

# Supporting Information

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## SI Materials and Methods

**Generation of *Mymx*<sup>loxP</sup> Mice.** Animal work described in this paper was approved and conducted under the oversight of the University of Texas Southwestern Institutional Animal Care and Use Committee. B6C3F1 mice were used as oocyte donors. Superovulated female B6C3F1 mice (6 wk old) were mated to B6C3F1 stud males. Zygotes were harvested and kept in M16 medium (Brinster's medium for ovum culture with 100 units/mL penicillin and 50 mg/mL streptomycin) at 37 °C for 1 h. Zygotes were transferred to M2 medium (M16 medium and 20 mM Hepes). A mixture of Cas9 mRNA, a pair of Myomixer sgRNAs (designed by [crispr.mit.edu](http://crispr.mit.edu)) and corresponding single-stranded oligodeoxynucleotide DNA (ssODNA, IDT Ultramer DNA Oligos) donors was injected into the pronucleus and cytoplasm. Sequences are as follows: sgRNA1, 5'-CGGGTTTTAGACTGTACCTC-3'; sgRNA2, 5'-GCCATTGCTGGGCGGGCCGC-3'; sgRNA3, 5'-AGTGATGCTGAATCCACCGC-3'; sgRNA4, 5'-CTACCGGTTGAGCTAATTCC-3'; ssODNA1, 5'-CAGTTGAAAAGTGGGAGTACAGAGGTATAAGCTGAAGGGAGGACTCCACACCCCGCACCCCTGCTTTGCCAGGTGAGGTGCGTGCTGAGGgtcgacATAACTTCGTATAGCATAACATTATACGAAGTTATGTACAGTCTAAAACCCGGGTTCCCTTTCAGGTGCA-3'; ssODNA2, 5'-CTTTGGATAAAGTTCATCTGTCTGTCCCTCGGTCCTCACAAAGCTACAAGTTTGGAAATTGTGTAAGTTCATTTAGAATGTCTCTGCGccgagATAACTTCGTATAGCATAACATTATACGAAGTTATGCCCCGCCAGCAATGGCTTAGATCTCTGTTGCAGGA-3'; ssODNA3, 5'-TTGCCCTCCTGCTCACCAGTTCTAACC-GGCCAGTGGCAGCTCACCAGTCCAGACAGTCTCAAGATCAATTTACAGGATGCTTTTGCCTGCGgtcgacATAACTTCGTATAATGTATGCTATAACGAAGTTATGTGGA-TTCAGCATCACTGGACATCACAAAGGGAGCCC-3'; and ssODNA4, 5'-CAGCTTCCCACAGATACCAACCCAGAGGTAGGGCAGAGTAGGGGTGGTGGAGATTTTCTGAGACACCTCAGTCATCCAACACAGAGCCTGGAccgagATAACTTCGTATAATGTATGCTATAACGAAGTTATATTAGCTCAACCGGTAGAGAGAGCGCTTGCTTGCG-3'.

