Muscleblind-like protein 3 deficit results in a spectrum of age-associated pathologies observed in myotonic dystrophy

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Running Title: Age-associated pathologies in *Mbnl3* deficient mice

SUPPLEMENTARY FIGURE AND TABLE LEGENDS

Supplementary Fig. S1. RT-PCR analysis of *Mbnl3* expression in adult mouse tissues.

RNA samples from 129Sv *Mbnl3*$^{+/+}$ and 129Sv *Mbnl3*$^{\Delta E2}$ E18 placenta and the indicated adult tissues/organs were subjected to RT-PCR (40 cycles) using primers located in Mbnl3 exon 2.

Supplementary Fig. S2. Splicing analysis in *Mbnl3*$^{\Delta E2}$ soleus muscle and heart.

a. *Insr* exon 10a inclusion was not significantly different in *Mbnl3*$^{+/+}$ and *Mbnl3*$^{\Delta E2}$ muscles at 7 months of age. Alternative splicing was analyzed for *Insr* by RT-PCR in male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ soleus muscles at 7 months of age. Band intensities were quantitated by densitometry. **b.** Splicing analyses for *Ldb3, Tnnt2, Insr* and *Mbnl1* performed by RT-PCR in male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ hearts at 7 months of age. *Tnnt2* exon 4 + 5, *Insr* exon 10a and *Mbnl1* exon 7 inclusion was not significantly different in *Mbnl3*$^{+/+}$ and *Mbnl3*$^{\Delta E2}$ hearts at 7 months of age. *Ldb3* exon 11 inclusion in *Mbnl3*$^{\Delta E}$ hearts was significantly decreased when compared to *Mbnl3*$^{+/+}$ hearts at 7 months of age. **c.** *Mbnl1* exon 7 and *Insr* exon 10a inclusion was not significantly different in male *Mbnl3*$^{+/+}$ and *Mbnl3*$^{\Delta E}$ hearts at 11 months of age. Alternative splicing was analyzed for *Mbnl1* and *Insr* by RT-PCR in male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ hearts at 11 months of age. Band intensities were quantitated by densitometry. The exon numbers are indicated and the alternatively spliced exons are shown as a gray box. Exon numbers are annotated based on Refseq from UCSC genome browser (NCBI37/mm9). The expected band sizes and p-values are shown. Data are standard error of mean.

Supplementary Fig. S3. Splicing analysis in *Mbnl3*$^{\Delta E2}$ hearts at 11 months of age.

Splicing analyses for *Pdlim3/Alp, Trim55/Murf2, Mapt/Tau, Pdlim5, Sorbs1, Sorbs2, Fhod1, Spag9, Mbnl2, Myom1, Clta, Stx2, Csda, Sirt2, Atp2a1, Atp11a and Gapdh* were performed by RT-PCR in male $Mbnl3^{+/+}$ and male $Mbnl3^{\Delta E2}$ hearts (n=3/genotype) at 11 months of age and E18 $Mbnl3^{+/+}$ hearts. Band intensities were quantitated by densitometry. The exon numbers are indicated and the alternatively spliced exons are shown as a green box. Exon numbers are annotated based on Refseq from UCSC genome browser (NCBI37/mm9). The expected band sizes and p-values are shown. Data are standard error of mean.

Supplementary Fig. S4. Splicing analysis in $Mbnl3^{\Delta E2}$ soleus muscles at 11 months of age.

Splicing analyses for *Pdlim3/Alp, Trim55/Murf2, Mapt/Tau, Pdlim5, Sorbs1, Sorbs2, Fhod1, Spag9, Mbnl2, Myom1, Clta, Stx2, Csda, Sirt2, Atp2a1, Atp11a and Gapdh* were performed by RT-PCR in male $Mbnl3^{+/+}$ and male $Mbnl3^{\Delta E2}$ soleus muscles (n=3/genotype) at 11 months of age and E18 $Mbnl3^{+/+}$ forelimbs. Band intensities were quantitated by densitometry. The exon numbers are indicated and the alternatively spliced exons are shown as a green box. Exon numbers are annotated based on Refseq from UCSC genome browser (NCBI37/mm9). The expected band sizes and p-values are shown. Data are standard error of mean.

Supplementary Fig. S5. Splicing analysis in $Mbnl3^{\Delta E2}$ whole lens at 8 months of age.

Splicing analyses for *Mapt/Tau, Pdlim5, Sorbs1, Sorbs2, Spag9, Mbnl2, Stx2, Csda, Sirt2, Atp11a and Gapdh* were performed by RT-PCR in the whole lens from male $Mbnl3^{+/+}$ and male $Mbnl3^{\Delta E2}$ mice (n=3/genotype) at 8 months of age and E18 $Mbnl3^{+/+}$ mice. Band intensities were quantitated by densitometry. The exon numbers are indicated and the alternatively spliced exons are shown as a green box. Exon numbers are annotated based on Refseq from UCSC genome

browser (NCBI37/mm9). The expected band sizes and p-values are shown. Data are standard error of mean.

Supplementary Table S1. a & b. Blood glucose levels (mg/dL) subsequent to dextrose injection at time 0 for male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ mice at 4 and 7-9 months of age are shown. p-values were calculated using the Student's t-test with significance set at $p \leq 0.05$. Values where $p \leq 0.05$ are shown in red. Data are standard error of mean.

Supplementary Table S2. Blood insulin levels (ng/ml) subsequent to dextrose injection at time 0 for male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ mice at 8-10 months of age are shown. p-values were calculated using the Student's t-test with significance set at $p \leq 0.05$. Values where $p \leq 0.05$ are shown in red. Data are standard error of mean.

