

Supplementary Data

Figure S1

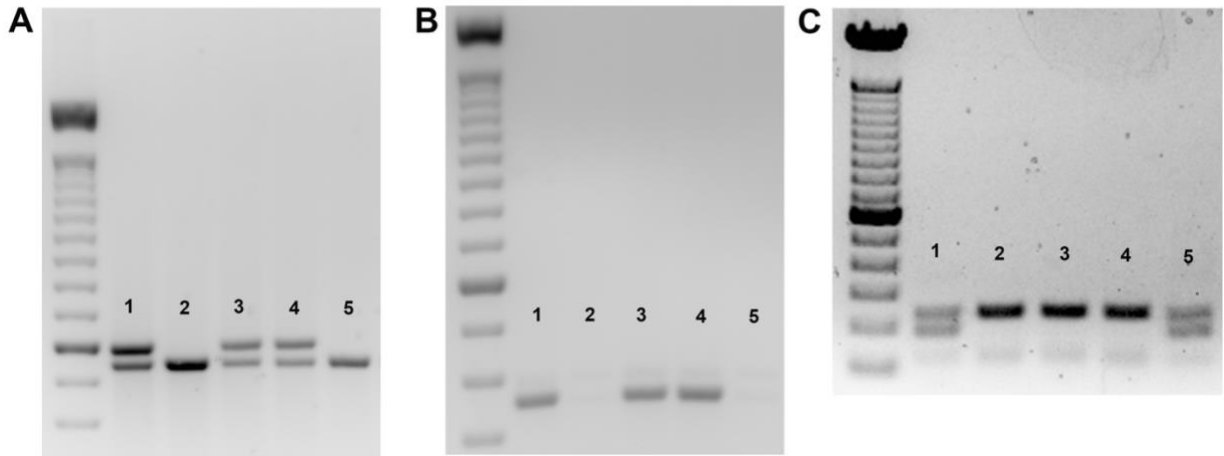


Fig. S1: Verification of *Myh3* targeting and deletion. (A) 5' LoxP targeting in *Myh3^{fl3-7/+}* mice was verified by PCR across the LoxP site; animal numbers 1, 3 and 4 show two bands, with the larger molecular weight band resulting from incorporation of the LoxP site and the smaller molecular weight band from the wild type homologous chromosome, with animals 2 and 5 being wild type. (B) 3' LoxP targeting in *Myh3^{fl3-7/+}* mice was verified by PCR for Neomycin cassette, which is part of the 3' targeted locus, in the same 5 animals tested in A for 5' LoxP targeting; animal numbers 1, 3 and 4 are positive for Neomycin while 2 and 5 are negative. (C) Deletion of *Myh3* exons 3-7 in the *Myh3^{Δ/+}* animals was verified by PCR with 3 primers, 2 amplifying across the deleted region to give a product and the third primer giving a product in cases where there is no deletion. Results show that animals 1 and 5 have the deletion (smaller molecular weight deleted band and larger molecular weight wild type band as these are heterozygotes), while animals 2, 3 and 4 are wild type (single larger molecular weight band).

Figure S2

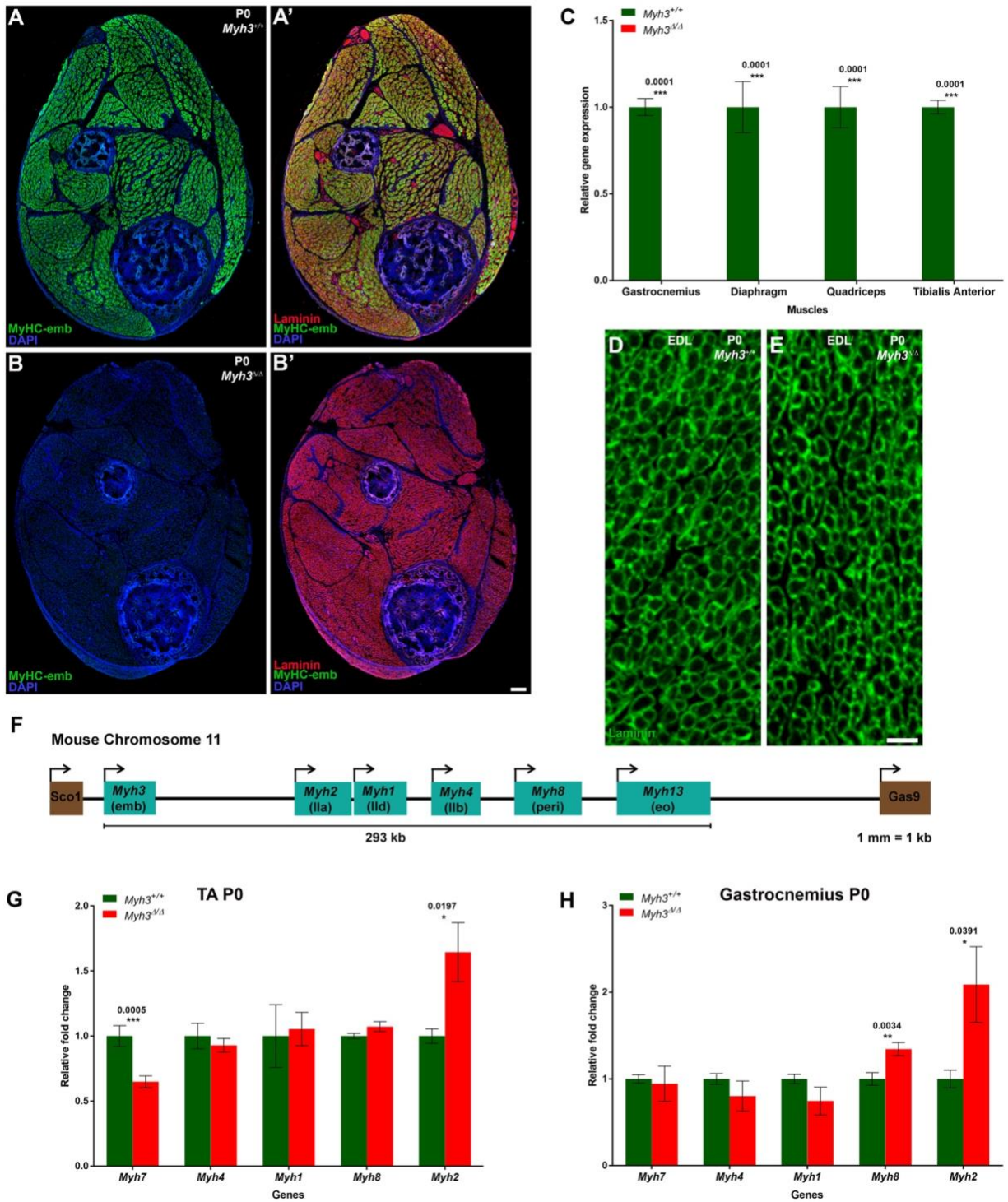


Fig. S2: Validation of *Myh3* knockout. (A-B') Cross sections through hind limbs of P0 *Myh3^{+/+}* (A, A') and *Myh3^{Δ/Δ}* (B, B') mice labeled by immunofluorescence for Laminin (red), MyHC-emb (green), and