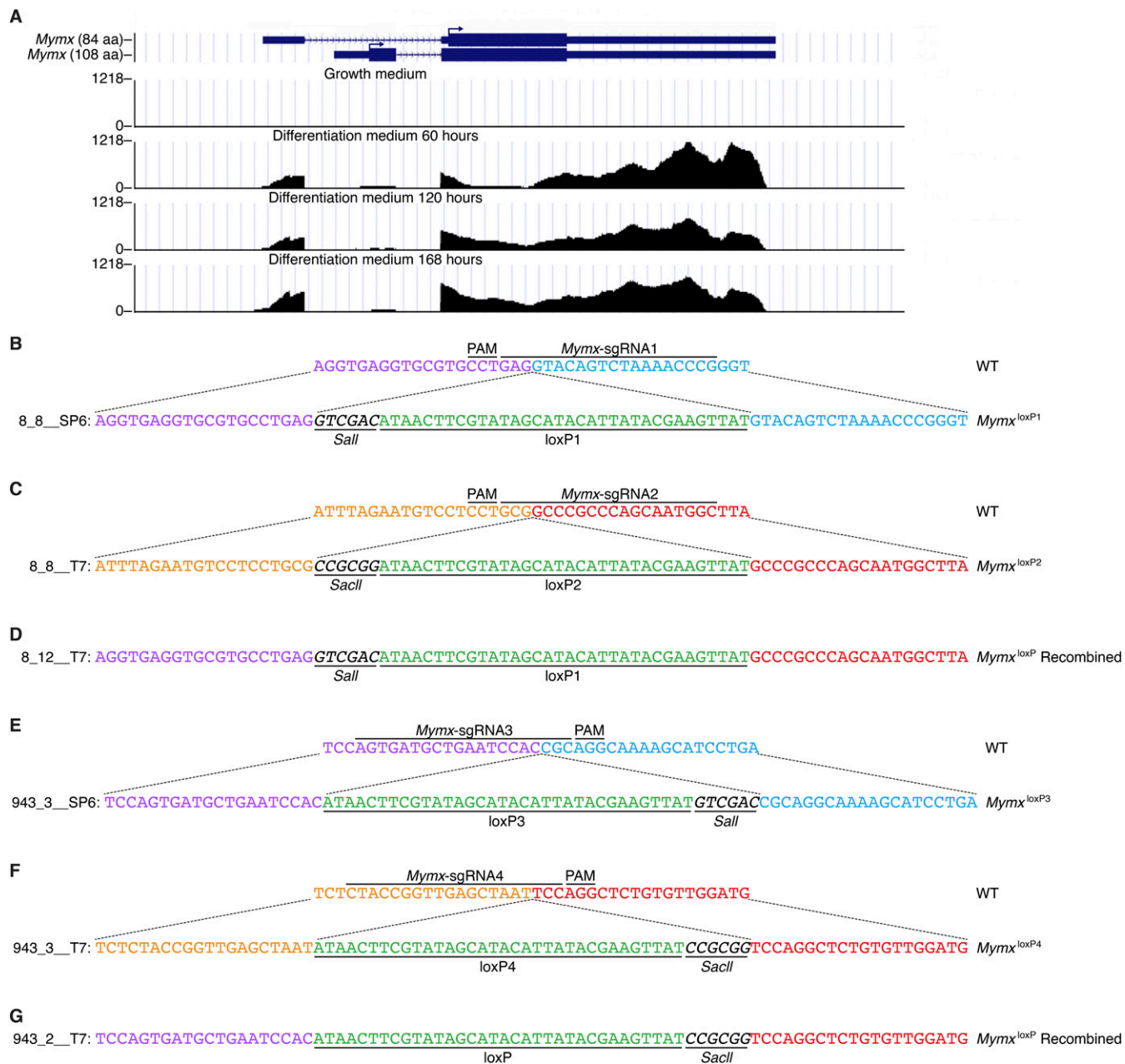
Injected zygotes were cultured in M16 medium for 1 h at 37 °C and then transferred into the oviducts of pseudopregnant ICR female mice. Three-week-old F0 mice were PCR genotyped with a forward primer: 5'-GGGCTCCCTTGTGATGTCC-3' and a reverse primer: 5'-TGCGTACACAAAAGTGCTCG-3' to test for simultaneous insertions of two loxP sites onto the same chromosome. This pair of primers detects a 2,075-bp wild-type band, a

2,155-bp *Mymx*<sup>loxP1/loxP2</sup>, or a 2,155-bp *Mymx*<sup>loxP3/loxP4</sup> band. PCR genotyping products were gel purified and cloned into the pCRII Topo vector (K460001, Thermo Fisher) and sequenced to verify the correct targeting.