Supplementary Table S3. a & b. Ultrasound echocardiographic values recorded from male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ mice at 4 months and 11 months of age are shown. Measurements of the left ventricle taken in this study include ventricular septal thickness (VST), end-diastolic dimension (EDD), posterior wall thickness (PWT), end-systolic dimension (ESD), and left ventricle mass (Lvmass). Heart function assessments included aorta ejection time (Ao-ET), left ventricle percent fractional shortening (Lv%FS), velocity of circumferential fiber shortening (Vcf), left ventricle ejection fraction (LvEF) and the E/A ratio. Data are mean and standard deviation. p-values were calculated using the Student's t-test with significance set at $p \leq 0.05$. Values where $p \leq 0.05$ are shown in red.

Supplementary Table S4. a & b. EKG values for male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ mice at 4 and 11 months of age are shown. Data are mean and standard deviation. p-values were calculated using the Student's t-test with significance set at $p \leq 0.05$.

Supplementary Table S5. Electroretinography values for male *Mbnl3*$^{+/+}$ and male *Mbnl3*$^{\Delta E2}$ mice at 13 months of age are shown. Data are mean and standard deviation. p-values were calculated using the Student's t-test with significance set at $p \leq 0.05$.

Supplementary Table S6. PCR conditions and primers.

Figure S1

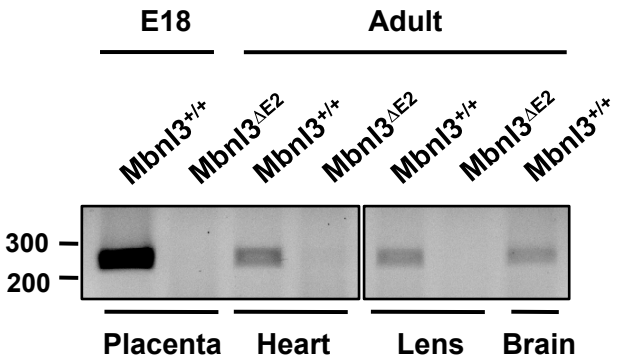
E18
Adult
Mbnl3+/+
Mbnl3ΔE2
Mbnl3+/+
Mbnl3ΔE2
Mbnl3+/+
Mbnl3ΔE2
Mbnl3+/+
300
200
Placenta
Heart
Lens
Brain

Figure S2

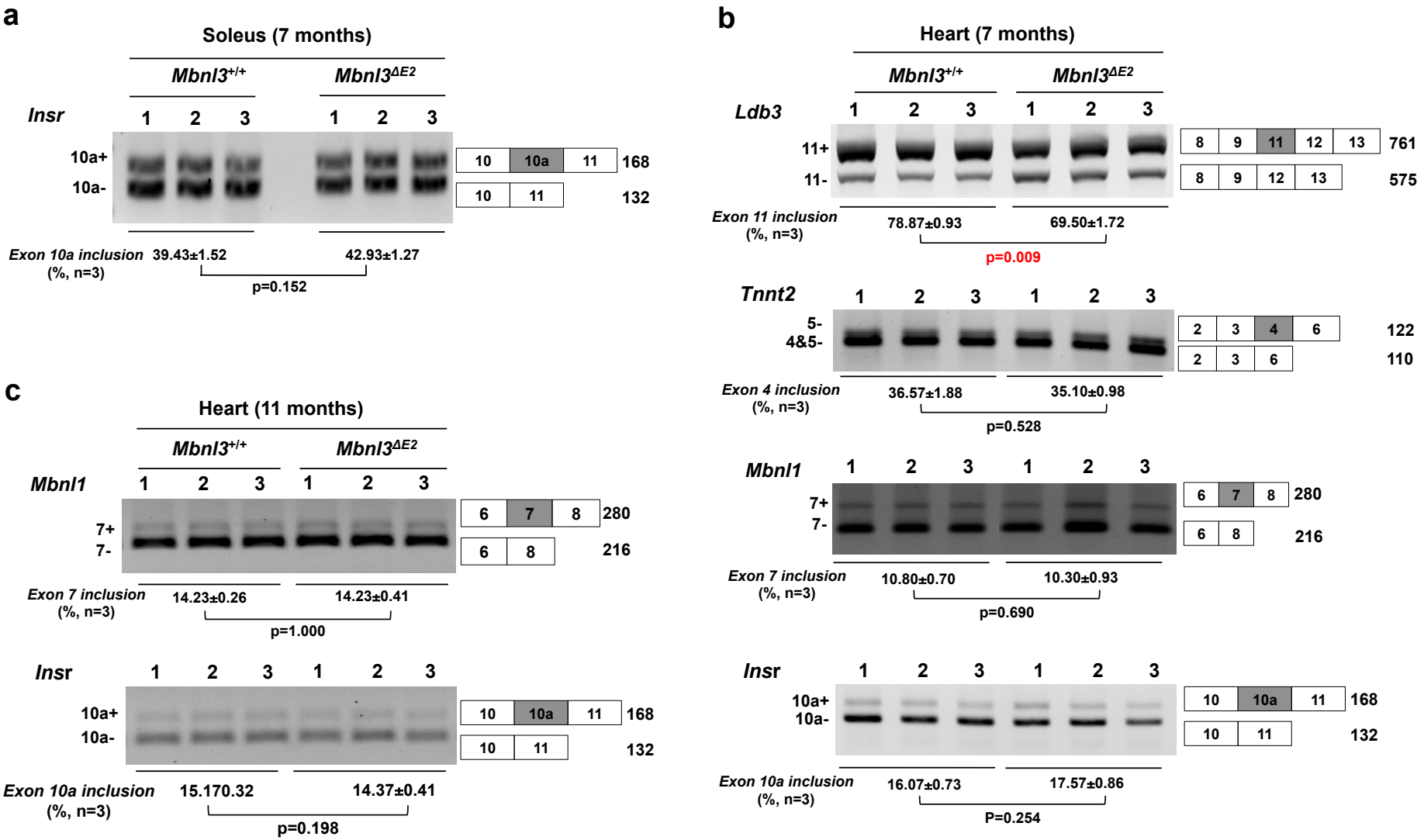
a
Soleus (7 months)
Mbnl3+/+
Mbnl3ΔE2
Insr
1 2 3 1 2 3
10a+
10a-
10 10a 11 168
10 11 132
Exon 10a inclusion (%, n=3)
39.43±1.52
42.93±1.27
p=0.152
b
Heart (7 months)
Mbnl3+/+
Mbnl3ΔE2
Ldb3
1 2 3 1 2 3
11+
11-
8 9 11 12 13 761
8 9 12 13 575
Exon 11 inclusion (%, n=3)
78.87±0.93
69.50±1.72
p=0.009
Tnnt2
1 2 3 1 2 3
5-
4&5-
2 3 4 6 122
2 3 6 110
Exon 4 inclusion (%, n=3)
36.57±1.88
35.10±0.98
p=0.528
Mbnl1
1 2 3 1 2 3
7+
7-
6 7 8 280
6 8 216
Exon 7 inclusion (%, n=3)
10.80±0.70
10.30±0.93
p=0.690
Insr
1 2 3 1 2 3
10a+
10a-
10 10a 11 168
10 11 132
Exon 10a inclusion (%, n=3)
16.07±0.73
17.57±0.86
P=0.254
c
Heart (11 months)
Mbnl3+/+
Mbnl3ΔE2
Mbnl1
1 2 3 1 2 3
7+
7-
6 7 8 280
6 8 216
Exon 7 inclusion (%, n=3)
14.23±0.26
14.23±0.41
p=1.000
Insr
1 2 3 1 2 3
10a+
10a-
10 10a 11 168
10 11 132
Exon 10a inclusion (%, n=3)
15.170.32
14.37±0.41
p=0.198

