Supporting Information

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SI Materials and Methods

Tamoxifen Injection and Cardiotoxin Injury. Tamoxifen (Sigma-Aldrich) was dissolved in 90% (vol/vol) sesame oil and 10% (vol/vol) ethanol at 25 mg/mL and delivered to both WT and mutant mice at 8 wk of age by i.p. injection at 0.1 mg/g for 5 d. Cardiotoxin (CTX) from *Naja mossambica mossambica* (Sigma-Aldrich) was dissolved in sterile saline to a final concentration of 10 μM and aliquoted and stored at –20 °C. Mice were anesthetized by i.p. injection of 2.5% Avertin at (15 μL/g). Mouse legs were shaved and cleaned. Tibialis anterior (TA) muscles were injected with 50 μL of CTX with a 26-gauge needle. After injection, animals were kept under a warming lamp until recovery. Barium chloride ($BaCl_2$) injury was performed by injecting 50 μL of 1.2% $BaCl_2$ (dissolved in sterile saline) into the TA muscle.

Immunohistochemistry. Frozen sections were fixed in freshly prepared 4% (vol/vol) paraformaldehyde for 20 min on ice and then treated with 0.3% Triton X-100/PBS at room temperature for 20 min. Sections were incubated with mouse IgG-blocking solution from the M.O.M. kit (Vector Lab) diluted in 0.01% Triton X-100/PBS at room temperature for 1 h. Sections were then incubated with 5% (vol/vol) goat serum (Sigma-Aldrich) in M.O.M. protein diluent for 30 min. Sections were incubated with primary antibodies diluted in M.O.M. protein diluent at 4 °C overnight. The next morning, slides were washed with PBS and incubated with secondary antibodies diluted in M.O.M. protein diluent at room temperature for 45 min. Sections were then washed and mounted with VectaShield Mounting Medium with DAPI. Pictures were taken with a Zeiss LSM 510 confocal microscope. Primary and secondary antibodies were as follows: Desmin (1:100; Dako), Laminin (1:200; Sigma-Aldrich), Alexa Fluor 594; goat anti-mouse IgG1 (1:400; Invitrogen), and Alexa Fluor 488 goat anti-rabbit IgG (1:400; Invitrogen).

Isolation of Satellite Cells by FACS. Isolation of satellite cells was performed as described. Briefly, hind limb muscles were pooled, minced, and digested with 0.2% Collagenase II (Gibco; Invitrogen), followed by trituration. Satellite cells were then isolated by further digestion of myofibers with 0.1% Dispase (Gibco; Invitrogen) and 0.05% Collagenase II (Gibco; Invitrogen). Cell suspension was filtered through a 40-μm cell strainer, and satellite cells were pelleted after centrifugation.

For FACS, satellite cells were counted and resuspended in PBS/3% (wt/vol) BSA at 1×10^6 cells per 50 μL. Cells were then incubated with the following antibodies for 1 h on ice: APC-CD29 (1:50) (AbD Serotec), PE-labeled rat anti-mouse CD45 (1:100), PE-labeled rat anti-mouse CD31 (1:100), PE-labeled anti-mouse Sca-1 (1:3,000) (all from BD Biosciences-Pharmingen). After incubation, cells were washed twice, filtered through a 40-μm cell strainer, and resuspended in PBS/3% (wt/vol) BSA at a concentration of 2×10^7 cells per mL. Cells were separated on a MoFlo Cytometer (Beckman Coulter). Sorting gates were strictly defined by the forward scatter and side scatter patterns of satellite cells as well as the positive control cells labeled with Alexa Fluor 488–CD34 and the negative control cells labeled with PE-CD45, PE-CD31, and PE–Sca-1. Cells positive for APC-CD29 and negative for PE-CD45, PE-CD31, and PE–Sca-1 were sorted to enrich for activated satellite cells.

Culture of Satellite Cell-Derived Myolbasts. CTX was injected into hind limb muscles and activated satellite cells were isolated 3 d after injection as described (1). Satellite cell-derived myoblasts were cultured on Matrigel-coated (BD Biosciences) plates in growth medium consisting of HAM's F-10 medium, 20% (vol/vol) bovine calf serum, and 5 ng/mL bFGF (Gibco; Invitrogen). Medium was changed daily. Cells were passaged at 70% confluence to prevent spontaneous differentiation. To induce differentiation, cells at 80% confluence were switched to differentiation medium containing DMEM and 2% (vol/vol) horse serum.