DAPI (Blue). (C) Quantitation of *Myh3* transcript levels by qPCR in P0 gastrocnemius, tibialis anterior, quadriceps and diaphragm muscles of *Myh3^{+/+}* and *Myh3^{Δ/Δ}* mice. (D-E) Laminin (green) labeling through the EDL muscles of P0 *Myh3^{+/+}* (D) and *Myh3^{Δ/Δ}* (E) mice. (F) Schematic showing the *Myh* gene cluster on mouse chromosome 11 which is ~293 kb in size, where *Myh3* is followed by *Myh2*, *Myh1*, *Myh4*, *Myh8* and *Myh13*. (G-H) Quantitation of transcript levels of other *Myh* isoforms by qPCR in the P0 TA (G) and gastrocnemius (H) muscles of *Myh3^{+/+}* and *Myh3^{Δ/Δ}* mice. The graphical data represent the mean ±SEM of a minimum of 3 independent experiments. (Scale bar in B' is 100 microns and E is 20 microns).

Figure S3

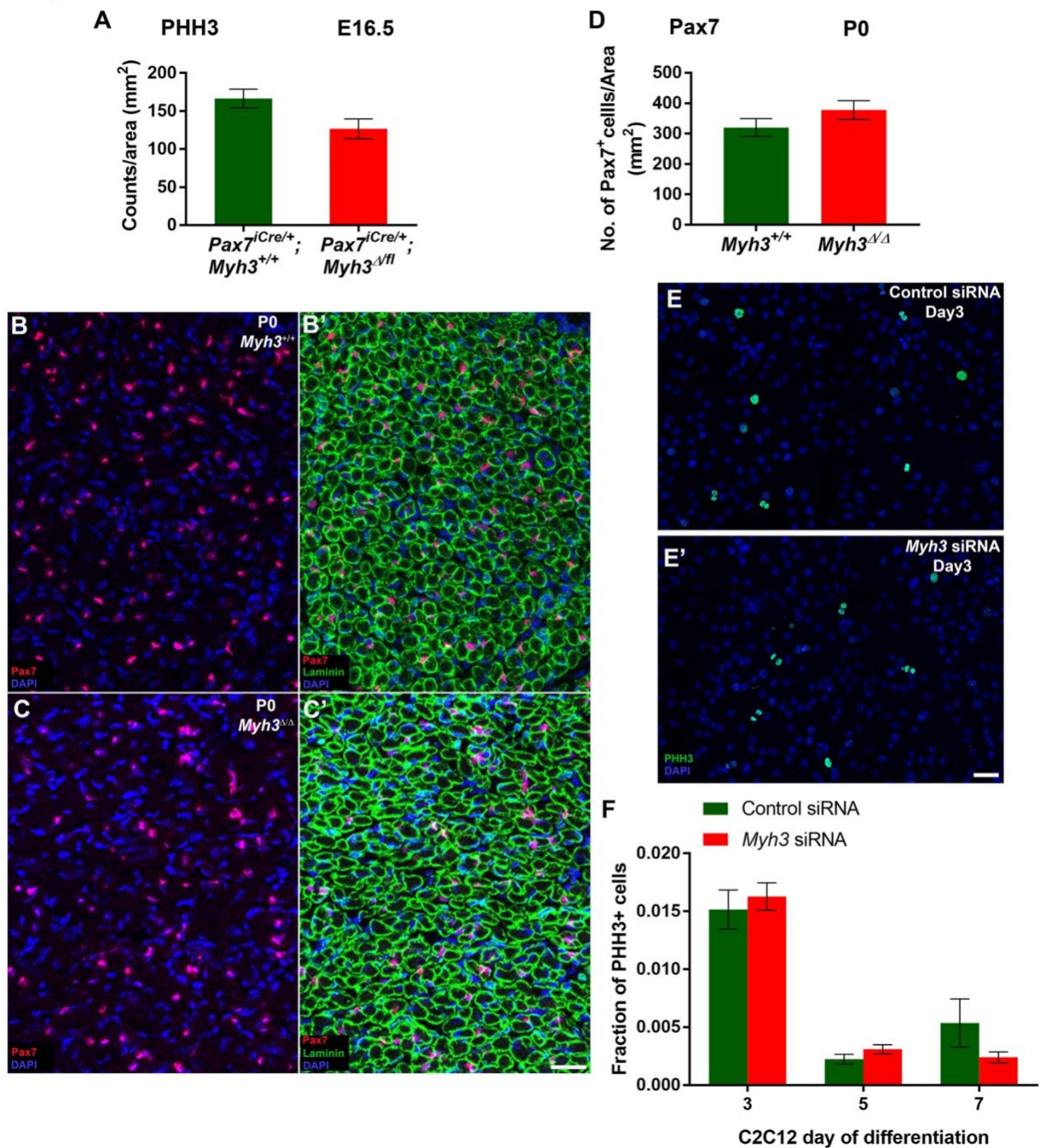


Fig. S3: Loss of MyHC-emb function does not affect cell proliferation. (A) Quantification of phospho-histone H3⁺ cells at E16.5 in *Pax7^{iCre/+};Myh3^{Δfl/3-7}* and control hind limbs normalized to total area. (B-C') Pax7 (red), Laminin (green) and DAPI (blue) immunofluorescence on *Myh3^{+/+}* (B-B') and *Myh3^{ΔΔ}* (C-C') knockout P0 hind limb sections. (D) Quantification of Pax7⁺ cell number normalized to total area in P0 *Myh3^{+/+}* and *Myh3^{ΔΔ}* hind limb sections. (E-E') Representative images of control siRNA (E) and *Myh3* siRNA (E') treated C2C12 cells labeled for PHH3 (green) and DAPI (blue) at day 3 of differentiation. (F)

Quantification of PHH3⁺ cells in control and *Myh3* siRNA treated C2C12 cells at days 3, 5 and 7 of differentiation. The graphical data represent the mean \pm SEM of a minimum of 3 independent experiments. (Scale bar in C' is 40 microns and E' is 50 microns).