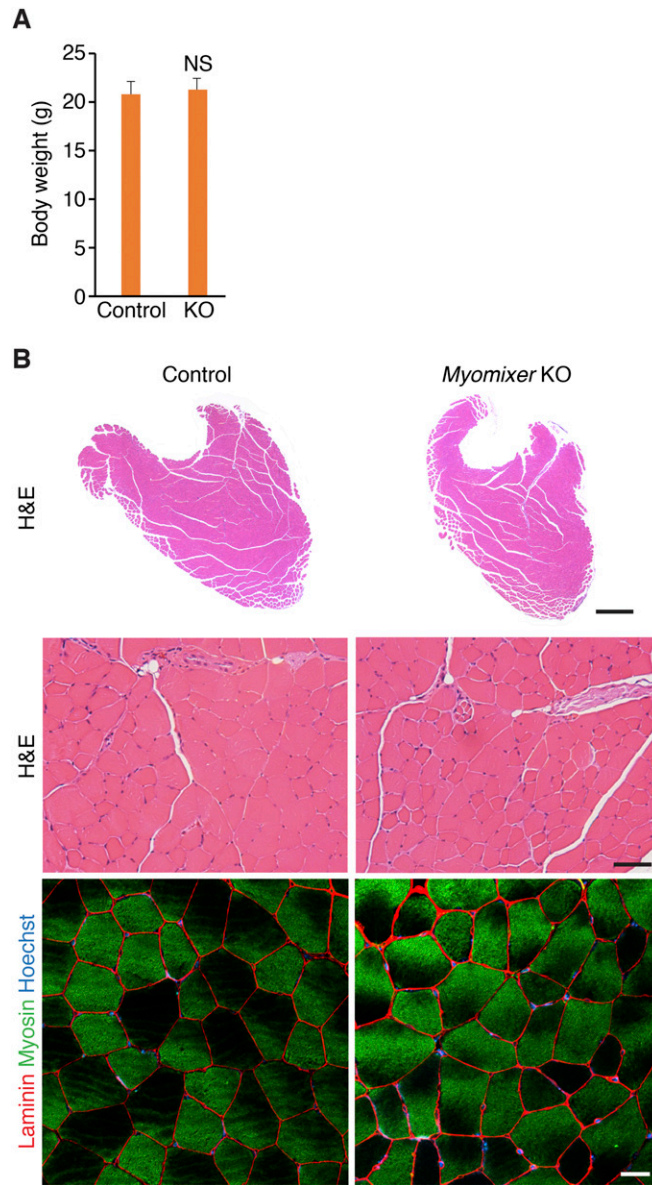
Correctly targeted F0 founder mice were crossed to *Pax7*<sup>CreERT2</sup> mice to achieve germline transmission of the targeted allele. The *Pax7*<sup>CreERT2</sup> mice were provided by C.-M. Fan, Carnegie Institution for Science, Baltimore (22). The F1 mice and mice of the following generations were genotyped with primers that are specific for each loxP site. LoxP1-F (5'-GCCAGAAGAAAGCTGCACTG-3') and loxP1-R (5'-TGTTGCTGGCTGAGGACAAA-3') detect a 349-bp wild-type band and a 389-bp knockin band. LoxP2-F (5'-CGGTCCTCACAAAGCTACAAGT-3') and loxP2-R (5'-ATGTCCCTAAAGGTCACGCA-3') detect a 232-bp wild-type band and a 272-bp knockin band. LoxP3-F (5'-AAGAGTCCACACGCTTCAGG-3') and loxP3-R (5'-AACTTCTTGCCCTCCTGCTC-3') detect a 347-bp wild-type band and a 387-bp knockin band. LoxP4-F (5'-GGTACGCAGCAGGTAGTCAA-3') and loxP4-R (5'-GTAGCTTGTGAGGACCGAGG-3') detect a 446-bp wild-type band and a 486-bp knockin band.