Immunostaining of Satellite Cell-Derived Myoblasts. For immunostaining, cells were grown on Matrigel-coated coverslips in either growth medium or differentiation medium. Cells were fixed on coverslips with 4% paraformaldehyde for 15 min, washed with PBS, and permeabilized with 0.3% Triton X-100/PBS for 5 min. Cells were blocked with 5% (vol/vol) goat serum/PBS/0.1% Triton X-100 for 3 min, followed by incubation with primary antibodies (diluted in 5% goat serum/PBS/0.1% Triton X-100) for 2 h. Cells were then washed in PBS and incubated with secondary antibodies for 1 h. After washing with PBS, coverslips were mounted on glass slides with VectoLab Mounting Medium (with DAPI). The antibodies used were as follows: anti-Pax7 (1:100, DSHB), MY32 (1:250, Sigma-Aldrich), Alexa Fluor 594 goat anti-mouse IgG1 (for Pax7) (1:400, Invitrogen), and Alexa Fluor 555 goat anti-mouse IgG (for MY32) (1:400, Invitrogen).

EdU Labeling and Detection. For cultured cells, EdU supplied in the Click-iT EdU cell proliferation assay kit (Invitrogen) was used as instructed with minor modifications: EdU was used at 10 μM for 8 h in culture media; for detection, we performed immunostaining for primary and secondary antibodies (see above) first, then the click chemical reaction using Alexa Fluor 647 as a reactive fluorophore for detection, followed by Hoescht staining.

Microarray Analysis and Bioinformatics. For microarray analysis, WT and MEF2-TKO myoblasts were cultured in differentiation medium for 3 d and total RNA was isolated by using TRIzol reagent (Invitrogen). Microarray analysis was performed by the University of Texas Southwestern Microarray Core Facility by using the MouseWG-6 v2.0 BeadChips (Illumina). Gene ontology (GO) analysis was performed by using DAVID with ILLUMINA_ID identifiers and Mus musculus as the background set (2, 3). For GO term enrichment, biological process level 4 was used. MEF2D ChIP-seq peaks from a published study (4) were compared with transcriptional start sites (TSS) of genes from the expression microarray. MEF2D binding sites were considered to be nearby if the ChIP-seq peak overlapped with a 50-bp window around the TSS using a custom script based on the HTSeq python package (www-huber.embl.de/users/anders/HTSeq/). PubMed searches were automatically conducted by using a custom script based on Biopython for significantly regulated genes using all known aliases to prevent underestimation of publication number.

Statistics. Data are presented as mean ± SEM. Differences between groups were tested for statistical significance by using the unpaired two-tailed Student *t* test. $P < 0.05$ was considered significant.

1. Liu N, et al. (2012) microRNA-206 promotes skeletal muscle regeneration and delays progression of Duchenne muscular dystrophy in mice. *J Clin Invest* 122(6):2054–2065.

2. Huang W, Sherman BT, Lempicki RA (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44–57.

3. Huang W, Sherman BT, Lempicki RA (2009) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37 (1):1–13.

4. Sebastian S, et al. (2013) Tissue-specific splicing of a ubiquitously expressed transcription factor is essential for muscle differentiation. *Genes Dev* 27(11): 1247–1259.

