PNAS
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Supplementary Information for

The RBFox2-miR-34a-Jph2 Axis Contributes to Cardiac Decompensation during Heart Failure

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www.pnas.org/cgi/doi/10.1073/pnas.1822176116

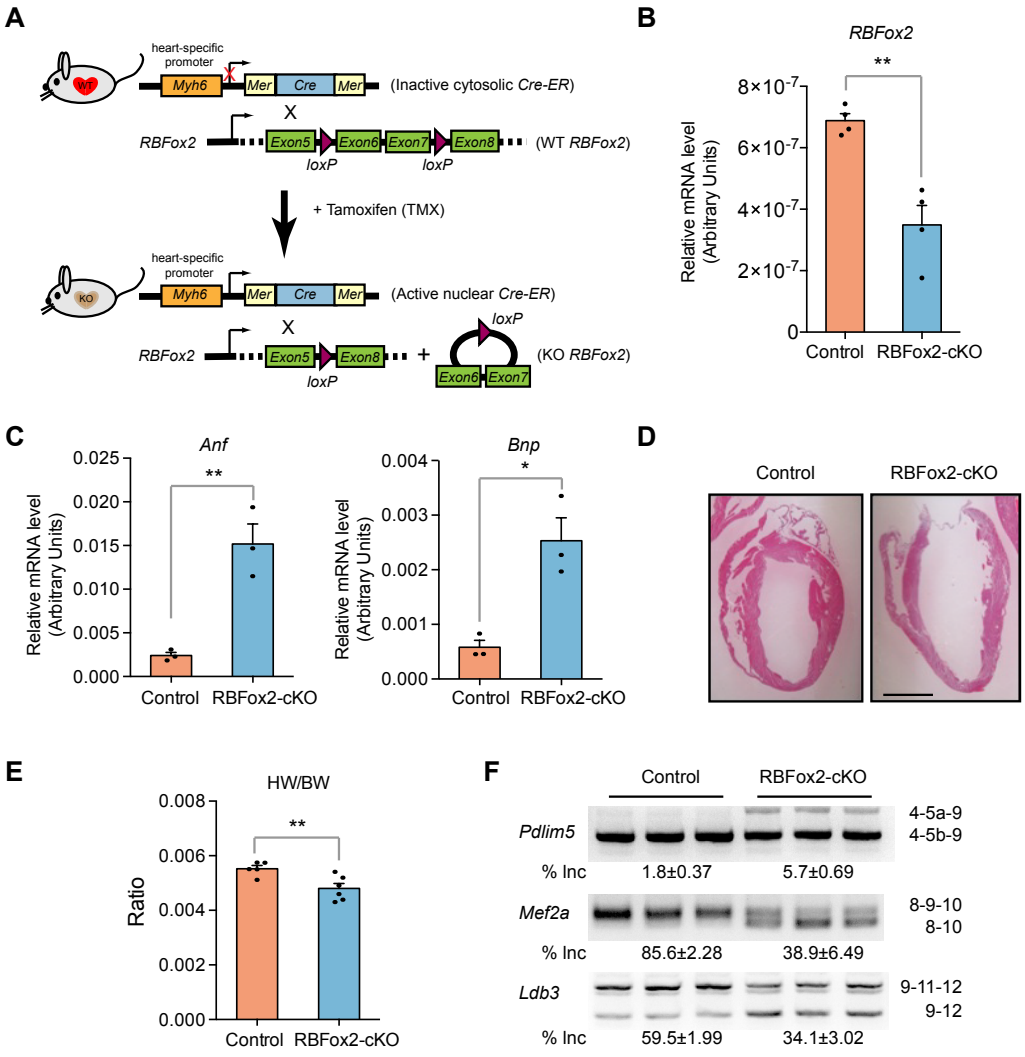

Fig. S1. Heart failure in tamoxifen-induced *RBFox2* knockout mice. (*A*) Scheme for generating *RBFox2-cKO* mice and induced recombination by tamoxifen. (*B*) Quantitative analysis of *RBFox2* mRNA expression in control and *RBFox2-cKO* groups; n=3 for each group, *$P<0.05$, **$P<0.01$. (*C*) Quantitative analysis of atrial natriuretic factor (*Anf*) and brain natriuretic protein (*Bnp*) mRNA expression in control and *RBFox2-cKO* groups; n=3 for each group, *$P<0.05$, **$P<0.01$. (*D-E)* Representative cross-sectional images of H&E stained heart (D) and analysis of heart weight (HW) / body weight (BW) ratio (E) from control and *RBFox2-cKO* mice after 2-week tamoxifen treatment; n=8 for control, n=9 for *RBFox2-cKO*, **$P<0.01$. Scale bar: 2mm. (*F)* Semi-quantitative RT-PCR analysis of alternative splicing of representative cardiac genes in WT and *RBFox2*-cKO cardiomyocytes. Shown at the bottom of each gel image are the percentages of splice-in (included exon divided by both isoforms) calculated based on three biological repeats.