Figure S4

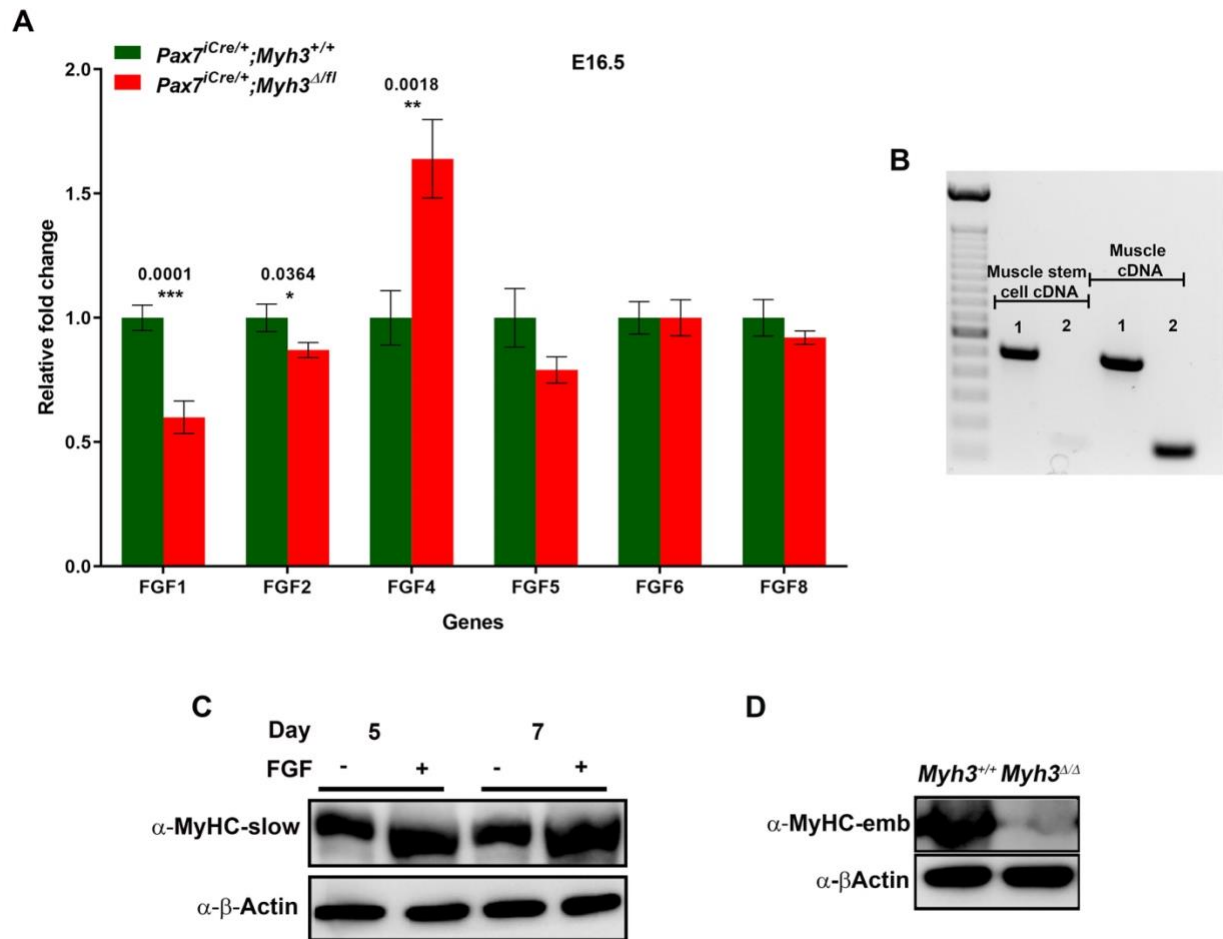


Fig. S4: FGF levels are misregulated upon loss of MyHC-emb function and FGF supplementation promotes differentiation. (A) Quantitation of transcript levels of FGFs known to bind FGFR4 by qPCR in E16.5 limb muscles of *Pax7^{iCre/+};Myh3^{+/+}* and *Pax7^{iCre/+};Myh3^{Δ/fl}* embryos. (B) MyHC-emb transcripts are not detectable in muscle stem cell cDNA as opposed to muscle cDNA by semi-quantitative RT-PCR; 1 denotes positive control (GAPDH) and 2 denotes *Myh3* PCR. (C) Western blots for MyHC-slow and beta-actin on *Myh3* siRNA treated C2C12 cells at days 5 and 7 of differentiation, grown in the presence or absence of FGF, with ‘-’ and ‘+’ denoting absence or presence of FGF in the media. (D) Western blots for MyHC-emb, and beta-actin from E16.5 *Myh3^{+/+}* and *Myh3^{Δ/Δ}* embryo heart protein lysates. The graphical data represent the mean \pm SEM of a minimum of 3 independent experiments.