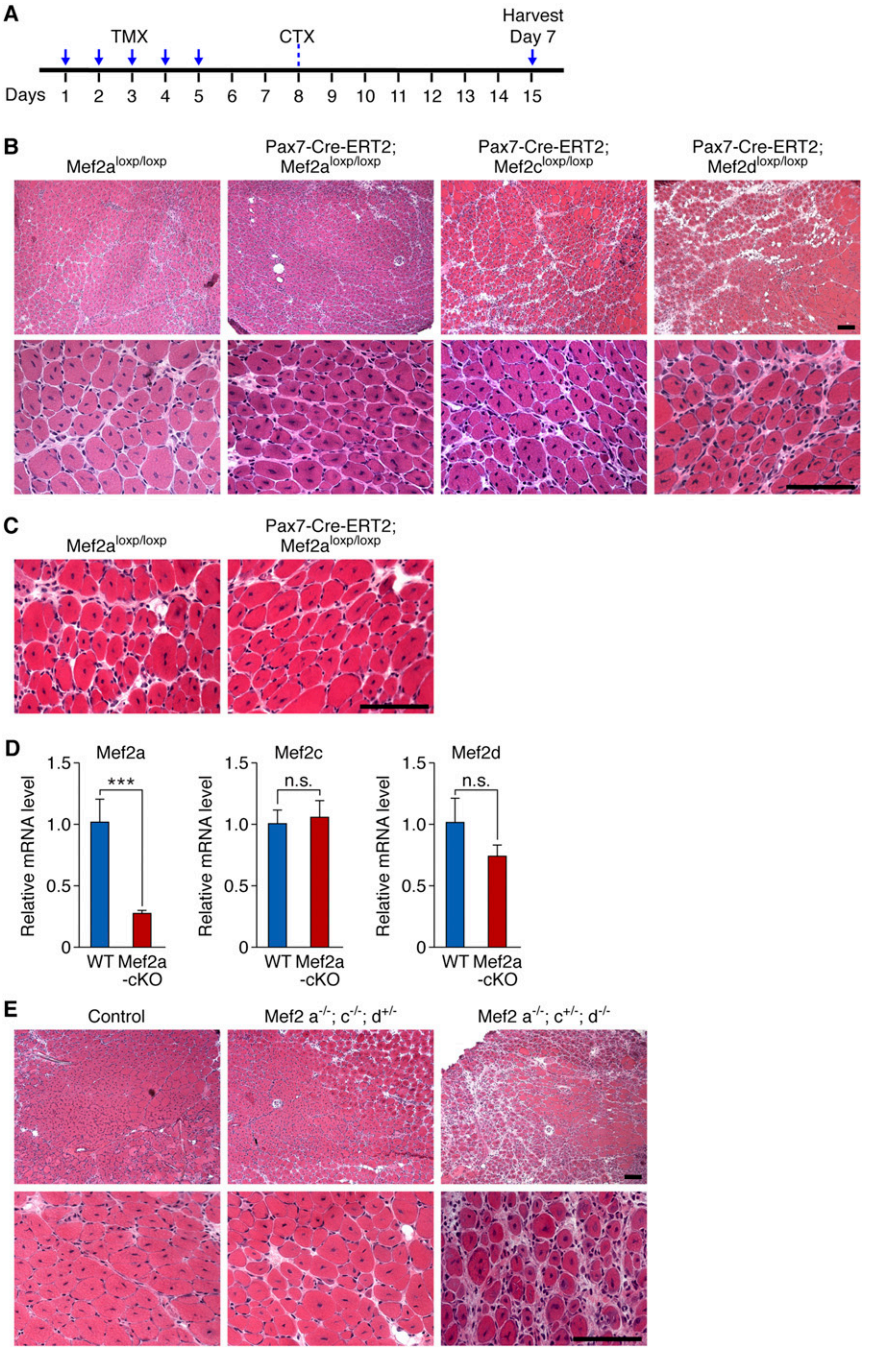


Fig. S1. MEF2A, C, and D play redundant roles in adult skeletal muscle regeneration. (*A*) Schematic of tamoxifen (TMX) and CTX treatment. (*B*) TA muscles from *Mef2a* $^{loxp/loxp}$; Pax7-Cre-ERT2; *Mef2a* $^{loxp/loxp}$; Pax7-Cre-ERT2; *Mef2c* $^{loxp/loxp}$; and Pax7-Cre-ERT2; *Mef2d* $^{loxp/loxp}$ mice were analyzed by H&E staining on day 7 after injury. (*C*) TA muscles from *Mef2a* $^{loxp/loxp}$ and Pax7-Cre-ERT2; *Mef2a* $^{loxp/loxp}$ mice were analyzed by H&E staining on day 7 after $BaCl_2$ injury. (*D*) TA muscle from WT and Pax7-Cre-ERT2; *Mef2a* $^{loxp/loxp}$ (Mef2a-cKO) mice was harvested 10 d after CTX injury