A

Statistics of CLIP-seqs

Sample	Raw	Trimmed	m1	Mapping Ratio	m1u1	Cell type
Ago2-RBFox2-WT-CM	39,666,870	26,566,050	22,178,243	0.83	4,381,810	Cardiomyocytes (W9)
Ago2-RBFox2-KO-CM	21,596,619	14,362,095	12,274,069	0.85	4,282,752	Cardiomyocytes (W9)

B

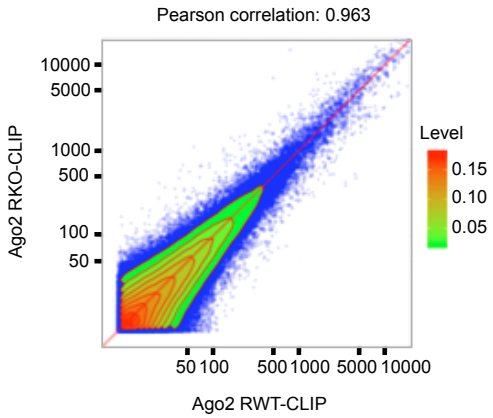

Fig. S2. Genome-wide analysis of Ago2-RNA interactions in the heart. (*A*) Ago2 crosslinking immunoprecipitation sequencing (CLIP-seq) performed on isolated WT or *RBFox2*$^{-/-}$ cardiomyocytes from 9 week-old mice. (*B*) Correlation between the Ago2 CLIP-seq data generated with WT and *RBFox2*$^{-/-}$ cardiomyocytes isolated from 9 week-old mice.