Figure S5

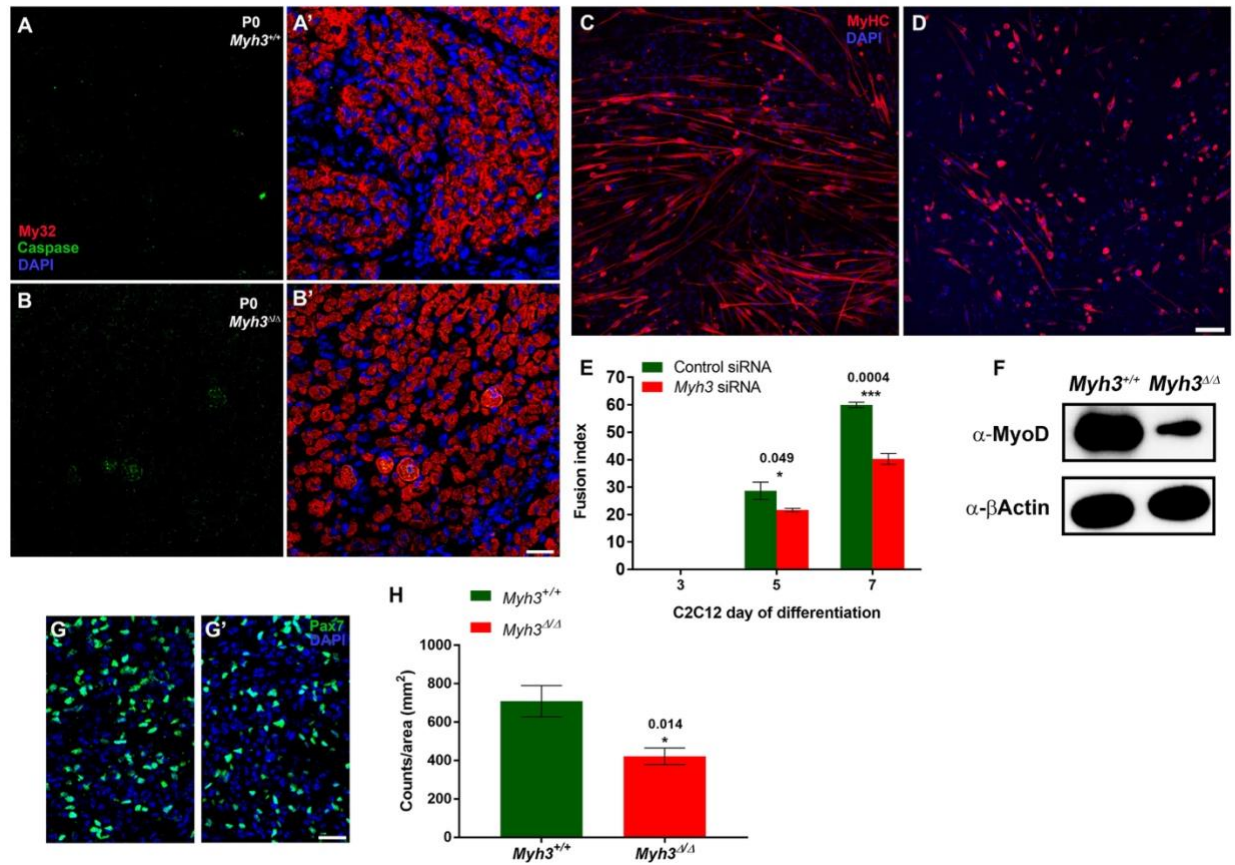


Fig. S5: *Myh3* null mice exhibit increased cell death, reduced fusion index and reduction in muscle progenitors. (A-B') Immunofluorescence for My32 (red), Caspase 3 (green), and DAPI (blue) on P0 *Myh3*^{+/+} (A-A') and *Myh3*^{-/-} (B-B') embryo hind limb cross sections; the left panels show Caspase 3 (A, B) and the right panels are the merge (A', B'). (C-D) Representative immunofluorescence images for MyHC (red) and DAPI (blue) on primary myoblasts, isolated from *Myh3*^{+/+} (C) and *Myh3*^{-/-} (D) P0 mice, differentiated for 7 days, for which fusion index is shown in Figure 5K. (E) Fusion index of control and *Myh3* siRNA treated C2C12 cells at days 3, 5 and 7 of differentiation. (F) Western blots for MyoD, and beta-actin from E16.5 *Myh3*^{+/+} and *Myh3*^{-/-} embryo hind limb protein lysates. (G-G') Immunofluorescence for Pax7 (green), and DAPI (blue) on cross sections through E16.5 *Myh3*^{+/+} (G) and *Myh3*^{-/-} (G') embryo hind limbs. (H) Quantification of Pax7⁺ cell number normalized to total area in E16.5 *Myh3*^{+/+} and *Myh3*^{-/-} hind limb sections. The graphical data represent the mean \pm SEM of a minimum of 3 independent experiments. (Scale bar in B' is 20 microns, D is 100 microns and G' is 25 microns respectively).

Table S1: List of primers

Gene	Direction	Primer sequence 5' to 3'	location	Product size (bp) using cDNA template
Primers used for qPCR analysis				
<i>Myh1</i> (MyHCIIx)	Forward	CGGTGGTGGAAAGAAAGG	Exon17	154bp
	Reverse	CAGGAGTCTTGGTTTCATT	Exon18	
<i>Myh2</i> (MyHCIIa)	Forward	CCAAGAAAGGTGCCAAGAAG	Exon17	147bp
	Reverse	CGGGAGTCTTGGTTTCATTG	Exon18	
<i>Myh3</i> (MyHCemb)	Forward	ATGAGTAGCGACACCGAGATG	Exon3	117bp
	Reverse	AAAGCAGTAGGTTTTGGCAT	Exon3	
<i>Myh4</i> (MyHCIIb)	Forward	GCTTGAAAACGAGGTGGAAA	Exon40	190bp
	Reverse	CCTCCTCAGCCTGTCTCTTG	Exon41	
<i>Myh7</i> (MyHCslow)	Forward	AGGGCGACCTCAACGAGAT	Exon32	92bp
	Reverse	CAGCAGACTCTGGAGGCTCTT	Exon32	
<i>Myh8</i> (MyHC-peri)	Forward	AACAGAAACGCAATGCTGAGG	Exon38	135bp
	Reverse	TCGCCTGTAATTTGTCCACCA	Exon39	
MyoD	Forward	GCTGCCTTCTACGCACCTG	Exon1	119bp
	Reverse	GCCGCTGTAATCCATCATGC	Exon2	
Myogenin	Forward	CAGTACATTGAGCGCCTACAG	Exon1	163bp
	Reverse	GGACCGAACTCCAGTGCAT	Exon2	
Myf5	Forward	CCTGTCTGGTCCCGAAAGAAC	Exon2	130bp
	Reverse	GACGTGATCCGATCCACAATG	Exon3	
Mrf4	Forward	ATCAGCTACATTGAGCGTCTACA	Exon1	173bp
	Reverse	CCTGGAATGATCCGAAACACTTG	Exon2	
Pax7	Forward	TGTTGGGCTCTTCAAGGTCT	Exon9	130bp
	Reverse	GGAATGTGGAGGAGGATGC	Exon9	
GAPDH	Forward	GACTTCAACAGCAACTCCCACT	Exon6	169bp
	Reverse	GGTCCAGGGTTTCTTACTCC	Exon7	
Fgf1	Forward	GTAGTTTCCTAGAGGCAGGTTG		
	Reverse	TGATAAAGTGGAGTGAAGAGAGC		
Fgf2	Forward	GAAACACTCTTCTGTAACACACTT		
	Reverse	GTCAAACACTACAACCTCCAAGCAG		
Fgf4	Forward	ACTCGTCGGTAAAGAAAGGC		
	Reverse	GACACGAGGGACAGTCTTC		
Fgf5	Forward	AACTCCTCGTATTCCTACAATCC		
	Reverse	CGGATGGCAAAGTCAATGG		
Fgf6	Forward	CTGTACACAACGCCAGCTT		
	Reverse	TTGTTTGGAAAGGAGGGTTTCTC		
Fgf8	Forward	CATGGCAGAAGACGGAGAC		
	Reverse	ACTCGGACTCTGCTTCCAAA		
Primers used for RNaseq validation				
Tpm3	Forward	CCCTGAGCCCAAACCTTAT	Exon9	173bp
	Reverse	GGCGATGAGATGATGTTC	Exon9	
Ppargc1a	Forward	TCACACCAAACCCACAGAAA	Exon5	127bp
	Reverse	GGTCAGAGGAAGAGATAAAG	Exon6	