for RNA analysis of Mef2a, c, and d transcripts by real-time RT-PCR. Values were normalized to 18s rRNA and then normalized to WT. ***$P < 0.001$. ns, not significant. (*E*) TA muscles from control (*Mef2a* $^{loxp/loxp}$; *2c* $^{loxp/loxp}$; *2d* $^{loxp/loxp}$); *Mef2a*$^{-/-}$; *c*$^{-/-}$; *d*$^{+/-}$ (Pax7-Cre-ERT2; *Mef2a* $^{loxp/loxp}$; *2c* $^{loxp/loxp}$; *2d* $^{loxp/+}$); and *Mef2a*$^{-/-}$; *c*$^{+/-}$; *d*$^{-/-}$ (Pax7-Cre-ERT2; *Mef2a* $^{loxp/loxp}$; *2c* $^{loxp/+}$; *2d* $^{loxp/loxp}$) mice were analyzed by H&E staining on day 7 after injury. (Scale bars: 100 μm.)

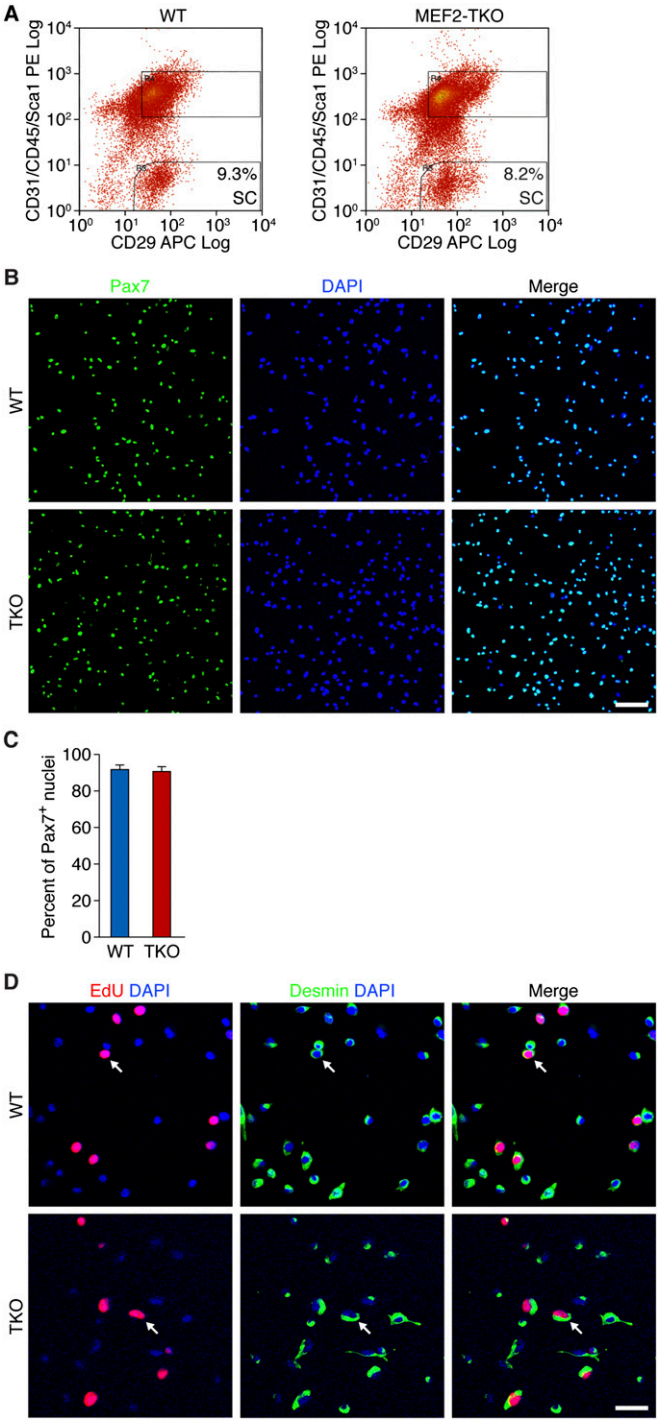
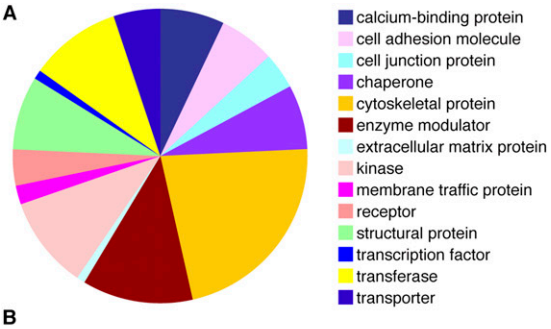


