Supporting information

Figure S1. Rbfox1 expression level increases during primary myoblast differentiation.

Primary myoblasts were isolated and in vitro differentiated. Cells were collected at the indicated time points and used for protein extraction. Western blot analysis for Rbfox1 showed increased protein expression during differentiation (top panel). Laminin B (middle panel) and Ponceau staining (bottom panel) show equal loading between samples.

Figure S2. *Rbfox1* depletion in satellite cells does not disrupt skeletal muscle regeneration.

(A) The genotype of control and *Rbfox1*$^{-/-}$ mice is schematically represented. In the control mouse (left), the floxed *Rbfox1* exon 11 and 12 are represented as red boxes, flanked by two loxP sites (green boxes). Wild-type alleles are represented as solid black bars. In *Rbfox1*$^{-/-}$ mice (right), expression of Cre recombinase under the control of the *Pax7* gene promoter (green box), drives recombination between the loxP sites and deletion of *Rbfox1* exons 11 and 12. (B) Scheme of tamoxifen administration, satellite cell isolation and differentiation. (C) Total RNA was isolated from differentiated (96 hours) myotubes. Quantitative RT-PCR was used to assess *Rbfox1* depletion efficiency. The results are expressed as the mean ± s.e.m. and the *p* values were estimated using two-tailed Student's T-test (** $p \leq 0.01$). (D) Hematoxylin and eosin staining of cross-sections of control and *Rbfox1*$^{-/-}$ tibialis anterior muscles at days 4, 14 and 30 after cardiotoxin injury. Scale bar 20 μm.

Figure S3. Analysis of regeneration markers upon *Rbfox1* depletion. (A) Immunostaining for laminin and MHC fast (top panels) or slow (bottom panel) on control and *Rbfox1*$^{-/-}$ tibialis anterior muscles. Scale bar 30 μm. (B) Top panel: immunostaining for desmin on control and *Rbfox1*$^{-/-}$ tibialis anterior muscles. Bottom panel: immunostaining for laminin and neonatal MHC. (C) Total RNA was extracted from control and *Rbfox1*$^{-/-}$ tibialis anterior muscles and quantitative RT-PCR analysis was performed in triplicate using TaqMan probes for Fbxo32 and Trim63. Data were normalized to the Rpl30 content and expressed as fold increase. The results were expressed as the mean ± s.e.m.

Figure S4. RT-PCR validation of alternative splicing events.

(A) RT-PCR validation (n = 2 biological replicates) for AS events (ΔPSI $\geq$ 10%) regulated by Rbfox1. PSI is indicated below each lane.

Figure S5. Depletion of Rbfox1 alters calcium kinetics and decreases muscle force generation.

(A) Immunostaining for Ryr1 and Serca1 in two month-old control and *Rbfox1*$^{-/-}$ gastrocnemius muscles shows early mislocalization of both calcium channels in *Rbfox1*$^{-/-}$ muscle (arrows). Scale bar 20 □m. (B) Top panel. Average time to the maximum response in FDB myofibers stimulated with 4-CMC. Bottom panel. Amplitude of the calcium transient stimulated with 1mM of 4-CMC. (C) Area under the curve (AUC) of the calcium transient evoked electrically with a 50 Hz (top) or 100 Hz train (bottom). **

indicates $p \leq 0.01$ as determined by two-tailed Student's T-test. (D) Force-frequency relationship obtained from *ex vivo* FBD muscle ($n = 6$ mice for each genotype) isolated from either male (left panel) or female (right panel) five month-old control and *Rbfox1*$^{-/-}$ mice.

Table S1. Parameters of RNA-Seq results on tibialis anterior muscle from control and *Rbfox1*$^{-/-}$.

Table S2. List of genes undergoing expression changes in *Rbfox1*$^{-/-}$.

The table also contains a list of genes sorted based on $p \leq 0.0010$ and fold change ≥ 1.5

Table S3. List of AS events in *Rbfox1*$^{-/-}$.