Table S1: List of primers

Lpin1	Forward Reverse	CAAACAAGCCAGTGACAACG AGGGAGATGGCGATGGAT	Exon10 Exon10	132bp
Ankrd2	Forward Reverse	CATTTTCTTTCCCTGGGCTTGG TTCTGCTCTGATTCTGGCTCGG	Exon7 Exon9	230bp
Myl2	Forward Reverse	GTCCACATCATTACCCACGG AGAGCCAAGACTTCCTG	Exon7 Exon7	118bp
Adamts15	Forward Reverse	GAGACACAACCCAAACAAGT CCTCGCAGTATTTCCACCG	Exon4 Exon5	152bp
Cebpb	Forward Reverse	CGGGTTTCGGGACTTGATGC ACCCCGCAGGAACATCTTTA	Exon1 Exon1	127bp
Csrp3	Forward Reverse	ACCACAAGCAACCCTTCCAAAT GTGTAAGCCCTCCAAACCCAAT	Exon4 Exon6	255bp
Btg2	Forward Reverse	GCTGCTTTGTATGGGTGGAT AAAATGGGGAAGGTTGCTCT	Exon2 Exon2	231bp
Myl3	Forward Reverse	CGGGAAGGAGTGGTTCGGAC AAAGGCAAGCACAGGTAGGT	Exon7 Exon7	104bp
Trim63	Forward Reverse	GGTGCCTACTTGCTCCTTGT ATTCCTTGGTCACTCTGC	Exon3 Exon5	190bp
Pde4d	Forward Reverse	GCTTTGGAGGCTGTGTTC CGAGTCCGAGTTTGTATTG	Exon13 Exon14	121bp
Primers used for Genotyping				
<i>Myh3</i>	Forward Reverse Reverse	CGTCTGAGAGGCTTCCATTC TAGGTTTTGGCATCAAAGGG TATCCTTCACGCTCTCCAC	Wildtype Floxed Delta	231bp 279bp 179bp

Table S2. Antibodies used for immunofluorescence and western blots

Antibody	Type	Source	Product No.	Working Concentration (ug/ml)	Antigen retrieval for tissue sections
Primary antibodies					
Pax7	Mouse IgG1	Developmental Studies Hybridoma Bank	PAX7	2.4	Yes
MyHCemb	Mouse IgG1	Developmental Studies Hybridoma Bank	F1.652	3	Yes
MyHCslow	Mouse IgG1	Sigma	M8421 (NOQ7.5.4D)	1.5 (IF), 6 (western)	Yes
MyHCfast	Mouse IgG1	Sigma	M4276 (MY-32)	10	Yes
MyoD	Mouse IgG1	Santa Cruz Biotechnology	sc-32758 (5.8A)	4 (IF), 2 (western)	Yes
MyoG	Mouse IgG1	Santa Cruz Biotechnology	sc-12732 (F5D)	2	Western only
Myf5	Rabbit polyclonal	Santa Cruz Biotechnology	sc-302 (C-20)	1	Western only
Caspase 3	Rabbit IgG	Cell Signaling Technology	9664 (Asp175) (5A1E)	0.02	No
Phospho-Histone H3	Rabbit IgG	Thermo Fisher Scientific	PA5-17869 (Ser10)	0.29	Yes
β -Actin	Mouse IgG2b	Cell Signaling Technology	3700 (810D10)	1.25	Western only
Laminin	Rabbit polyclonal	Sigma	L9393	2.5	Yes
Phospho-p42/44 MAPK (Thr202/Tyr204)	Rabbit IgG	Cell Signaling Technology	4370	0.01	Western only
Phospho-p38 MAPK (Thr180/Tyr182)	Rabbit IgG	Cell Signaling Technology	4511	0.02	Western only
Phospho-Akt (Ser473)	Rabbit IgG	Cell Signaling Technology	4060	0.02	Western only
Phospho-Stat3 (Tyr705)	Mouse IgG1	Cell Signaling Technology	4113	0.01	Western only

Secondary antibodies, fluorescent coupled conjugates					
Cy2/Cy3 conjugated Goat anti-mouse	Goat	Jackson ImmunoResearch Laboratories	115-225-146/ 115-165-146	7.5	IF only
Cy2/Cy3 conjugated Goat anti-rabbit	Goat	Jackson ImmunoResearch Laboratories	111-225-144/ 111-165-144	7.5	IF only
Biotin conjugated Goat anti-mouse	Goat	Jackson ImmunoResearch Laboratories	115-065-020	2.8	IF only
Biotin conjugated Goat anti-rabbit	Goat	Jackson ImmunoResearch Laboratories	111-065-144	2.8	IF only
Cy2 conjugated streptavidin	-	Jackson ImmunoResearch Laboratories	016-220-084	3.6	IF only
Cy3 conjugated streptavidin	-	Jackson ImmunoResearch Laboratories	016-160-084	3.6	IF only
Oregon Green 488 Phalloidin	-	Life Technologies	O7466	0.6 Units/ml	IF only
Peroxidase-AffiniPure Goat anti-rabbit	Goat	Jackson ImmunoResearch Laboratories	111-035-144	0.08	Western only
Peroxidase-AffiniPure Goat anti-mouse	Goat	Jackson ImmunoResearch Laboratories	111-035-003	0.08	Western only

Table S3: List of RNAseq selected genes and their log2fold change values in Diaphragm, Gastrocnemius, Quadriceps and TA muscles. Only genes with statistically significant log2 fold change values are shown.