Fig. S2. Normal proliferation of MEF2-TKO satellite cell-derived myoblasts. (*A*) FACS plots of WT and MEF2-TKO–activated satellite cells are shown. Cells were harvested at day 3 after injury. $CD29^+$ $Sca1^-$ $CD31^-CD45^-$ cells were collected as activated satellite cells. There was no significant difference in the percentage of activated satellite cells between WT and MEF2-TKO mice. SC, satellite cells. (*B*) Immunostaining for Pax7 in WT and MEF2-TKO myoblasts showed that most cells are $Pax7^+$ myoblasts. (*C*) Percentage of $Pax7^+$ cells in WT and MEF2-TKO were counted and plotted. n.s, not significant. (*D*) Immunostaining for desmin and EdU in WT and MEF2-TKO myoblasts showed that EdU^+ myoblasts express desmin (arrows). (Scale bars: 100 μm.)

A



B

Gene Symbol	Description	Fold Down
	muscle contraction	
Myh4	Skeletal muscle myosin heavy chain II-b	6.962
Myl6b	myosin light chain 6B	6.234
Myom1	myomesin-1 isoform 1	6.157
Actn2	alpha-actinin-2	5.959
Mybpc1	myosin binding protein C, slow type	5.688
Myh1	skeletal muscle myosin heavy chain II-d/x	4.782
Mybpc2	myosin-binding protein C, fast-type isoform 2	3.569
Myh8	myosin, heavy polypeptide 8, skeletal muscle, perinatal	3.191
Ttn	titin	2.662
Tpm2	tropomyosin beta chain	2.603
Ryr1	ryanodine receptor 1, skeletal muscle	2.550
Myom2	myomesin 2	2.496
Trim63	E3 ubiquitin-protein ligase TRIM63	2.335
	myofibril assembly	
Tcap	telethonin	21.750
Xirp1	xin actin-binding repeat-containing protein 1	4.649
Capn3	calpain 3	2.911
Neurl2	neuralized-like protein 2	2.619
Murc	muscle-related coiled-coil protein	2.557
Myl2	myosin regulatory light chain 2,	2.476
Tmod1	tropomodulin-1	2.001
	muscle organ development	
Myf6	myogenic factor 6	7.801
Trp63	tumor protein 63 isoform c	7.217
Ky	kyphoscoliosis peptidase	5.350
Csrp3	cysteine and glycine-rich protein 3	3.915
Myo18b	myosin 18b	3.442
Rxrg	retinoic acid receptor RXR-gamma isoform 1	3.067
Tnni1	troponin I, slow skeletal muscle	2.867
Jph1	junctophilin-1	2.704
Mef2c	myocyte-specific enhancer factor 2C isoform 2	2.232
Cryab	alpha-crystallin B chain	2.231
Csrp3	cysteine and glycine-rich protein 3	2.106
Erbb2	receptor tyrosine-protein kinase erbB-2	2.005
	response to heat	
Hspb6	Mus musculus 13 days embryo brain cDNA	2.442
Hspb7	heat shock protein beta-7	8.671
Hspb2	heat shock protein beta-2 isoform 2	2.633
Hspb3	heat shock protein beta-3	4.426
Hspb8	heat shock protein beta-8	2.409
Cryab	alpha-crystallin B chain	2.231
	regulation of transport	
Pacsin1	protein kinase C and casein kinase substrate in	11.555
Pfkm	6-phosphofructokinase, muscle type	4.604
Pacsin3	protein kinase C and casein kinase II substrate	3.101
Mybph	myosin-binding protein H	2.879
Pea15a	astrocytic phosphoprotein PEA-15 isoform 2	2.788
Stim1	Mus musculus 3 days neonate thymus cDNA	2.601
Cav3	caveolin-3	2.223
Uaca	uveal autoantigen with coiled-coil domains and	2.194
Orai1	calcium release-activated calcium channel	2.127
Sln	sarcolipin	2.125
Tgfb3	transforming growth factor beta-3 preproprotein	2.073
Rab3d	ras-related protein Rab-3D	2.018
Tac4	tachykinin-4 precursor	2.004
	negative regulation of microtubule depolymerization	
Mtap4	SubName: Full=Putative uncharacterized protein;	2.389
Mid1ip1	mid1-interacting protein 1	2.277
Mtap1b	microtubule-associated protein 1B	2.471
Trim54	tripartite motif-containing protein 54	2.161
DQ722155	Mus musculus adult male hippocampus cDNA	3.124
	negative regulation of cytoskeleton organization	
Spnb1	spectrin beta chain, erythrocyte	4.121
DQ722155	Mus musculus adult male hippocampus cDNA	3.124
Kank1	KN motif and ankyrin repeat domain-containing	2.917
Mtap1b	microtubule-associated protein 1B	2.471
Mtap4	SubName: Full=Putative uncharacterized protein;	2.389
Mid1ip1	mid1-interacting protein 1	2.277
Trim54	tripartite motif-containing protein 54	2.161