Figure S3

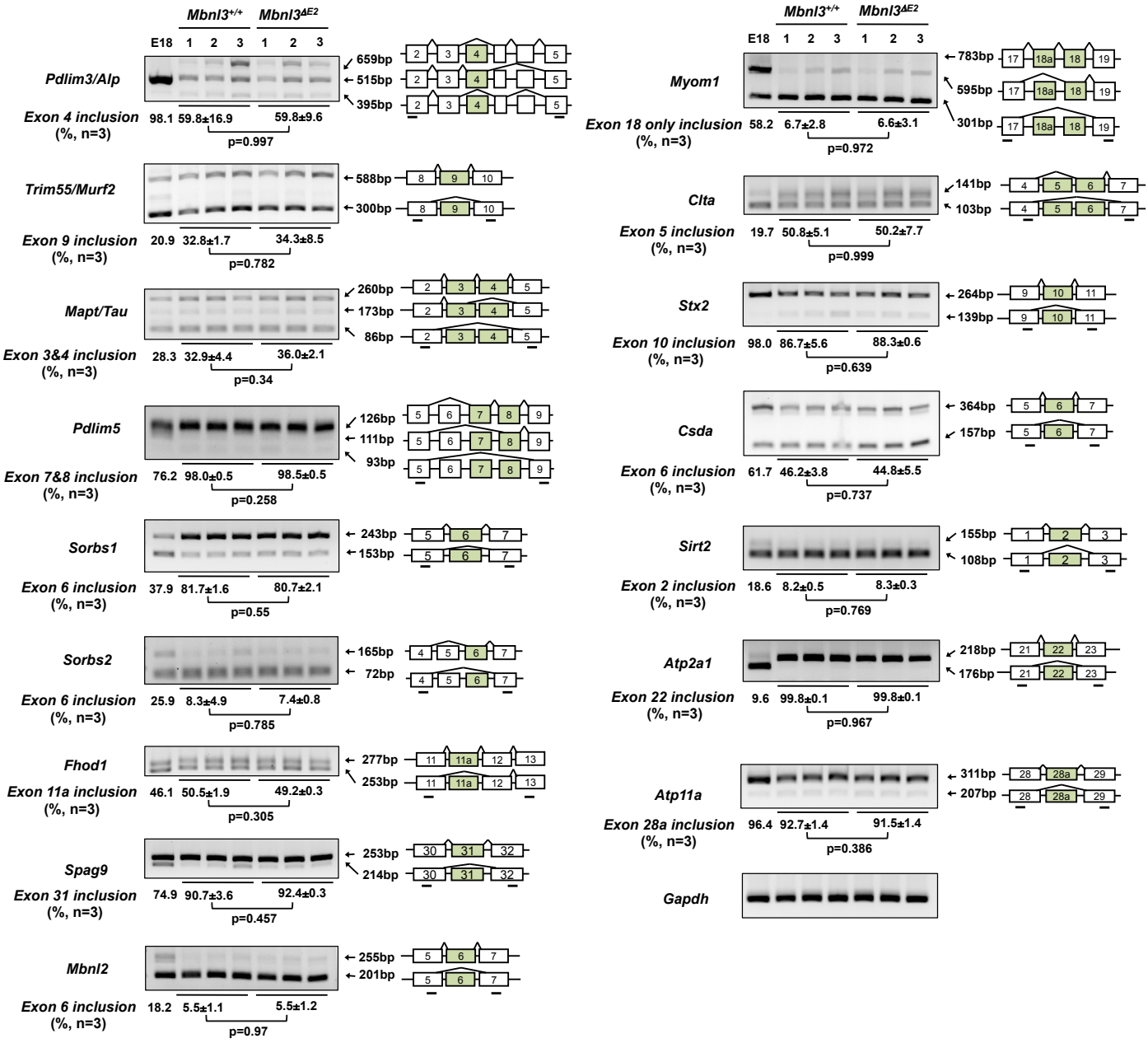
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Pdlim3/Alp
659bp
515bp
395bp
Exon 4 inclusion (%, n=3)
98.1 59.8±16.9 59.8±9.6
p=0.997
Trim55/Murf2
588bp
300bp
Exon 9 inclusion (%, n=3)
20.9 32.8±1.7 34.3±8.5
p=0.782
Mapt/Tau
260bp
173bp
86bp
Exon 3&4 inclusion (%, n=3)
28.3 32.9±4.4 36.0±2.1
p=0.34
Pdlim5
126bp
111bp
93bp
Exon 7&8 inclusion (%, n=3)
76.2 98.0±0.5 98.5±0.5
p=0.258
Sorbs1
243bp
153bp
Exon 6 inclusion (%, n=3)
37.9 81.7±1.6 80.7±2.1
p=0.55
Sorbs2
165bp
72bp
Exon 6 inclusion (%, n=3)
25.9 8.3±4.9 7.4±0.8
p=0.785
Fhod1
277bp
253bp
Exon 11a inclusion (%, n=3)
46.1 50.5±1.9 49.2±0.3
p=0.305
Spag9
253bp
214bp
Exon 31 inclusion (%, n=3)
74.9 90.7±3.6 92.4±0.3
p=0.457
Mbnl2
255bp
201bp
Exon 6 inclusion (%, n=3)
18.2 5.5±1.1 5.5±1.2
p=0.97
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Myom1
783bp
595bp
301bp
Exon 18 only inclusion (%, n=3)
58.2 6.7±2.8 6.6±3.1
p=0.972
Clta
141bp
103bp
Exon 5 inclusion (%, n=3)
19.7 50.8±5.1 50.2±7.7
p=0.999
Stx2
264bp
139bp
Exon 10 inclusion (%, n=3)
98.0 86.7±5.6 88.3±0.6
p=0.639
Csda
364bp
157bp
Exon 6 inclusion (%, n=3)
61.7 46.2±3.8 44.8±5.5
p=0.737
Sirt2
155bp
108bp
Exon 2 inclusion (%, n=3)
18.6 8.2±0.5 8.3±0.3
p=0.769
Atp2a1
218bp
176bp
Exon 22 inclusion (%, n=3)
9.6 99.8±0.1 99.8±0.1
p=0.967
Atp11a
311bp
207bp
Exon 28a inclusion (%, n=3)
96.4 92.7±1.4 91.5±1.4
p=0.386
Gapdh