A total of 210 events are listed with BF ≥ 2.0 and ΔPSI (MPD) ≥ 0.10)

Table S4. List of primers used to validate splicing events

Figure S1

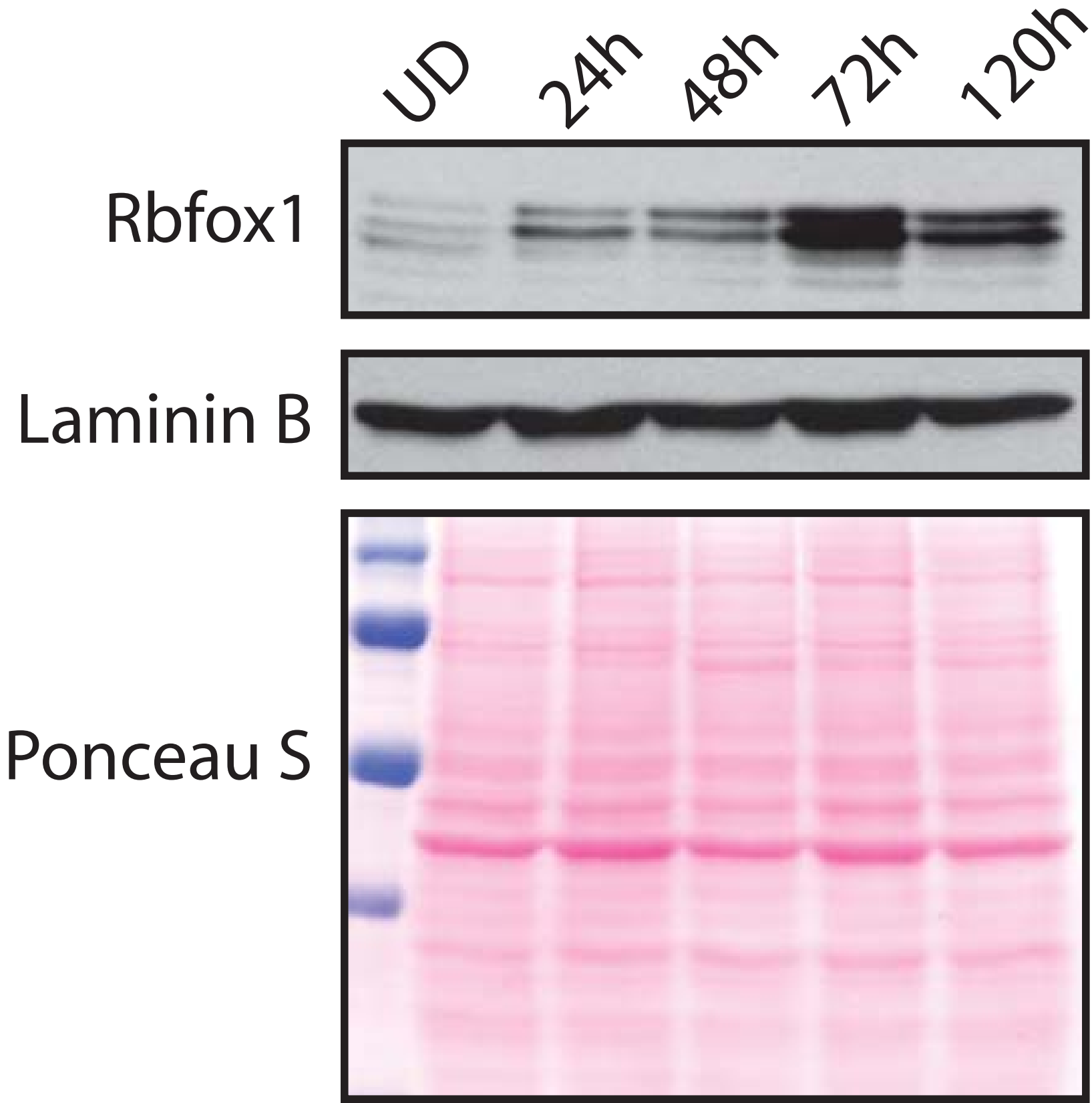

Figure S2

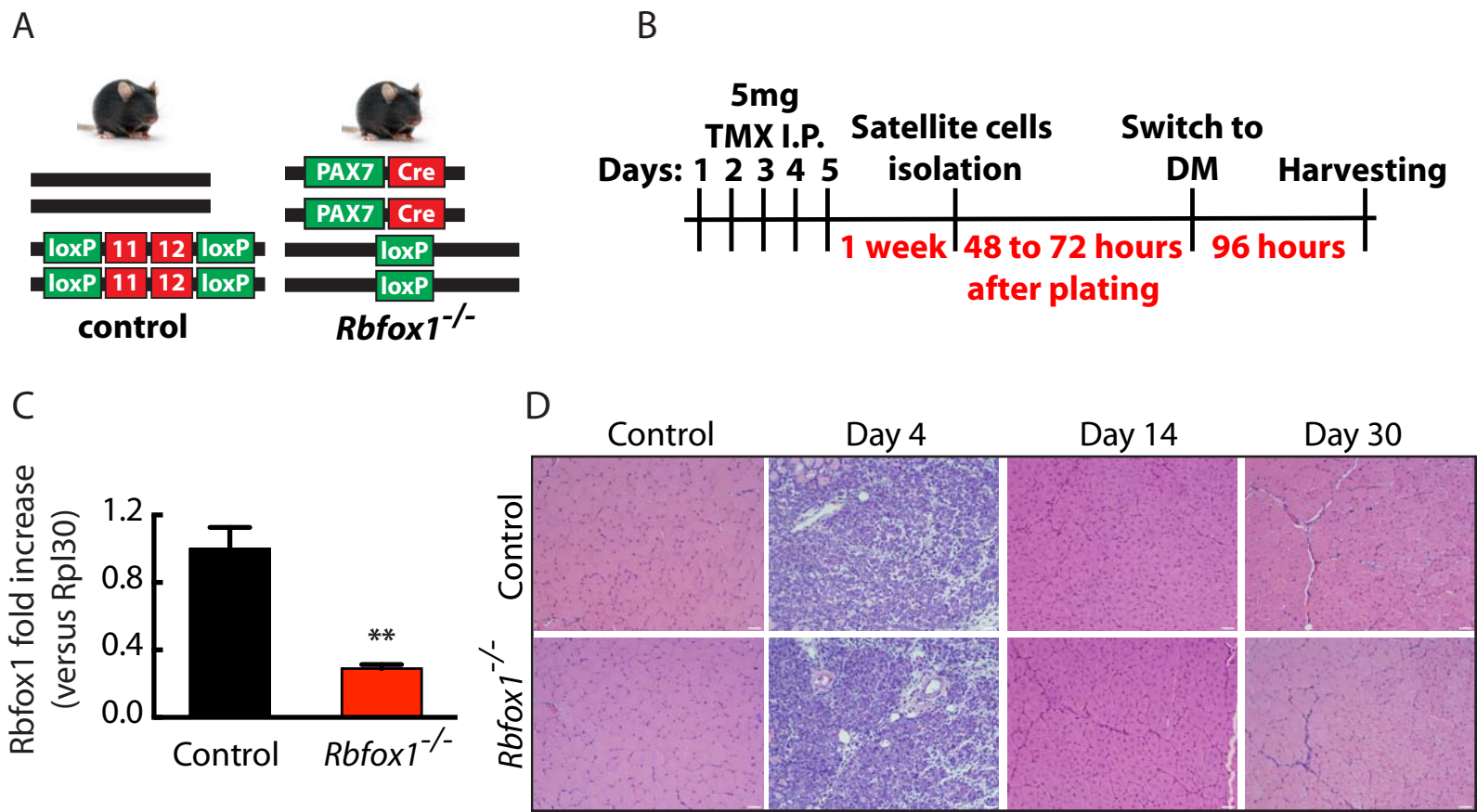
A
loxP
11
12
loxP
loxP
11
12
loxP
control
PAX7
Cre
PAX7
Cre
loxP
loxP
Rbfox1-/-
B
5mg
TMX I.P.
Days: 1 2 3 4 5
Satellite cells
isolation
Switch to
DM
Harvesting
1 week
48 to 72 hours
after plating
96 hours
C
Rbfox1 fold increase
(versus Rpl30)
1.2
0.8
0.4
0.0
**
Control
Rbfox1-/-
D
Control
Day 4
Day 14
Day 30
Control
Rbfox1-/-