Genes	Gene Symbol	Diaphragm	Gastrocnemius	Quadriceps	Tibialis anterior
B cell translocation gene 2, anti-proliferative	Btg2	0.77	0.47	0.63	0.41
Nuclear factor, interleukin 3, regulated	Nfil3	0.51	0.38	0.48	0.47
Cysteine and glycine-rich protein 3	Csrp3	0.76	0.67	0.71	-
Tribbles homolog 1 (Drosophila)	Trib1	0.56	0.50	0.47	-
Phosphodiesterase 4D, cAMP specific	Pde4d	0.56	-	0.55	0.69
CCR4 carbon catabolite repression 4-like (<i>S. cerevisiae</i>)	Ccrn4l	0.53	-	0.52	0.45
Cell adhesion molecule 3	Cadm3	-	-0.55	-0.43	-0.39
CCAAT/enhancer binding protein (C/EBP), beta	Cebpb	-	0.63	0.56	0.50
Myosin, light polypeptide 3	Myl3	-	-0.46	-0.68	-0.66
Calmodulin binding transcription activator 1	Camta1	-	0.44	0.38	0.38
Early B cell factor 2	Ebf2	-	-0.30	-0.31	-0.36
Solute carrier family 2 (facilitated glucose transporter), member 1	Slc2a1	-	0.53	0.57	0.66
RIKEN cDNA C130074G19 gene	C130074G19Rik	-	-0.31	-0.33	-0.26
Peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	Ppargc1a	-	0.42	0.58	0.52
Tripartite motif-containing 63	Trim63	-	0.45	0.69	0.55
Tropomyosin 3, gamma	Tpm3	-	-0.34	-0.47	-0.41
Family with sequence similarity 107, member A	Fam107a	-	0.48	0.52	0.69
a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 15	Adamts15	-	0.34	0.55	0.43
Lipin 1	Lpin1	-	0.46	0.59	0.75
a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 1	Adamts1	-	0.31	0.40	0.29
Ankyrin repeat domain 2 (stretch responsive muscle)	Ankrd2	1.01	0.61	-	-

ST3 beta-galactoside alpha-2,3-sialyltransferase 5	St3gal5	0.42	0.54	-	-
Uridine-cytidine kinase 2	Uck2	0.31	0.35	-	-
Ankyrin repeat domain 1 (cardiac muscle)	Ankrd1	0.54	0.54	-	-
v-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)	Maff	0.63	-	0.48	-
salt inducible kinase 1	Sik1	0.54	-	0.39	-
xin actin-binding repeat containing 2	Xirp2	-	0.87	0.48	-
shisa family member 3	Shisa3	-	0.74	0.74	-
collagen, type XXII, alpha 1	Col22a1	-	0.53	0.35	-
regulator of cell cycle	Rgcc	-	0.67	0.46	-
heat shock protein family, member 7 (cardiovascular)	Hspb7	-	0.63	0.45	-
major facilitator superfamily domain containing 2A	Mfsd2a	-	0.72	0.48	-
peptidase inhibitor 16	Pi16	-	-0.42	-0.46	-
family with sequence similarity 214, member B	Fam214b	-	0.48	0.40	-
stathmin-like 4	Stmn4	-	0.59	0.46	-
ATP-binding cassette, sub-family B (MDR/TAP), member 4	Abcb4	-	0.56	0.46	-
BarH-like homeobox 2	Barx2	-	-0.49	-0.54	-
progesterin and adipoQ receptor family member VIII	Paqr8	-	0.53	0.55	-
dual specificity phosphatase 5	Dusp5	-	0.53	0.59	-
musculoskeletal, embryonic nuclear protein 1	Mustn1	-	0.42	0.47	-
solute carrier family 35, member F5	Slc35f5	-	0.39	0.41	-
BCL2-associated athanogene 3	Bag3	-	0.29	0.31	-
G protein-coupled receptor 133	Gpr133	-	-0.37	-0.45	-
meningioma 1	Mn1	-	0.48	-	0.67
solute carrier family 43, member 2	Slc43a2	-	0.44	-	0.40
hypoxia inducible factor 3, alpha subunit	Hif3a	-	0.49	-	0.49

maestro heat-like repeat family member 1	Mroh1	-	0.33	-	0.43
ankyrin repeat and SOCS box-containing 2	Asb2	-	0.29	-	0.32
nuclear receptor subfamily 4, group A, member 1	Nr4a1	-	0.49	-	0.46
ankyrin repeat and zinc finger domain containing 1	Ankzf1	-	0.37	-	0.43
ubiquitin specific peptidase 2	Usp2	-	-	0.28	0.34
solute carrier family 15, member 4	Slc15a4	-	-	0.39	0.38
transmembrane protein 37	Tmem37	-	-	0.46	0.46
solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	Slc7a8	-	-	0.39	0.37
cytochrome b-561 domain containing 1	Cyb561d1	-	-	-0.46	-0.58
kelch-like 38	Klhl38	-	-	0.53	0.74
carbonic anhydrase 3	Car3	-	-	-0.38	-0.57
galactosidase, beta 1-like 2	Glb1l2	-	-	0.54	0.47
solute carrier family 25 (mitochondrial carrier, phosphate carrier), member 25	Slc25a25	-	-	0.51	0.54
deiodinase, iodothyronine, type II	Dio2	-	-	0.56	0.54
RIKEN cDNA 2410131K14 gene	2410131K14Rik	-	-	0.45	0.34
myosin, light polypeptide 2, regulatory, cardiac, slow	Myl2	-	-	-0.77	-1.21
pyridoxal (pyridoxine, vitamin B6) kinase	Pdxk	0.47	-	-	-
heat shock 105kDa/110kDa protein 1	Hsph1	0.37	-	-	-
ATPase, class II, type 9A	Atp9a	0.42	-	-	-
TCDD-inducible poly(ADP-ribose) polymerase	Tiparp	0.54	-	-	-
chemokine (C-C motif) ligand 21A (serine)	Ccl21a	0.54	-	-	-
ADAMTS-like 2	Adamtsl2	0.53	-	-	-
serum/glucocorticoid regulated kinase 1	Sgk1	0.45	-	-	-
ADP-ribosylation factor-like 4D	Arl4d	0.59	-	-	-

