)	Fwd	AAATATGAGAGGGTCACGGATG	741
	Rev	TCATCTTGAGCATAGCGG	
Gapdh	Fwd	TGTCAAGCTCATTCCTGG	157
	Rev	TTGGGGGCCGAGTTGGATA	
Gapdh (Standard)	Fwd	GAAGGTGGTGAAGCAGGCATCT	387
	Rev	GTATTCAAGAGAGTAGGGAGGG	
MyoD	Fwd	CCCCGGCGGCAGAATGGCTACG	174
	Rev	ACTCTGGTGGTGCATCTG	
MyoD (Standard)	Fwd	CCCCGGCGGCAGAATGGCTACG	234
	Rev	GGTCTGGGTTCCCTGTTCTGTT	

Supplemental Figure legends

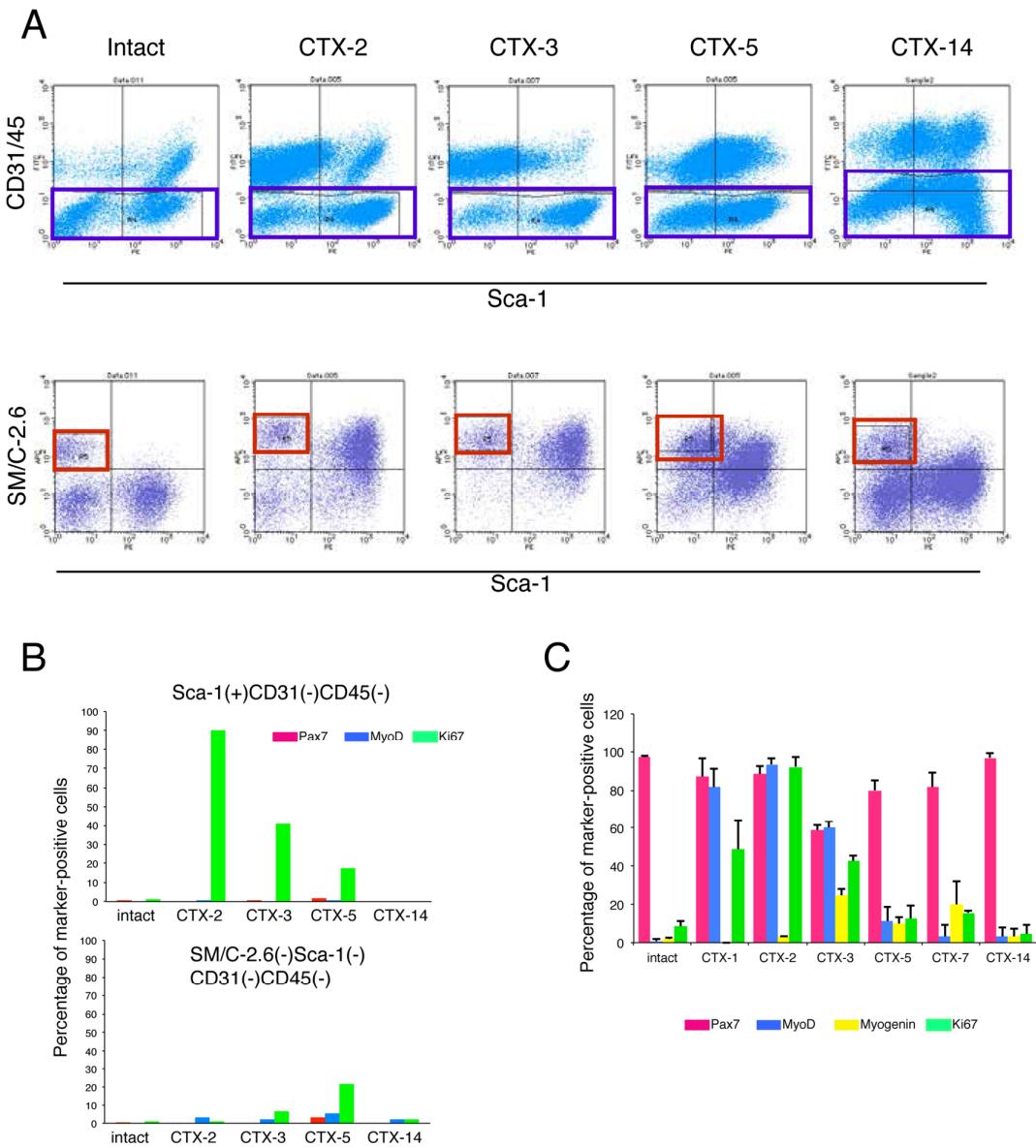


Fig. S1

- (A) Representative FACS profiles of intact or injured muscles. The lower profiles are gated in blue box in the upper profile. Myogenic population is enriched in the red box.
- (B) The frequencies of Pax7, MyoD, and Ki67-expressing cells in Sca-1⁺CD31⁻CD45⁻ or SM/C-2.6⁻Sca-1⁻CD31⁻CD45⁻ population during skeletal muscle regeneration.
- (C) The frequencies of Pax7, MyoD, myogenin, and Ki67-expressing cells during skeletal muscle regeneration.