Fig. S3. (*A*) GO analysis was performed with DAVID. Microarray data from WT and MEF2-TKO myotubes were used in the analysis. Protein classes were assigned to the genes that fall in the most significantly enriched biological process "muscle contraction" as presented in Fig. 5*D*. (*B*) List of most down-regulated in MEF2-TKO myotubes. Genes were grouped according to GO terms of biological processes by DAVID.

Gene Symbol	Description	Fold Down	Mef2D Site 50bp Window
Myadml2	myeloid-associated differentiation marker-like	11.639	Yes
2310002L09Rik	hypothetical protein LOC71886 isoform 2	9.165	No
Dysfip1	dysferlin-interacting protein 1	7.062	No
Tceal5	transcription elongation factor A protein-like	5.905	No
Fam78a	hypothetical protein LOC241303	5.675	No
1500017E21Rik	putative noncoding RNA	4.901	No
Gm10406	predicted gene 10406	4.614	No
Gm3696	alpha25-takusan	4.609	No
AK008705	RIKEN cDNA 2210011C24 gene	4.330	Yes
Yipf7	protein YIPF7	4.293	Yes
Dhrs7c	Dhrs7c dehydrogenase/reductase (SDR family) member 7C	4.147	Yes
Adamtsl5	ADAMTS-like protein 5 isoform 2	4.139	Yes
Gm1973	predicted gene 1973	4.094	No
Klhl30	kelch-like protein 30	3.996	Yes
Prelid2	PRELI domain-containing protein 2	3.928	No
Fsd2	fibronectin type III and SPRY domain-containing	3.846	Yes
AK014378	putative noncoding RNA	3.806	No
Ablim2	actin-binding LIM protein 2 isoform 1	3.768	Yes
AK031097	protein phosphatase 1 (formerly 2C)-like	3.533	No
AK006812	putative noncoding RNA	3.442	No
Tceal7	transcription elongation factor A (SII)-like 7	3.401	No
Afap1l1	actin filament-associated protein 1-like 1	3.367	No
Myom3	myomesin-3	3.339	No
Zdbf2	DBF4-type zinc finger-containing protein 2	3.171	No
Dennd2c	DENN domain-containing protein 2C	3.139	No
DQ722155	integrator complex subunit 6	3.124	No
AK165804	putative noncoding RNA	3.109	No
Igfn1	immunoglobulin-like and fibronectin type III domain containing 1	3.039	No
Kbtbd13	kelch repeat and BTB domain-containing protein	2.990	No
Cacng6	voltage-dependent calcium channel gamma-6	2.987	No
AK009335	putative noncoding RNA	2.878	No
Adamtsl5	ADAMTS-like protein 5 isoform 2	2.861	Yes
Stac2	SH3 and cysteine-rich domain-containing protein	2.826	No
A830080H07RIK	putative noncoding RNA	2.768	No
Zfp385c	zinc finger protein 385C	2.765	No
Ttc39b	tetratricopeptide repeat protein 39B	2.759	No
Ntn5	netrin-5	2.714	No
Rftn2	raftlin-2	2.704	No
Gm13305	interleukin 11 receptor, alpha chain 2-like	2.680	No
NCTC1	putative noncoding RNA	2.643	No
Kbtbd5	kelch repeat and BTB domain-containing protein	2.620	Yes
Lrrc14b	leucine-rich repeat-containing protein 14B	2.616	Yes
1700113I22Rik	hypothetical protein LOC73635	2.510	Yes