Figure S3

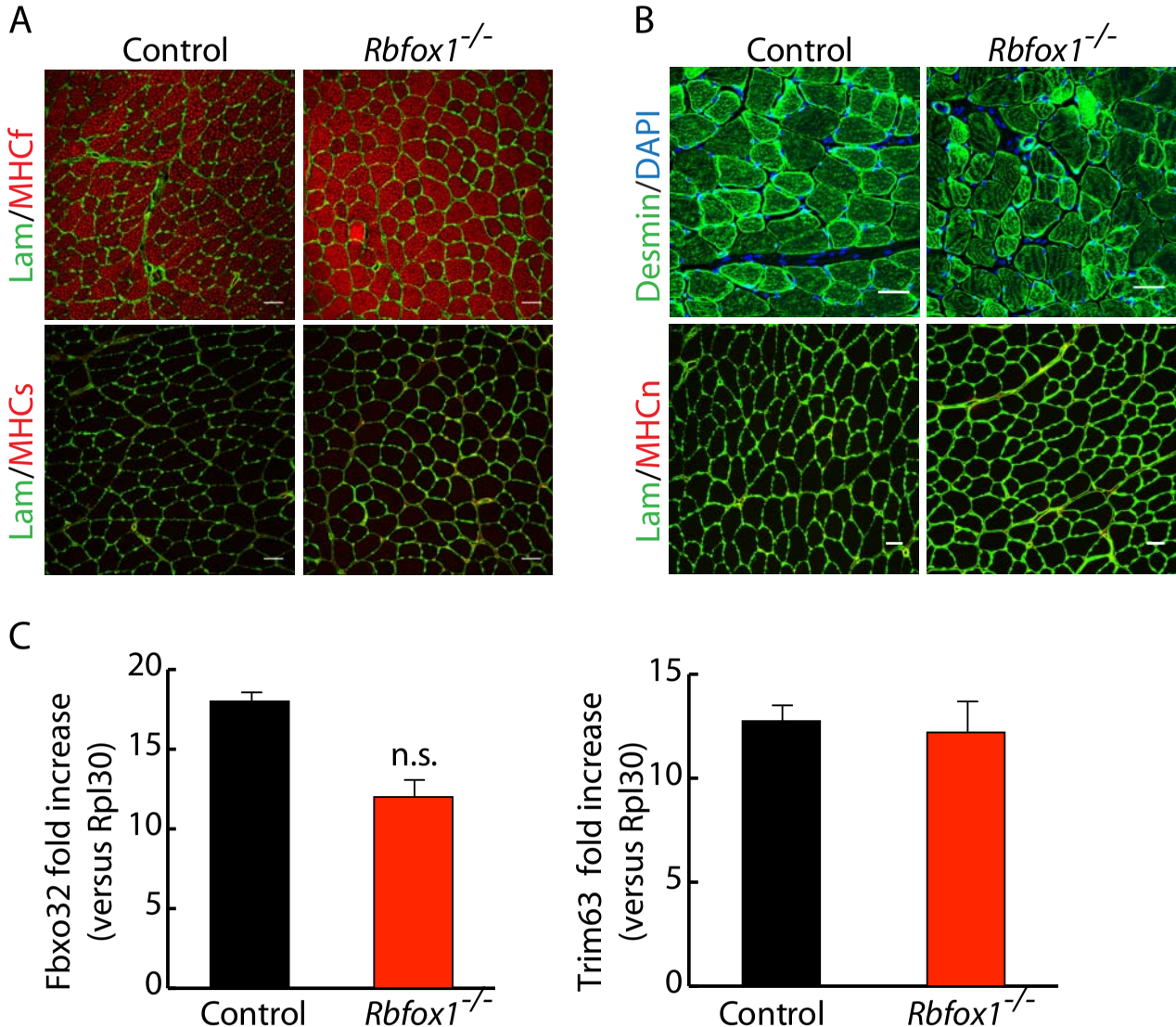
A
Control
Rbfox1-/-
Lam/MHCf
Lam/MHCs
B
Control
Rbfox1-/-
Desmin/DAPI
Lam/MHCn
C
Fbxo32 fold increase (versus Rpl30)
20
15
10
5
0
n.s.
Control
Rbfox1-/-
Trim63 fold increase (versus Rpl30)
15
10
5
0
Control
Rbfox1-/-