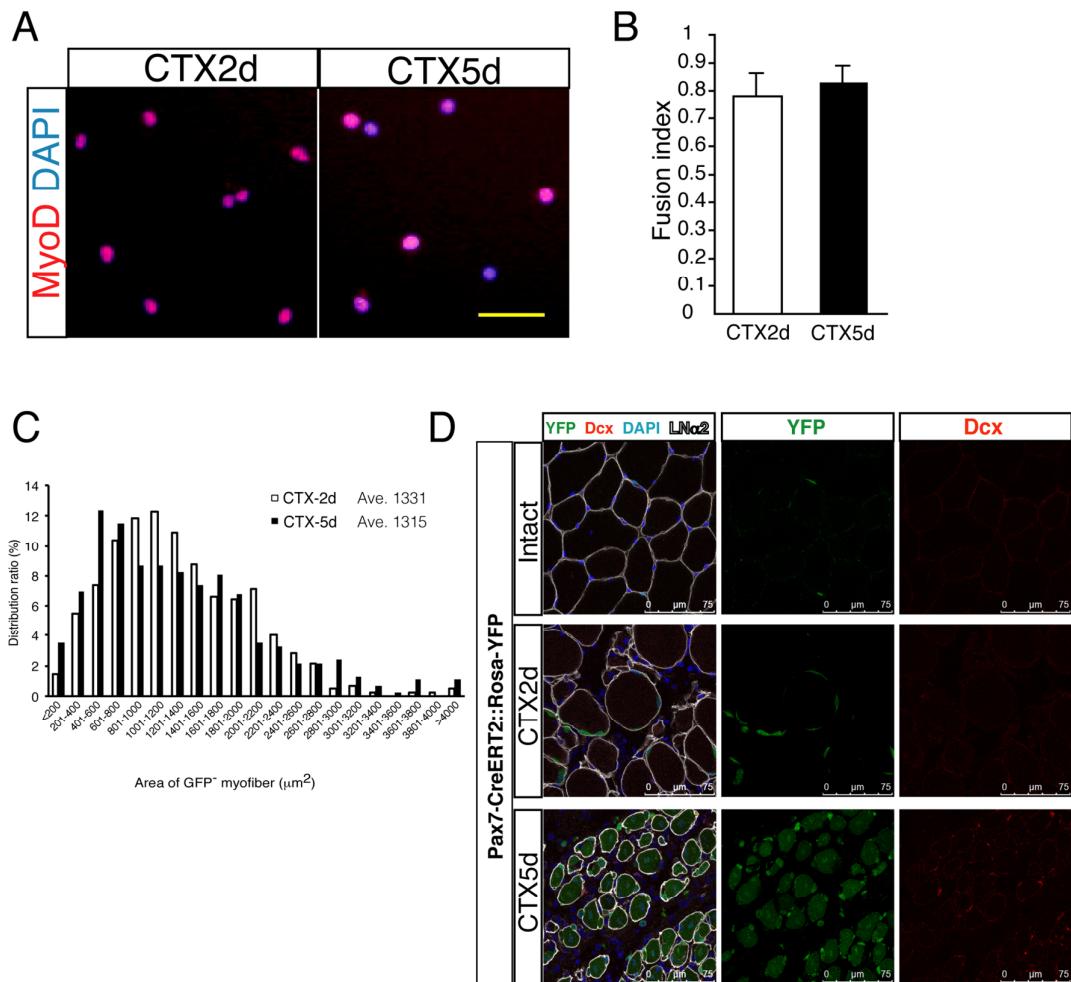


Fig. S2

- (A) Expression of MyoD in CTX-2d or CTX-5d cells after 3 d culture.
- (B) In vitro fusion index of primary myoblasts derived from CTX-2d (white) or CTX-5d (black) muscles. The y axis shows the mean value with S.D.
- (C) The GFP⁺ myofibers were measured (Figure 2E). The histograms show the frequency of myofibers in the indicated area on the x-axis per total GFP⁺ myofibers. More than 200 myofibers from independent samples were counted in each group.
- (D) Expression of DCX in YFP⁺ cells in uninjured, CTX-2d, or CTX-5d muscles.