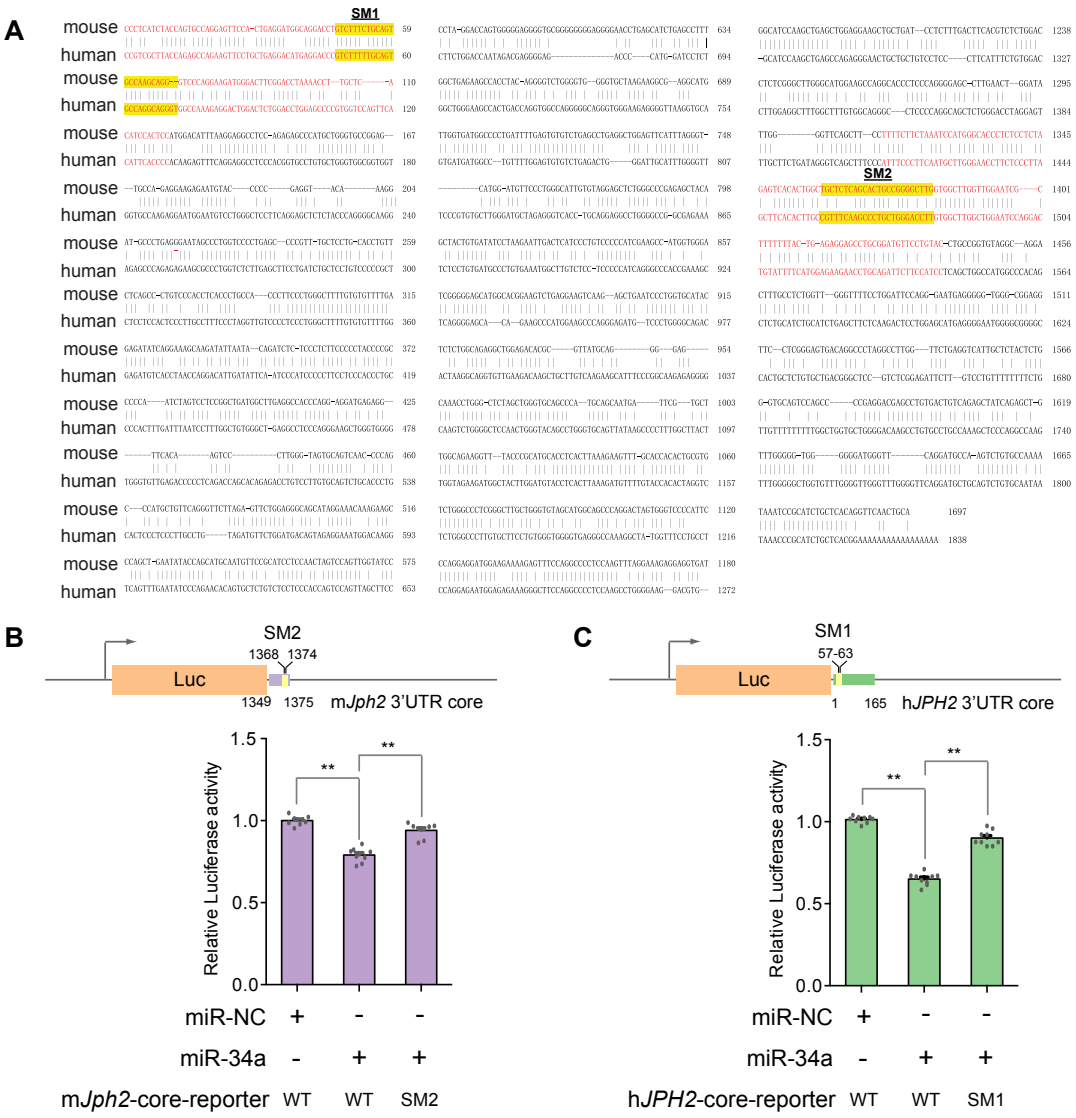

Fig. S3. Regulation of mouse and human *JPH2* 3'UTRs by miR-34a. (*A*) Comparison between the 3'UTRs of mouse and human *JPH2* genes. Red regions are those bound by Ago2 and yellow highlightes the predicted miR-34a target sites. (*B-C*) The luciferase assays of the reporters containing the core miR-34a target site in the 3'UTR of mouse (B) or human (C) *JPH2* gene in response to miR-34a overexpression; n=8 for each group, ***P<0.01*.