Figure S4

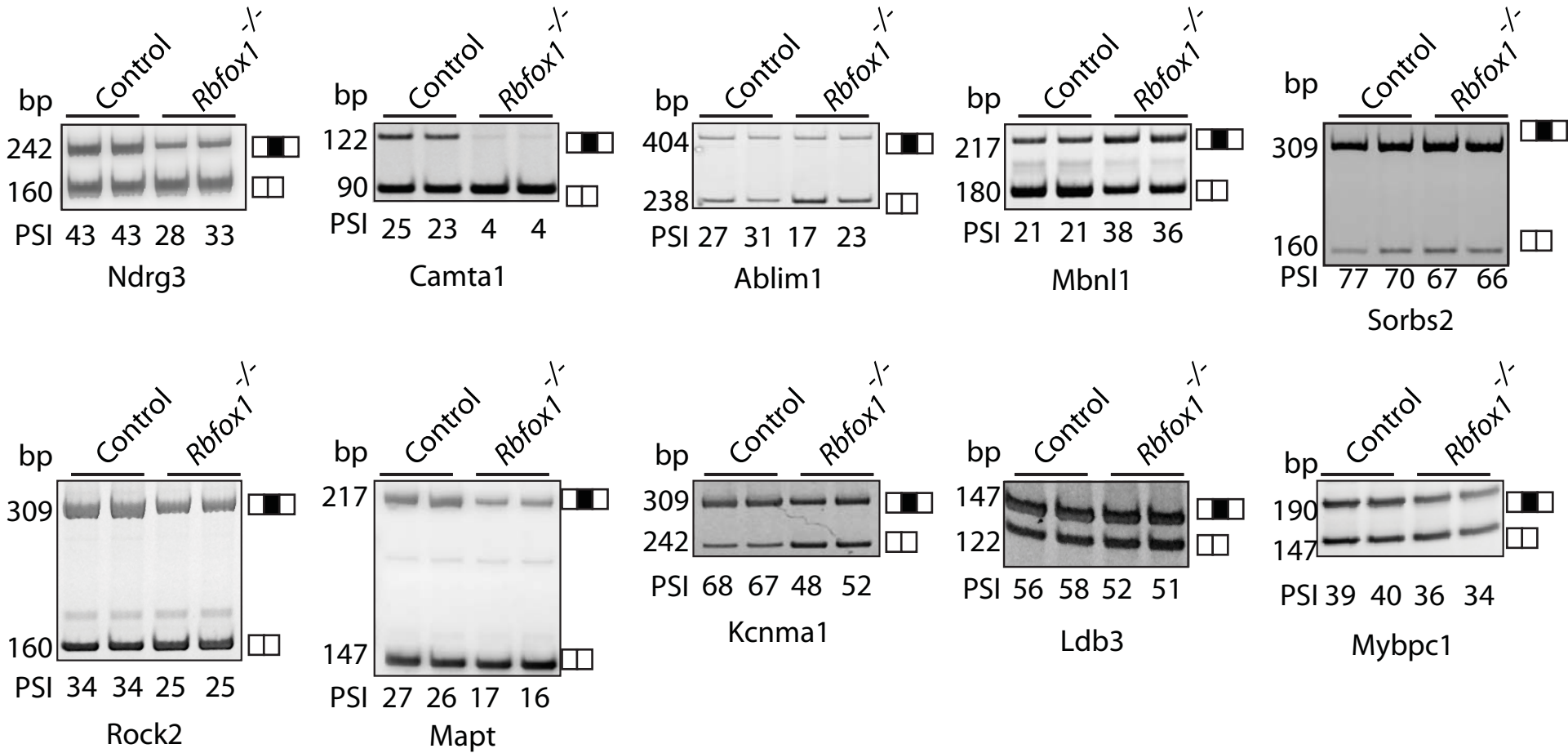
bp
Control
Rbfox1 -/-
242
160
PSI 43 43 28 33
Ndrg3
bp
Control
Rbfox1 -/-
122
90
PSI 25 23 4 4
Camta1
bp
Control
Rbfox1 -/-
404
238
PSI 27 31 17 23
Ablim1
bp
Control
Rbfox1 -/-
217
180
PSI 21 21 38 36
Mbnl1
bp
Control
Rbfox1 -/-
309
160
PSI 77 70 67 66
Sorbs2
bp
Control
Rbfox1 -/-
309
160
PSI 34 34 25 25
Rock2
bp
Control
Rbfox1 -/-
217
147
PSI 27 26 17 16
Mapt
bp
Control
Rbfox1 -/-
309
242
PSI 68 67 48 52
Kcnma1
bp
Control
Rbfox1 -/-
147
122
PSI 56 58 52 51
Ldb3
bp
Control
Rbfox1 -/-
190
147
PSI 39 40 36 34
Mybpc1