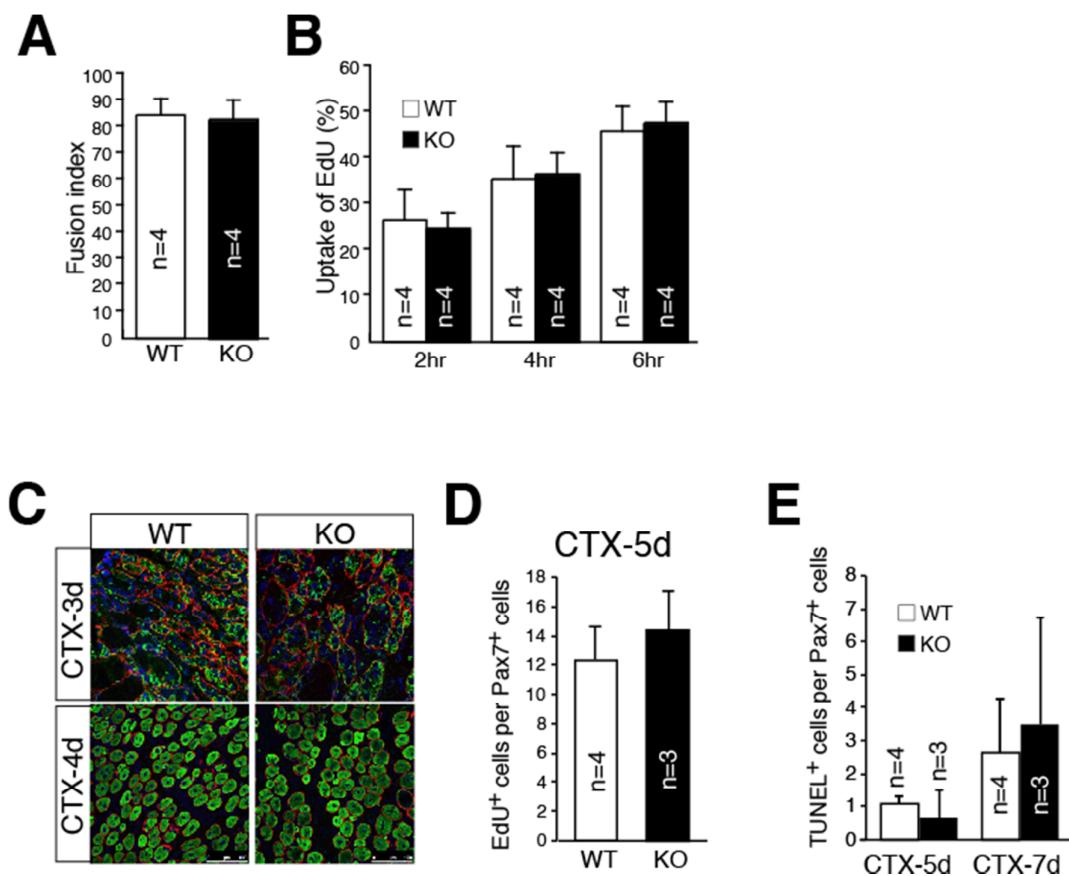


Fig. S3

In vitro fusion index (A) or EdU uptake (B) of primary myoblasts derived from WT or KO mice. The y-axis shows the mean value with S.D. The x-axis (B) shows the time of EdU treatment.

(C) Immunostaining of M-cadherin (green; upper panels), embryonic myosin heavy chain (green; lower panels) LN α 2 (red), and DAPI (blue) in injured muscle 3 or 4 d after CTX injection. Scale bar: 100 μ m.

(D) EdU uptake of *in vivo* Pax7⁺ cells in injured muscle 5 d after CTX injection of WT or KO mice. The y-axis shows the mean percentage of EdU⁺ cells in Pax7⁺ cells with S.D.

(E) The frequency of TUNEL⁺ cells in Pax7⁺ cells of WT or KO mice in injured muscle 5 or 7 d after CTX injection. The y-axis shows the mean percentage of TUNEL⁺ cells in Pax7⁺ cells with S.D.