sodium channel, voltage-gated, type V, alpha	Scn5a	0.42	-	-	-
family with sequence similarity 220, member A	Fam220a	0.33	-	-	-
keratocan	Kera	0.51	-	-	-
OTU deubiquitinase with linear linkage specificity	Otulin	0.36	-	-	-
atonal homolog 8 (Drosophila)	Atoh8	0.49	-	-	-
RIKEN cDNA 6030419C18 gene	6030419C18Rik	0.44	-	-	-
phosphodiesterase 4B, cAMP specific	Pde4b	0.49	-	-	-
NA	NA	0.35	-	-	-
myosin binding protein C, cardiac	Mybpc3	-	0.66	-	-
microtubule-associated protein tau	Mapt	-	0.74	-	-
carboxylesterase 5A	Ces5a	-	0.80	-	-
epidermal growth factor-containing fibulin-like extracellular matrix protein 1	Efemp1	-	-0.58	-	-
myosin, heavy polypeptide 2, skeletal muscle, adult	Myh2	-	0.76	-	-
tripartite motif-containing 9	Trim9	-	0.72	-	-
T cell lymphoma invasion and metastasis 2	Tiam2	-	0.59	-	-
ubiquitin carboxy-terminal hydrolase L1	Uchl1	-	0.45	-	-
calmodulin-like 3	Calml3	-	0.59	-	-
microtubule associated tumor suppressor candidate 2	Mtus2	-	0.60	-	-
runt related transcription factor 2	Runx2	-	0.60	-	-
methyltransferase like 21C	Mettl21c	-	0.58	-	-
family with sequence similarity 81, member A	Fam81a	-	0.56	-	-
MAP/microtubule affinity-regulating kinase 1	Mark1	-	-0.30	-	-
proline synthetase co-transcribed	Prosc	-	0.26	-	-

secreted frizzled-related protein 4	Sfrp4	-	-0.57	-	-
tenascin N	Tnn	-	0.57	-	-
connector enhancer of kinase suppressor of Ras 1	Cnksr1	-	0.56	-	-
DnaJ (Hsp40) homolog, subfamily B, member 2	Dnajb2	-	0.42	-	-
myoglobin	Mb	-	0.40	-	-
immunoglobulin superfamily, member 21	Igsf21	-	0.47	-	-
dachshund 1 (Drosophila)	Dach1	-	-0.38	-	-
transmembrane protein 125	Tmem125	-	0.45	-	-
chondroitin sulfate proteoglycan 4	Cspg4	-	0.43	-	-
calcium/calmodulin-dependent protein kinase I gamma	Camk1g	-	0.52	-	-
solute carrier family 6 (neurotransmitter transporter), member 18	Slc6a18	-	0.52	-	-
myelocytomatosis oncogene	Myc	-	0.32	-	-
tubby candidate gene	Tub	-	-0.49	-	-
plasminogen activator, tissue	Plat	-	-0.27	-	-
tetratricopeptide repeat domain 9	Ttc9	-	0.38	-	-
spleen focus forming virus (SFFV) proviral integration oncogene	Spi1	-	-0.43	-	-
laminin, gamma 2	Lamc2	-	0.46	-	-
splA/ryanodine receptor domain and SOCS box containing 2	Spsb2	-	0.44	-	-
regulator of G-protein signaling 5	Rgs5	-	-0.31	-	-
tetraspanin 18	Tspan18	-	-0.29	-	-
Mir17 host gene (non-protein coding)	Mir17hg	-	0.36	-	-
extracellular matrix protein 1	Ecm1	-	-0.37	-	-
netrin 1	Ntn1	-	-0.37	-	-
WD40 repeat domain	Wdr95	-	0.43	-	-

coagulation factor II (thrombin) receptor-like 3	F2rl3	-	-0.45	-	-
wingless-type MMTV integration site family, member 10B	Wnt10b	-	-0.46	-	-
melanophilin	Mlph	-	0.42	-	-
Fras1 related extracellular matrix protein 1	Frem1	-	0.31	-	-
RNA, 7SK, nuclear	Rn7sk	-	0.42	-	-
vacuolar protein sorting 37B (yeast)	Vps37b	-	-0.26	-	-
sodium channel, voltage-gated, type III, alpha	Scn3a	-	0.40	-	-
C1q and tumor necrosis factor related protein 2	C1qtnf2	-	-0.39	-	-
CD34 antigen	Cd34	-	-0.15	-	-
family with sequence similarity 174, member B	Fam174b	-	-0.36	-	-
FAD-dependent oxidoreductase domain containing 2	Foxred2	-	-	0.61	-
ring finger protein 144B	Rnf144b	-	-	0.38	-
gap junction protein, delta 4	Gjd4	-	-	0.39	-
angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	Agt	-	-	0.47	-
dynamamin 3, opposite strand	Dnm3os	-	-	0.23	-
solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	Slc3a2	-	-	0.26	-
TSC22 domain family, member 1	Tsc22d1	-	-	0.39	-
homeobox C8	Hoxc8	-	-	-0.39	-
NA	NA	-	-	0.46	-
Solute carrier family 35, member E1	Slc35e1	-	-	0.38	-
NCK associated protein 1 like	Nckap11	-	-	0.39	-

pleckstrin homology domain containing, family F (with FYVE domain) member 1	Plekhf1	-	-	0.38	-
sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A	Sema6a	-	-	-0.19	-
growth associated protein 43	Gap43	-	-	-0.41	-
kinesin family member 26A	Kif26a	-	-	-0.29	-
carbonic anhydrase 14	Car14	-	-	0.46	-
low density lipoprotein receptor-related protein 11	Lrp11	-	-	-0.46	-
R-spondin 3 homolog (<i>Xenopus laevis</i>)	Rspo3	-	-	-0.35	-
RIKEN cDNA 2500002B13 gene	2500002B13Rik	-	-	0.43	-
twinfilin, actin-binding protein, homolog 2 (<i>Drosophila</i>)	Twf2	-	-	0.25	-
sestrin 2	Sesn2	-	-	0.43	-
troponin C, cardiac/slow skeletal	Tnnc1	-	-	-	-0.55
melanin-concentrating hormone receptor 1	Mchr1	-	-	-	0.81
phosphoserine aminotransferase 1	Psat1	-	-	-	-0.45
myosin, light polypeptide kinase 2, skeletal muscle	Mylk2	-	-	-	0.57
serine hydroxymethyltransferase 1 (soluble)	Shmt1	-	-	-	0.41
sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	Sema3c	-	-	-	-0.36
SH2B adaptor protein 2	Sh2b2	-	-	-	0.48
ankyrin repeat and SOCS box-containing 11	Asb11	-	-	-	0.53
myosin, heavy polypeptide 7, cardiac muscle, beta	Myh7	-	-	-	-0.43
solute carrier family 2 (facilitated glucose transporter), member 4	Slc2a4	-	-	-	0.42
troponin T1, skeletal, slow	Tnnt1	-	-	-	-0.32
family with sequence similarity 214, member A	Fam214a	-	-	-	0.43
PHD finger protein 10	Phf10	-	-	-	-0.28