Figure S5

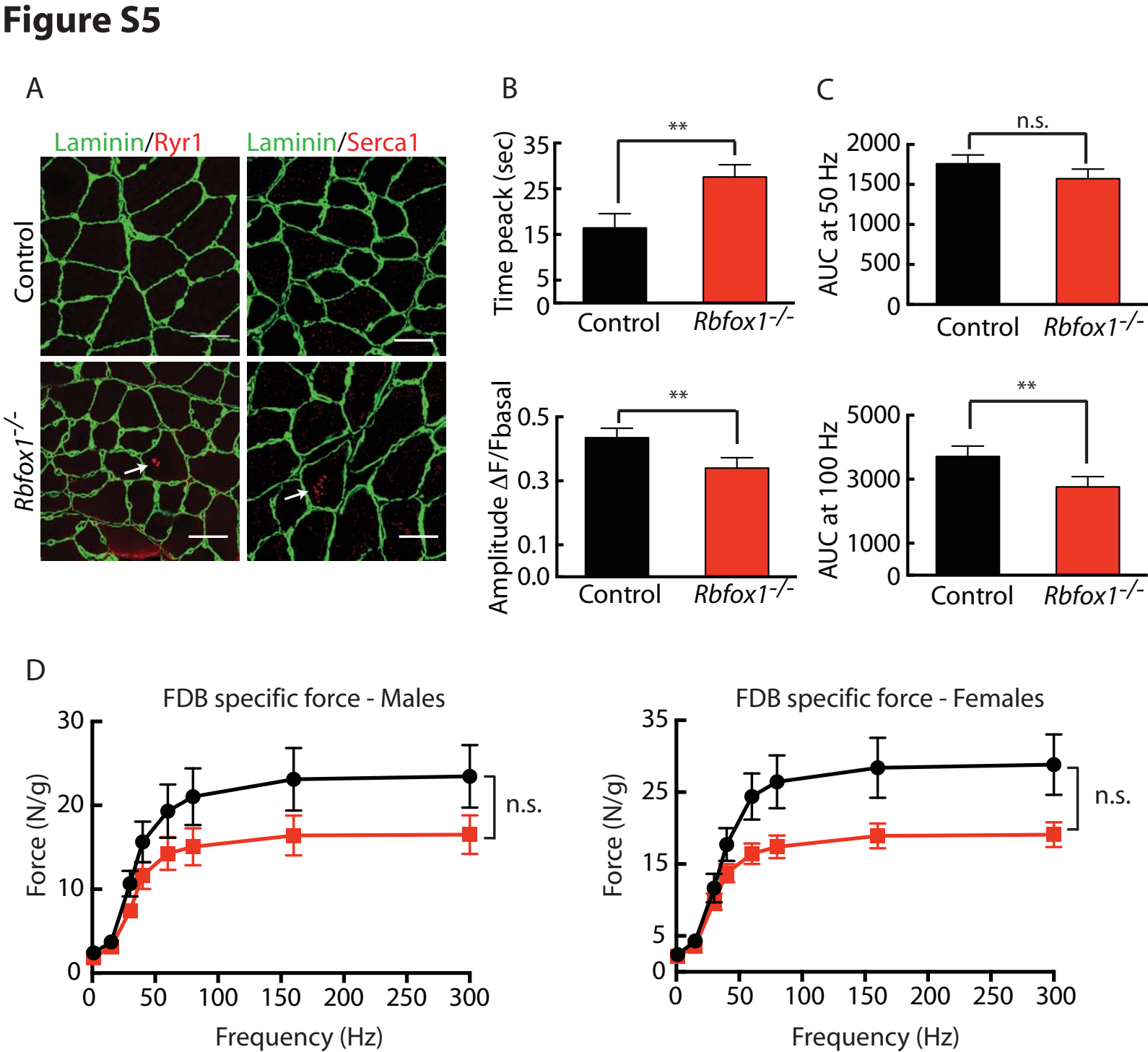
A
Laminin/Ryr1
Laminin/Serca1
Control
Rbfox1-/-
B
Time peack (sec)
**
Control
Rbfox1-/-
Amplitude ΔF/Fbasal
**
Control
Rbfox1-/-
C
AUC at 50 Hz
n.s.
Control
Rbfox1-/-
AUC at 100 Hz
**
Control
Rbfox1-/-
D
FDB specific force - Males
Force (N/g)
n.s.
Frequency (Hz)
FDB specific force - Females
Force (N/g)
n.s.
Frequency (Hz)