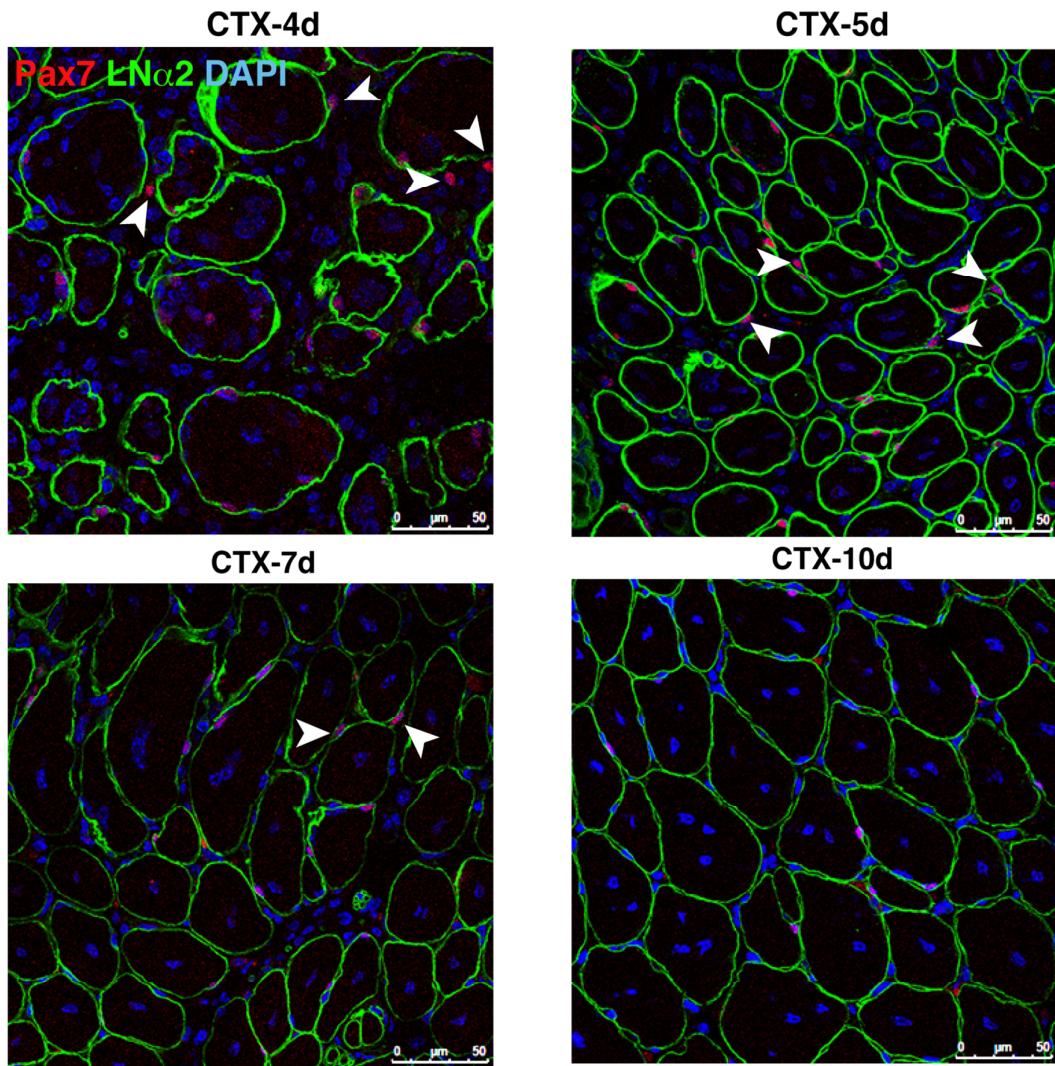


Fig. S4

Immunostaining of Pax7 (red) and laminin α 2 (LN α 2: green) during skeletal muscle regeneration. Arrow indicates interstitial Pax7⁺ cell. Scale bar: 50 μ m.