**Tamoxifen and Cardiotoxin Treatment.** Tamoxifen (T5648, Sigma-Aldrich) was dissolved at 10 mg·mL<sup>-1</sup> in ethanol. Before injection, this stock solution was diluted with seven-time volumes of sesame oil (S3547, Sigma-Aldrich). Two milligrams of tamoxifen was administered by i.p. injection to 2-mo-old adult mice as schematized in Figs. 2*A* and 4*A* and *D*. Cardiotoxin (CTX) (*Naja mossambica mossambica*, Sigma-Aldrich) was dissolved in sterile saline at 10 μM concentration. For muscle injury, mice were anesthetized and 50 μL CTX (10 μM) was delivered by intramuscular injection to TA muscle or 100 μL CTX was delivered to gastrocnemius muscle with a 28-gauge needle. As a control, muscle from contralateral hind limb was injected with the same volume of saline. Muscles were harvested at days 3, 7, and 14 postinjury.

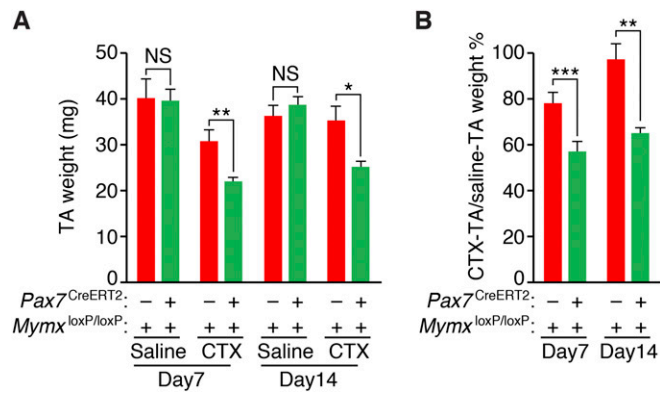
**Primers Used for qPCR.** The following primers were used for qPCR: *Mymx*-F, 5'-CTGAGCTGTCTGCTCTTTGT-3'; *Mymx*-R, 5'-TCTCCTTCTCTGGGAGTG-3'; *Mymk*-F, 5'-CCTGCTGCTCTCCCAAG-3'; *Mymk*-R, 5'-AGAACCAGTGGGTCCCTAA-3'; *Myod1*-F, 5'-CCACTCCGGGACATAGACTTG-3'; *Myod1*-R, 5'-AAAAGCGCAGGTCTGGTGGAG-3'; *Myog*-F, 5'-GAGACATCCCCCTATTTCTACCA-3'; *Myog*-R, 5'-GCTCAGTCCGCTCATAGCC-3'; *Gapdh*-F, 5'-GAAGGTCCGGTGTGAACGG-3'; and *Gapdh*-R, 5'-TGAAGGGGTGCTTGATGG-3'.



**Fig. S1.** (A) University of California Santa Cruz Genome Browser track hub displaying RNA sequencing peaks (GSE20846) to show expression of the short and long isoforms of Myomixer during C2C12 differentiation. Arrows indicate the translation start sites. (B–G) Sequencing results of genotyping PCR products shown in Fig. 1. Numbers before sequences are sample identification numbers.



**Fig. S2.** (A) Body weight of mice ( $n = 5$  for control and *Myomixer* KO mice) and (B) histology analysis of control and *Myomixer* KO mice at day 4 post last tamoxifen injection. (Scale bars: Top H&E staining image, 500  $\mu\text{m}$ ; Bottom H&E staining image, 50  $\mu\text{m}$ ; fluorescence image, 20  $\mu\text{m}$ .) NS, not significant. Student's  $t$  test. Data are mean  $\pm$  SEM.



**Fig. S3.** Muscle weight measurement (A) and weight ratio (B) of saline or CTX-injected TA muscles of the indicated genotype at day 7 postinjury ( $n = 5$  for control and  $n = 7$  for *Myomixer* KO mice) and day 14 postinjury ( $n = 5$  for control and *Myomixer* KO mice).  $*P < 0.05$ ;  $**P < 0.01$ ;  $***P < 0.001$ . Student's *t* test. Data are mean  $\pm$  SEM. NS, not significant.