Table S1. **Parameters of RNA-seq experiments on tibialis anterior muscle from control and *Rbfox1*$^{-/-}$.**

RNA-Seq on tibialis anterior muscle (poly A selected)		
Sample	**# reads**	**Mapping rate**
*Control (*replicate 1)	153,405,533	91.00%
Control (replicate 2)	139,796,822	95.10%
Rbfox1$^{-/-}$ (replicate 1)	141,703,878	95.10%
Rbfox1$^{-/-}$ (replicate 2)	134,271,609	95.20%

Table S4. **List of primers used to validate splicing events**

Gene name	Alternative exon genomic coordinates	Forward primer	Revers primer	exon length (nt)	PCR product sizes
Ablim1	**chr19:57059085-57059204**	GGTTATCAAGATGTCCGGGATCGGA	CTGGAACATGGAAATGTTTAGGGGC	120	355-235
Ablim2	**chr5:35848804-35848905**	CTGGTACTGTGAGTGTTGGTACCAG	CGTGCTGTTTGTAGATAGGGGGTTT	102	255-153
Ablim2	**chr5:35849516-35849569**	GGGGATCAGGATGATCGGTCCTACA	CTTCCTGCTGTCCTGGTCAAAGCTG	54	228-174
Ambra1	**chr2:91805024-91805110**	GTGGGGGTGGCCTTTAACCAGGAGA	CTTGGAACAAATCGTCCAAGT	87	485-398
Camk2b	**chr11:5979673-5979721:-@chr11:5972813-5972888:-**	GAATCTTCCGACAGCACCAACACAA	TGGTCTTGATGATTTCCTGCTTCC	114, 129, 129	448-76
Camk2d	**chr3:126805857-126805916**	GGCGCCATCTTGACAACTATGCTGG	TGGTGTTTGAGCTCTCAGTTGACTC	38	208-148
Camk2g	**chr14:20747819-20747851**	CTGCCAAAAGCCTATTGAACAAGAA	CATGGCCGTCTGCAAGGGCGC	33	146-113
Camta1	**chr4:151071426-151071456**	GAGCAGTTTGCTGACCAAAAAGCAG	CCTTGGCCTTTTTCAATTCTTTCACT	31	132-101
Clmn	**chr12:104773182-104773274**	GACCATTTTAGCTATGTTCAATTGA	AGCCGGCTGACATCAAGCTGTGGGAA	93	332-239
Kcnma1	**chr14:23336040-23336120**	GATTCACACCTCCTGGAATGGACAG	CGCGCTCATGAGTGAGTCCAGGACG	81	326-245
Kcnq5	**chr1:21461551-21461607**	GAAAGAACAAGGGGAGGCATCAAGC	GAGCACTGACATCACTGCCGAGGGC	57	207-150
Ldb3	**chr14:34569713-34569727**	CACCCCGATCAGCATGTACTCACAG	GAGAGGCGCTGTCCACCGCCAAGTC	15	140-125
Mapt	**chr11:104287123-104287209**	GCTTTGAACCAGTATGGCTGACCCT	TGTCTCCGATGCCTGCTTCTTCGG	87	224-137
Mark2	**chr19:7279111-7279278**	AAATCTGTCTTTCAGGTTTGCCAGAAG	TCTTTGCTTTCAGGTTCATTCAGGTTC	168	222-54
Mast2	**chr4:116333413-116333433**	GTAACAGTCCCTTGGACAGCCCC	AGAGACTGTAGAGCTAGGAGTGTT	21	176-155