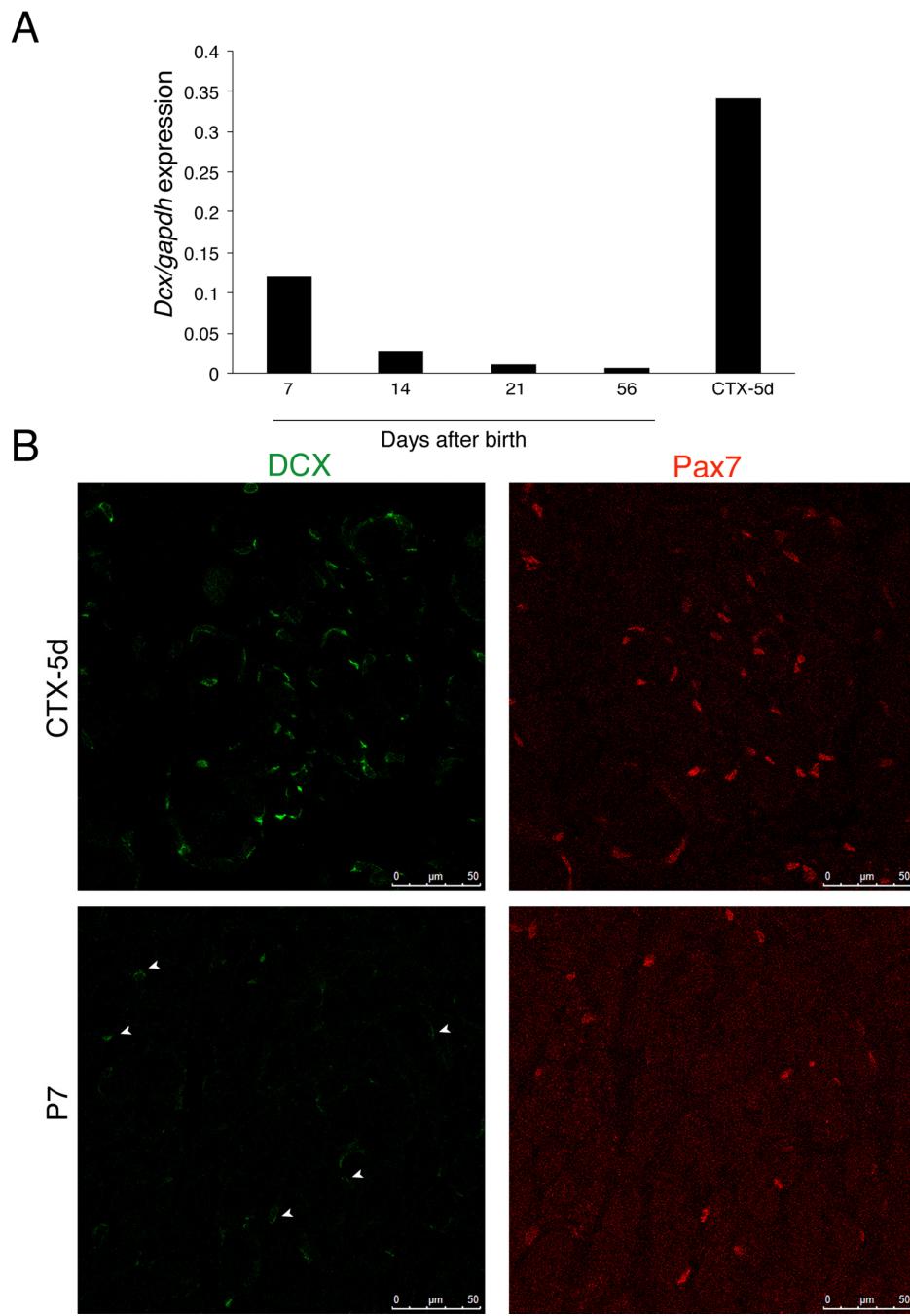


Fig. S5

- (A) Transcripts of *doublecortin* genes were quantified by qRT-PCR in freshly isolated myogenic cells during postnatal development or CTX-5d myogenic cells.
- (B) Immunostaining of Pax7 (red) and DCX (green) in