Ccdc141	coiled-coil domain containing 141	2.504	Yes
Fam171a2	hypothetical protein LOC217219 precursor	2.456	No
Lrtm1	SubName: Full=Leucine-rich repeats and transmembrane domains 1;	2.444	No
Gm10341	SubName: Full=Putative uncharacterized protein Gm2974;	2.437	No
Mfsd7	RecName: Full=Major facilitator superfamily domain-containing protein 7;	2.415	No
Lrrc30	leucine-rich repeat-containing protein 30	2.409	Yes
Gm3696	alpha25-takusan	2.404	No
BC046404	RecName: Full=Leucine-rich repeat-containing protein C17orf76 homolog;	2.400	No
Fam171b	family with sequence similarity 171, member B	2.398	No
Btbd6	BTB/POZ domain-containing protein 6 isoform 2	2.392	No
Calr4	calreticulin 4	2.364	No
Riiad1	RIIa domain-containing protein C1orf230 homolog	2.353	No
9930013L23Rik	KIAA1199 homolog precursor	2.349	No
Gpr146	probable G-protein coupled receptor 146 isoform	2.349	No
2610019F03Rik	hypothetical protein LOC72148	2.345	No
Tmem119	transmembrane protein 119 precursor	2.343	Yes
Enho	adropin precursor	2.337	No
Chst13	carbohydrate sulfotransferase 13	2.334	No
Enho	adropin precursor	2.312	No
Ablim3	actin-binding LIM protein 3	2.289	Yes
Tmem179	transmembrane protein 179	2.283	No
Zfp385b	zinc finger protein 385B isoform 3	2.280	No
1110028C15Rik	hypothetical protein LOC68691 isoform 2	2.279	No
AK165889	predicted gene 10419p	2.266	No
Zfp773	putative noncoding RNA	2.266	No
Tmem62	transmembrane protein 62	2.247	No
Gm13305	interleukin 11 receptor, alpha chain 2-like	2.235	No
Myom3	myomesin-3	2.231	No
PAPK-A	Mus musculus mRNA for polyploidy associated protein kinase PAPK-A, complete cds.	2.219	No
7030401E22RIK	putative noncoding RNA	2.202	No
Sel1l3	protein sel-1 homolog 3	2.178	No
2900026A02Rik	hypothetical protein LOC243219	2.173	No
Tmem117	transmembrane protein 117	2.168	Yes
Mospd1	motile sperm domain-containing protein 1	2.165	No
BC046404	RecName: Full=Leucine-rich repeat-containing protein C17orf76 homolog;	2.150	No
PAPK-A	Mus musculus mRNA for polyploidy associated protein kinase PAPK-A, complete cds.	2.123	No
Gm3219	Mus musculus predicted pseudogene 3219 (Gm3219), non-coding RNA.	2.096	No
Gm2506	chemokine (C-C motif) ligand 27b isoform 3	2.094	No
Ccdc92	coiled-coil domain-containing protein 92	2.080	No
Gm889	hypothetical protein LOC380755	2.025	No
BC024659	hypothetical protein LOC108934	2.022	Yes
C1qtnf4	complement C1q tumor necrosis factor-related	2.011	No
Shisa4	protein shisa-4 precursor	2.006	Yes

Fig. S4. List of uncharacterized or minimally characterized genes that are down-regulated in MEF2-TKO myotubes. Genes that contain MEF2-binding sites near their promoters are indicated as yes.