asparagine synthetase	Asns	-	-	-	-0.50
F-box protein 31	Fbxo31	-	-	-	0.39
cysteinyl-tRNA synthetase	Cars	-	-	-	-0.42
tetraspanin 13	Tspan13	-	-	-	-0.29
adenosine monophosphate deaminase 3	Ampd3	-	-	-	0.56
lysine (K)-specific demethylase 4B	Kdm4b	-	-	-	0.28
patatin-like phospholipase domain containing 7	Pnpla7	-	-	-	0.47
myosin VC	Myo5c	-	-	-	0.55
pantothenate kinase 1	Pank1	-	-	-	0.53
dihydrolipoamide branched chain transacylase E2	Dbt	-	-	-	0.47
nicotinamide phosphoribosyltransferase	Nampt	-	-	-	0.35
DDB1 and CUL4 associated factor 11	Dcaf11	-	-	-	0.24
potassium voltage gated channel, Shaw-related subfamily, member 4	Kcnc4	-	-	-	-0.48
glycyl-tRNA synthetase	Gars	-	-	-	-0.36
inscuteable homolog (Drosophila)	Insc	-	-	-	-0.53
aldehyde dehydrogenase 18 family, member A1	Aldh18a1	-	-	-	-0.42
nitric oxide synthase 1, neuronal	Nos1	-	-	-	0.50
proteolipid protein (myelin) 1	Plp1	-	-	-	0.32
pyruvate dehydrogenase phosphatase catalytic subunit 2	Pdp2	-	-	-	0.52
myosin, light polypeptide 6B	Myl6b	-	-	-	-0.34
ADP-ribosylhydrolase like 1	Adprh1	-	-	-	-0.32
transport and golgi organization 2	Tango2	-	-	-	0.48
ankyrin repeat and SOCS box-containing 14	Asb14	-	-	-	0.49
glycoprotein galactosyltransferase alpha 1, 3	Ggta1	-	-	-	0.34
protein phosphatase 1K (PP2C domain containing)	Ppm1k	-	-	-	0.51
RIKEN cDNA 1110018N20 gene	1110018N20Rik	-	-	-	-0.47

corneodesmosin	Cdsn	-	-	-	-0.49
WD repeat domain 1	Wdr1	-	-	-	-0.30
Smith-Magenis syndrome chromosome region, candidate 8 homolog (human)	Smcr8	-	-	-	0.42
Kruppel-like factor 15	Klf15	-	-	-	0.50
tyrosyl-tRNA synthetase	Yars	-	-	-	-0.32
NA	NA	-	-	-	0.49
glutamate-ammonia ligase (glutamine synthetase)	Glul	-	-	-	0.45
epithelial membrane protein 1	Emp1	-	-	-	-0.27
methionine-tRNA synthetase	Mars	-	-	-	-0.34
Rho GTPase activating protein 36	Arhgap36	-	-	-	-0.20
mitochondrially encoded 12S rRNA	mt-Rnr1	-	-	-	0.44
ankyrin repeat and SOCS box-containing 10	Asb10	-	-	-	0.47
transmembrane protein 52	Tmem52	-	-	-	0.41
exportin, tRNA (nuclear export receptor for tRNAs)	Xpot	-	-	-	0.29
ankyrin repeat domain 9	Ankrd9	-	-	-	-0.26
cystathionase (cystathionine gamma-lyase)	Cth	-	-	-	0.43
host cell factor C1 regulator 1 (XPO1-dependent)	Hcfc1r1	-	-	-	-0.23
solute carrier family 44, member 2	Slc44a2	-	-	-	0.22
F-box protein 32	Fbxo32	-	-	-	0.44
myosin, heavy polypeptide 3, skeletal muscle, embryonic	Myh3	-	-	-	-0.32
tetratricopeptide repeat domain 38	Ttc38	-	-	-	0.45
myosin, light polypeptide 9, regulatory	Myl9	-	-	-	-0.33
zinc finger, FYVE domain containing 21	Zfyve21	-	-	-	0.35
solute carrier family 25, member 38	Slc25a38	-	-	-	0.38
RIKEN cDNA 4933431E20 gene	4933431E20Rik	-	-	-	0.37

SLIT and NTRK-like family, member 4	Slitrk4	-	-	-	-0.43
peroxisome proliferator activated receptor alpha	Ppara	-	-	-	0.46
paired related homeobox 1	Prrx1	-	-	-	-0.22
transmembrane protein 131	Tmem131	-	-	-	0.21
phosphodiesterase 7A	Pde7a	-	-	-	0.33
methyltransferase like 21E	Mettl21e	-	-	-	0.46

Table S4: Table showing details of candidate genes common between MyHC-emb^{ΔΔ} RNA-seq analysis and transcription factors regulating myogenesis found by Rajan et al (2012).

Genes	Rajan S et al (2012)			MyHC-emb ^{ΔΔ} RNA-seq	
	Log Fold change	Expression stage and effect	Validated	Log2 fold change	Muscles
Nr4a1	Max 3.29 Min -0.08	Early upregulated	yes	0.492792(G) 0.460666(TA)	Gastrocnemius and TA
Btg2	Max 0.94 Min 0.00	Early upregulated	yes	0.774867(D) 0.473973 (G) 0.631396(Q) 0.412594(TA)	Diaphragm, Gastrocnemius, Quadriceps and TA
Ccrn41	Max 1.52 Min 0.00	Early upregulated	No	0.537682(D) 0.525946(Q) 0.451526(TA)	Diaphragm, Quadriceps and TA
Ankrd1	Max 1.22 Min -0.14	Early upregulated	yes	0.542368 (D) 0.541384(G)	Diaphragm and Gastrocnemius
Maff	Max 0.83 Min -0.06	Early upregulated	yes	0.631282(D) 0.484519(Q)	Diaphragm and Quadriceps
Ankrd2	Max 4.12 Min	Late upregulated	yes	1.016406(D) 0.616889(G)	Diaphragm and Gastrocnemius
Cebpb	Max 0 Min-0.09	Late downregulated	No	0.634893(G) 0.56011(Q) 0.509648(TA)	Gastrocnemius, Quadriceps and TA
Asb2	-	-	yes	0.298487(G) 0.326305(TA)	Gastrocnemius and TA
Myc	Max 0.99 Min -0.22	Early upregulated	yes	0.321544(G)	Gastrocnemius
Prrx1	Max 0.00 Min -1.23	Early and late downregulated	No	-0.22888(TA)	TA
Hoxc8	Max 0.00 Min -1.13	Early downregulated	No	-0.39671 (Q)	Quadriceps
Runx2	Max 0.03 Min -1.24	Late downregulated	No	0.60068(G)	Gastrocnemius