Figure S4

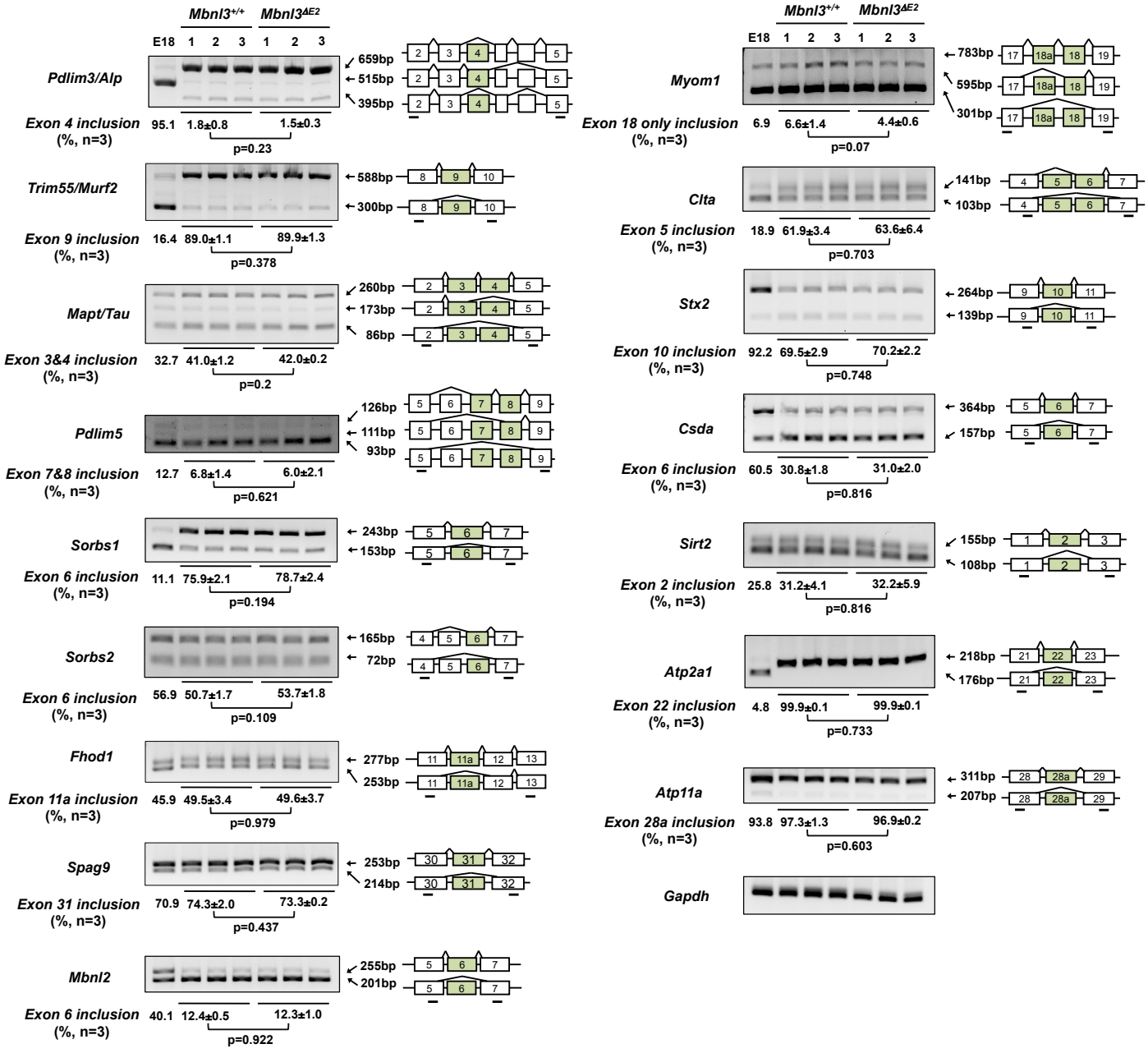
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Pdlim3/Alp
659bp
515bp
395bp
Exon 4 inclusion (%, n=3)
95.1 1.8±0.8 1.5±0.3
p=0.23
Trim55/Murf2
588bp
300bp
Exon 9 inclusion (%, n=3)
16.4 89.0±1.1 89.9±1.3
p=0.378
Mapt/Tau
260bp
173bp
86bp
Exon 3&4 inclusion (%, n=3)
32.7 41.0±1.2 42.0±0.2
p=0.2
Pdlim5
126bp
111bp
93bp
Exon 7&8 inclusion (%, n=3)
12.7 6.8±1.4 6.0±2.1
p=0.621
Sorbs1
243bp
153bp
Exon 6 inclusion (%, n=3)
11.1 75.9±2.1 78.7±2.4
p=0.194
Sorbs2
165bp
72bp
Exon 6 inclusion (%, n=3)
56.9 50.7±1.7 53.7±1.8
p=0.109
Fhod1
277bp
253bp
Exon 11a inclusion (%, n=3)
45.9 49.5±3.4 49.6±3.7
p=0.979
Spag9
253bp
214bp
Exon 31 inclusion (%, n=3)
70.9 74.3±2.0 73.3±0.2
p=0.437
Mbnl2
255bp
201bp
Exon 6 inclusion (%, n=3)
40.1 12.4±0.5 12.3±1.0
p=0.922
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Myom1
783bp
595bp
301bp
Exon 18 only inclusion (%, n=3)
6.9 6.6±1.4 4.4±0.6
p=0.07
Clta
141bp
103bp
Exon 5 inclusion (%, n=3)
18.9 61.9±3.4 63.6±6.4
p=0.703
Stx2
264bp
139bp
Exon 10 inclusion (%, n=3)
92.2 69.5±2.9 70.2±2.2
p=0.748
Csda
364bp
157bp
Exon 6 inclusion (%, n=3)
60.5 30.8±1.8 31.0±2.0
p=0.816
Sirt2
155bp
108bp
Exon 2 inclusion (%, n=3)
25.8 31.2±4.1 32.2±5.9
p=0.816
Atp2a1
218bp
176bp
Exon 22 inclusion (%, n=3)
4.8 99.9±0.1 99.9±0.1
p=0.733
Atp11a
311bp
207bp
Exon 28a inclusion (%, n=3)
93.8 97.3±1.3 96.9±0.2
p=0.603
Gapdh