postnatal 7 day (P7) or CTX-5d muscle. Arrowhead indicates DCX^{dim} cell in P7 muscle. Scale bar: 50 μm.

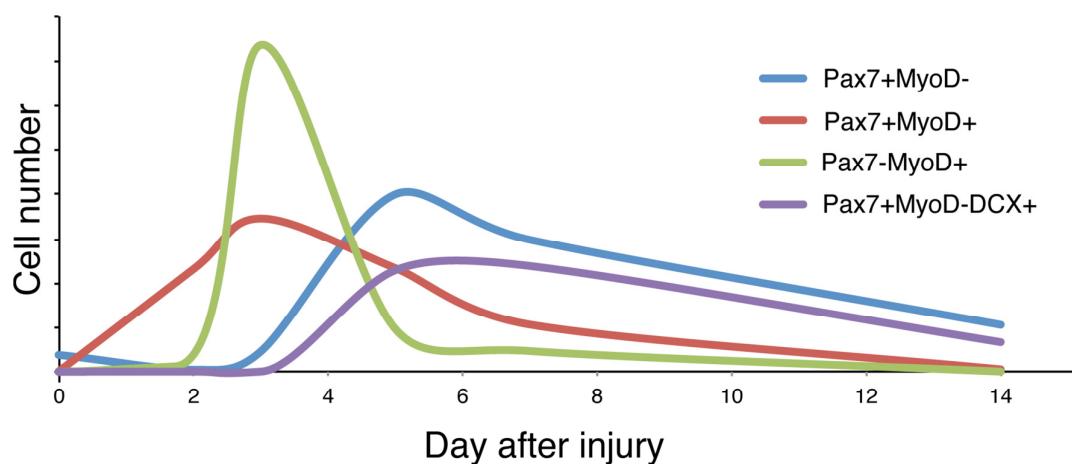


Fig. S6

The kinetics of Pax7⁺MyoD⁻, Pax7⁺MyoD⁺, Pax7⁻MyoD⁺, Pax7⁺MyoD⁻DCX⁺ cells during muscle regeneration.