Figure S5

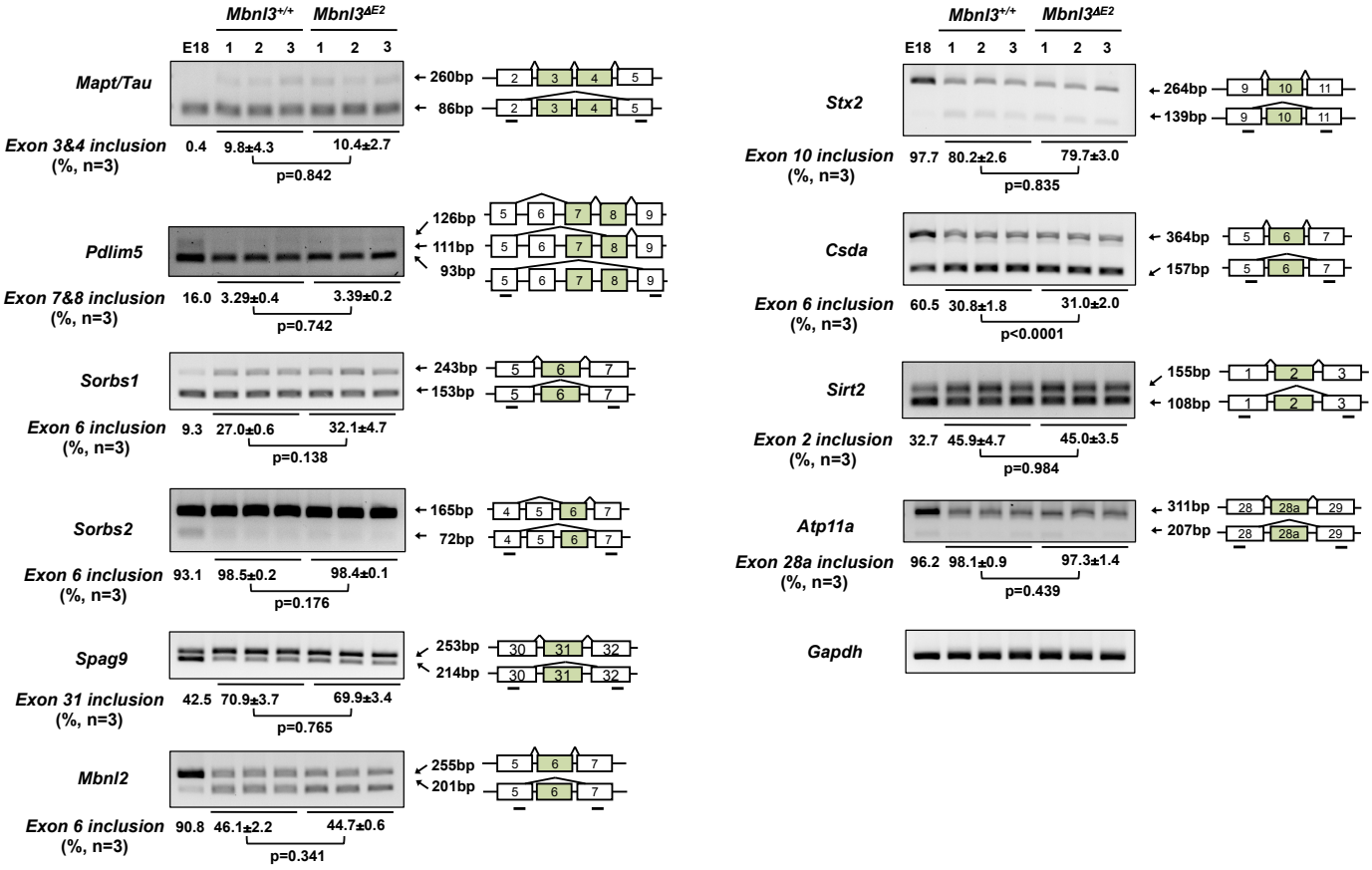
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Mapt/Tau
260bp
86bp
Exon 3&4 inclusion (%, n=3)
0.4 9.8±4.3 10.4±2.7
p=0.842
Pdlim5
126bp
111bp
93bp
Exon 7&8 inclusion (%, n=3)
16.0 3.29±0.4 3.39±0.2
p=0.742
Sorbs1
243bp
153bp
Exon 6 inclusion (%, n=3)
9.3 27.0±0.6 32.1±4.7
p=0.138
Sorbs2
165bp
72bp
Exon 6 inclusion (%, n=3)
93.1 98.5±0.2 98.4±0.1
p=0.176
Spag9
253bp
214bp
Exon 31 inclusion (%, n=3)
42.5 70.9±3.7 69.9±3.4
p=0.765
Mbnl2
255bp
201bp
Exon 6 inclusion (%, n=3)
90.8 46.1±2.2 44.7±0.6
p=0.341
Mbnl3+/+
Mbnl3ΔE2
E18 1 2 3 1 2 3
Stx2
264bp
139bp
Exon 10 inclusion (%, n=3)
97.7 80.2±2.6 79.7±3.0
p=0.835
Csda
364bp
157bp
Exon 6 inclusion (%, n=3)
60.5 30.8±1.8 31.0±2.0
p<0.0001
Sirt2
155bp
108bp
Exon 2 inclusion (%, n=3)
32.7 45.9±4.7 45.0±3.5
p=0.984
Atp11a
311bp
207bp
Exon 28a inclusion (%, n=3)
96.2 98.1±0.9 97.3±1.4
p=0.439
Gapdh

Table S1

a

Genotype	Gender	Age (months)	n	0 min	10 mins	20 mins	30 mins	60 mins	90 mins	120 mins	180 mins
***Mbnl3*$^{+/+}$**	Male	4	4	93.13±22.99	164.00±26.75	179.88±37.84	168.13±37.60	123.50±30.18	123.38±19.60	108.00±21.04	88.50±16.69
***Mbnl3*$^{\Delta E2}$**	Male	4	3	101.33±15.67	179.67±17.75	143.67±45.17	126.33±35.11	128.50±17.21	117.83±23.06	123.67±30.15	113.20±25.25
p-value				0.468	0.239	0.128	0.056	0.732	0.636	0.273	0.083

b

Genotype	Gender	Age (months)	n	0 min	10 mins	20 mins	30 mins	60 mins	90 mins	120 mins	180 mins
***Mbnl3*$^{+/+}$**	Male	7-9	5	74.72±4.87	134.64±11.21	152.80±4.97	99.20±7.27	92.09±3.08	77.70±4.72	76.90±6.74	62.20±5.62
***Mbnl3*$^{\Delta E2}$**	Male	7-9	7	111.88±3.33	163.13±8.53	191.43±14.88	178.64±14.88	164.72±12.50	142.57±7.65	130.57±7.46	108.40±3.90
p-value				*<0.001	*0.021	*0.045	*<0.001	*<0.001	*<0.001	*<0.001	*<0.001

Table S2

Genotype	Gender	Age (months)	n	0 min	8 mins	15 mins	30 mins	60 mins	90 mins
***Mbnl3*$^{+/+}$**	Male	8-10	4	0.74±40.10	1.29±0.12	1.02±0.09	1.08±0.13	0.86±0.09	0.85±0.06
***Mbnl3*$^{\Delta E2}$**	Male	8-10	5	1.62±0.21	1.78±0.21	1.58±0.19	1.93±0.20	1.76±0.23	1.81±0.25
p-value				*0.005	0.275	*0.013	*0.048	*0.014	*0.008

Table S3

a

Genotype	Gender	Age (weeks)	n	Heart Rate	VST (mm)	EDD (mm)	PWT (mm)	ESD (mm)	Ao-ET (ms)	Lv%FS	Vcf	LvEF	Lvmass	E/A
Mbnl3$^{+/+}$	Male	17	4	476±64	0.55±0.01	4.25±0.10	0.54±0.05	2.68±0.25	54.3±2.1	37.0±5.7	6.80±0.86	73.8±7.7	78.1±3.5	1.81±0.15
Mbnl3$^{\Delta E2}$	Male	17	7	475±48	0.56±0.04	4.20±0.23	0.50±0.05	3.04±0.21	52.1±3.4	27.5±4.6	5.29±0.87	59.9±7.8	76.0±11.2	2.10±0.65
p-value				0.97	0.64	0.72	0.3	*0.03	0.26	*0.01	*0.02	*0.02	0.72	0.48

b

Genotype	Gender	Age (weeks)	n	Heart Rate	VST (mm)	EDD (mm)	PWT (mm)	ESD (mm)	Ao-ET (ms)	Lv%FS	Vcf	LvEF	Lvmass	E/A
Mbnl3$^{+/+}$	Male	46	4	468±50	0.46±0.02	4.00±0.20	0.46±0.01	2.85±0.44	48.9±4.6	29.0±7.8	5.98±1.69	63.9±9.9	58.7±6.9	1.50±0.16
Mbnl3$^{\Delta E2}$	Male	46	6	525±38	0.53±0.04	4.48±0.35	0.56±0.05	3.37±0.42	47.1±8.1	24.1±6.0	5.19±1.45	55.6±9.9	88.5±11.2	1.79±0.35
p-value				0.07	*0.02	*0.04	*0.003	0.09	0.69	0.37	0.57	0.22	*0.001	0.16

Table S4

a

Genotype	Gender	Age (weeks)	n	PR (ms)	QRS (ms)	QT (ms)
***Mbnl3*+/+**	Male	17	4	42.9±2.2	8.9±0.8	45.8±1.8
***Mbnl3*$^{\Delta E2}$**	Male	17	4	45.2±2.3	8.3±0.2	51.0±7.1
p-value				0.198	0.199	0.228

b

Genotype	Gender	Age (weeks)	n	PR (ms)	QRS (ms)	QT (ms)
***Mbnl3*+/+**	Male	46	4	48.9±3.4	10.0±3.4	49.9±0.9
***Mbnl3*$^{\Delta E2}$**	Male	46	4	44.2±1.0	9.7±2.2	55.8±4.1
p-value				0.085	0.908	0.068

Table S5

Genotype	Gender	Age (months)	n	Scotopic Max (μV)		Mesopic (μV)		Photopic (μV)								
								1 min		4 min		7 min		10 min		10 Hz
				a-wave	b-wave	a-wave	b-wave	a-wave	b-wave	a-wave	b-wave	a-wave	b-wave	a-wave	b-wave	Flicker
Mbnl3 $^{\Delta E2}$	Male	13	4	51.8±13.7	640.5±175.1	419.0±50.8	1065.0±357.9	21.3±4.3	288.3±31.7	19.5±13.9	310.0±77.4	23.8±5.4	336.8±83.0	26.8±8.6	350.3±85.0	144.5±70.6
Mbnl3 $^{+/+}$	Male	13	4	36.0±16.1	734.0±204.8	370.5±69.1	1234.3±249.2	18.3±3.2	262.3±68.0	21.5±1.7	277.0±69.6	21.8±3.1	287.8±54.8	17.8±2.9	292.8±65.4	109.5±26.9
p-value				0.187	0.514	0.301	0.467	0.371	0.524	0.785	0.549	0.543	0.363	0.095	0.325	0.39

Table S6

PCR primers and conditions:

RT-PCR primers	PCR Conditions:
Muscleblind-like protein 3 (*Mbnl3*)	
Forward: 5'-TCCTTGAACCATCTGCAGTCA-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-GTGAATCAAAACAGGCCACCA-3'	45 SEC AT 72C X35], 10 MIN AT 72C
Insulin receptor (*Insr*)	
Forward: 5'-GAGGATTACCTGCACAACG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 55C,
Reverse: 5'-CACAATGGTAGAGGAGACG-3'	45 SEC AT 72C X35], 10 MIN AT 72C
Cardiac troponin T (*Tnnt2*)	
Forward: 5'-GCCGAGGAGGTGGTGGAGGAGTA-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 65C,
Reverse: 5'-GTCTCAGCCTCACCCTCAGGCTCA-3'	45 SEC AT 72C X35], 10 MIN AT 72C
LIM domain binding protein 3 (*Ldb3*)	
Forward: 5'-GGAAGATGAGGCTGATGAGTGG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-TGCTGACAGTGGTAGTGCTCTTTC-3'	45 SEC AT 72C X35], 10 MIN AT 72C
Muscleblind-like protein 1 (*Mbnl1*)	
Forward: 5'-GCTGCCCAATACCAGGTCAAC-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-TGGTGGGAGAAATGCTGTATGC-3'	45 SEC AT 72C X35], 10 MIN AT 72C
PDZ and LIM domain 5 (*Pdlim3/Alp*)	
Forward: 5'- AGC TGC CAA CCT GTG TCC TG -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- GAT CCT GCA GCA CCC TGA AG -3	45 SEC AT 72C X35], 10 MIN AT 72C
ATPase, Ca++ transporting, cardiac muscle, fast twitch 1 *(Atp2a1)*	
Forward: 5'- GCT CAT GGT CCT CAA GAT CTC AC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- GGG TCA GTG CCT CAG CTT TG -3	45 SEC AT 72C X35], 10 MIN AT 72C
Clathrin, light chain A (*Clta*)	
Forward: 5'- GGC GAT AAA GGA GCT GGA AGA -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- AAT GTC GTT TAC AAA GGC TTC -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Cold shock domain protein A/Y-box binding portein 3 (*Csda/Ybx3*)	
Forward: 5'- GGT GCT GAA GCA GCA AAC GT -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- GGA CTC CAT CCT TCA TCT CCC C -3'	45 SEC AT 72C X35], 10 MIN AT 72C

Continued

RT-PCR primers	**PCR Conditions:**
Muscleblind-like protein 2 (*Mbnl2*)	
Forward: 5'- ACC GTA ACC GTT TGT ATG GAT TAC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- CTT TGG TAA GGG ATG AAG AGC AC -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Myomesin 1 *(Myom1*)	
Forward: 5'- CAT GGA CTG ACG ACT GCT CAG AGC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 55C,
Reverse: 5'- CTG CAT CGC TGA CGG CCT TGA TG -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Sorbin and SH3 domain-containing protein 2 (*Sorbs2*)	
Forward: 5'- CTG AAG AAG TTA ATA ACA AAC CCT TCA AGG TG -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 65C,
Reverse: 5'- CTG AAT CTG GAG ACT GAG ACT CAC G -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Syntaxin 2 (S*tx2*)	
Forward: 5'- GCC AAG GAA GAG ACG AAG AAA GC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-GGT ACG GTT GCT ATG ACA ATG CTG -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Tripartite motif containing 55 (*Trim55/Murf-2*)	
Forward: 5'- GGA GAG GAT GCA GTA GAA GTA -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- CAA CCA GGA GAA GGA GAA GAC -3'	45 SEC AT 72C X35], 10 MIN AT 72C
ATPase , class VI, type 11A (Atp11a)	
Forward: 5'- CCG TTC CTC AGT TAC CAG AGG AT -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- CAG AGG GGT GAA TTC TGA AAT GCT G -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Formin homolog 2 domain containing 1 (*Fhod1)*	
Forward: 5'- CGC CTA CAA ATC CAG CCT TC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- TCC CTT GTG CCT GAT GAT CC -3'	45 SEC AT 72C X35], 10 MIN AT 72C
PDZ and LIM domain 5 (*Pdlim5*)	
Forward: 5'- CAG GGT GAC ATT AAG CAG CAA AAT GG -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- GGC ATC ACT GTG AGT GGG TAT GTG -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Sirtuin 2 (*Sirt2*)	
Forward: 5'- CAG AGC AGT CGG TGA CAG TCC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- TCT CTG CCT CTC CAC CAG TG -3'	45 SEC AT 72C X35], 10 MIN AT 72C

Continued

RT-PCR primers	**PCR Conditions:**
Sorbin and SH3 domain containing 1 (*Sorbs1*)	
Forward: 5'- CTG GCA ACC ATG GAC CAG AC -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'- CTG ACT TGC TTT CAT GCT TCG -3	45 SEC AT 72C X35], 10 MIN AT 72C
C-jun-amino-terminal kinase-interacting protein (*Spag9*)	
Forward: 5'- GGA CTG GAA ATG GTGTCATTA TCT CCA T -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 55C,
Reverse: 5'-ACT GCC ACA AAG AAT TTC ACA G -3'	45 SEC AT 72C X35], 10 MIN AT 72C
Glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*)	
Forward: 5'-AGA GAC GGC CGC CGC ATC TTC TTG TG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 65C,
Reverse: 5'-TCT GGG TCC CAG TGA TGG CAT GG-3'	45 SEC AT 72C X35], 10 MIN AT 72C

PCR primers and conditions:

qPCR primers	**PCR Conditions:**
Insulin Receptor (*Insr*) qPCR Exon 9-10	
Forward: 5'-GAGAGGCAAGCAGAGGACAG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-ACCAGGTCCGTGAAGGGA-3'	30 SEC AT 72C X35], 10 MIN AT 72C
Insulin Receptor (*Insr*) qPCR Exon 10-10a	
Forward: 5'-TGGTGCCGAGGACAGTAGG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-GTGTGGTGGCTGTCACATTC-3'	30 SEC AT 72C X35], 10 MIN AT 72C
LIM domain binding protein 3 (*Ldb3*) qPCR Exon 16-17	
Forward: 5'-CCTGGCATGACACCTGCTT-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-TGCACAGTGGTTTGTCTTTCTTAG-3'	30 SEC AT 72C X35], 10 MIN AT 72C
LIM domain binding protein 3 (*Ldb3*) qPCR Exon 11-12	
Forward: 5'-GCTCTGCGAAGGTCAAGCA-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 60C,
Reverse: 5'-CGGGCGACTGGGCAGAAG-3'	30 SEC AT 72C X35], 10 MIN AT 72C
Cardiac troponin T (*Tnnt2*) qPCR 3-6	
Forward: 5'-GGAACAGGAAGAGCAAGAGG-3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 65C,
Reverse: 5'-CAGGTTCAGCCCCACCAG-3'	45 SEC AT 72C X35], 10 MIN AT 72C
Cardiac troponin T (*Tnnt2*) qPCR Exon 7-9	
Forward: 5'-GCTGAAGAAGGTCCAGTAGAGG -3'	5 MIN AT 95C, [30 SEC AT 95C, 30 SEC AT 65C,
Reverse: 5'-GCACCAAGTTGGGCATGAAG-3'	45 SEC AT 72C X35], 10 MIN AT 72C