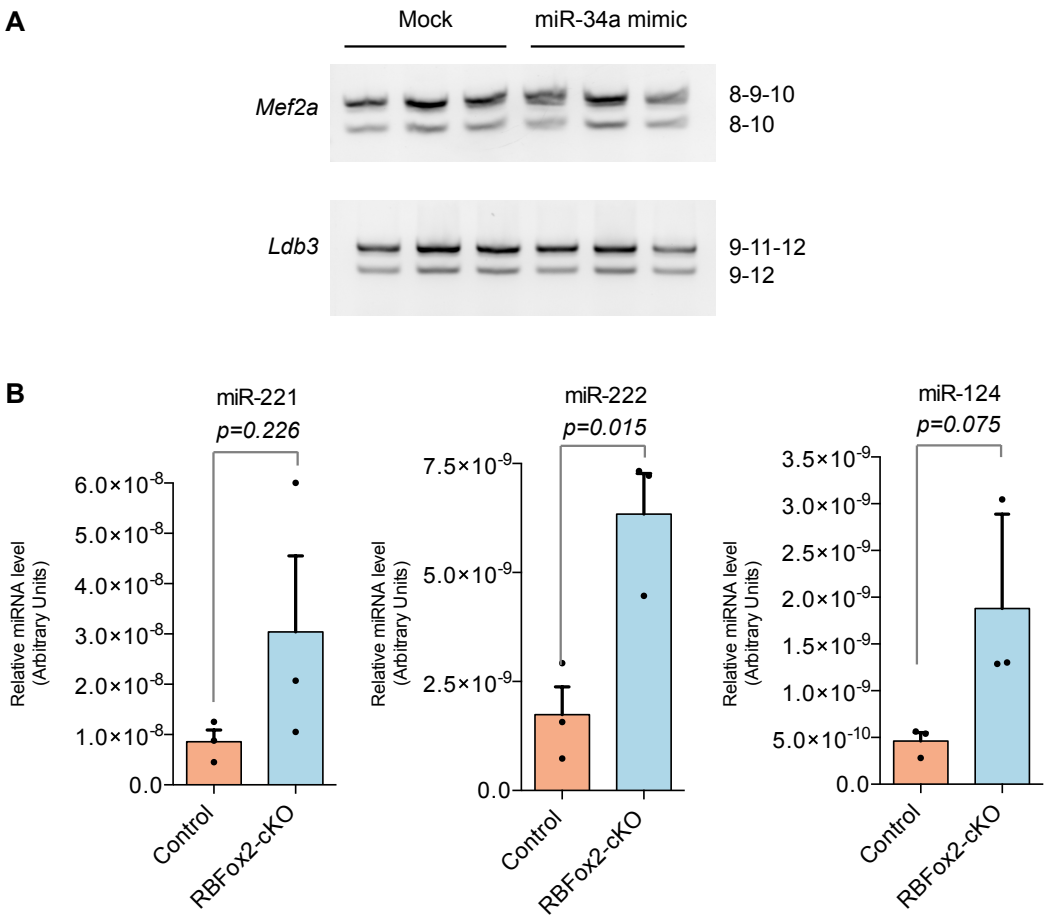

Fig. S4. Impact of miR-34a mimic on alternative splicing and induction of additional miRNAs in the heart of *RBFox2-cKO* mice. (*A*) Alternative splicing of two RBFox2-regulated genes in the hearts treated with mock or miR-34a mimic. (*B*) RT-qPCR analysis of three representative miRNAs in the heart of *RBFox2-cKO* mice; n=3 for each group, *P*-value was labeled individually. The trends of elevation of miRNAs are shown although some *P*-values are not significant.

Table S1. Expression profiling of 160 microRNAs from *RBFox2*$^{-/-}$mouse cardiomyocytes at 5 -week, 9-week and 18-week age points.

miRNA	5W-KO/WT(Log_2)	9W-KO/WT(Log_2)	18W-KO/WT(Log_2)
miR-1983	1.34	2.44	2.86
miR-34a	1.82	1.91	2.52
miR-34b	2.09	1.55	2.24
miR-222	1.25	1.50	2.01
miR-32	1.57	1.56	1.60
miR-34c	1.43	1.39	1.92
miR-208b	0.48	0.98	2.70
miR-221	0.96	1.14	1.82
miR-449a	0.70	1.12	1.42
miR-21a	0.92	0.85	1.11
miR-124a	0.45	0.93	1.43
miR-199a*	1.00	0.09	1.32
miR-215	1.02	0.83	0.41
miR-301a	0.44	0.93	0.62
miR-214	0.44	0.03	1.45
miR-744	0.37	0.95	0.46
miR-23b	0.66	0.51	0.53
miR-484	0.73	0.53	0.39
miR-541	0.50	0.70	0.39
miR-501	0.69	0.04	0.83
miR-361	0.01	0.86	0.65
miR-31	0.36	0.50	0.65
miR-199a	0.44	0.42	0.62
miR-142	0.79	0.13	0.54
miR-103	0.57	0.50	0.22
miR-300	-0.25	0.45	1.09
miR-107	0.52	0.56	0.19
miR-27b	0.35	0.53	0.36
miR-23a	0.50	0.41	0.31
miR-142*	-0.06	0.61	0.62
miR-339	0.46	0.42	0.24
miR-183	0.12	0.23	0.66
miR-27a	0.41	0.27	0.24
miR-15b	0.57	0.25	0.04
miR-210	-0.11	0.39	0.50
miR-33	-0.25	0.20	0.82
miR-302b	0.91	0.68	-0.84
miR-497	-0.26	0.59	0.38
miR-100	0.64	0.25	-0.19

miR-1306	0.36	0.24	0.11
miR-24	0.21	0.25	0.20
miR-320	0.15	0.47	0.04
miR-208a	0.44	0.44	-0.24
miR-18a	0.44	0.15	-0.04
miR-486	0.30	0.31	-0.13
miR-99a	0.66	-0.23	0.04
miR-125b	0.38	0.11	-0.03
miR-152	0.27	0.10	0.06
miR-494	1.28	-0.07	-0.80
miR-187	0.03	0.22	0.12
miR-185	-0.20	0.39	0.12
miR-378c	0.15	0.46	-0.33
miR-148a	0.26	0.06	-0.05
miR-127	0.82	-0.09	-0.53
miR-28a	0.52	-0.29	-0.04
miR-500	0.28	-0.04	-0.06
miR-362	0.56	-0.31	-0.09
miR-29b	0.55	0.04	-0.44
miR-423	-0.75	0.68	0.21
miR-133b	-0.04	0.31	-0.16
miR-132	0.22	-0.09	-0.06
miR-139	0.57	-0.08	-0.44
miR-350	0.26	-0.25	0.01
miR-582	0.77	-0.40	-0.39
miR-365	0.32	-0.21	-0.18
Let-7b	-0.06	-0.02	0.00
miR-133a	0.31	-0.04	-0.36
miR-652	-0.08	0.28	-0.33
miR-342	-0.08	0.61	-0.66
miR-106b	0.44	0.00	-0.57
miR-128	-0.14	0.12	-0.13
miR-193a	0.18	0.35	-0.69
miR-302a	-1.33	0.63	0.54
miR-98	0.18	0.06	-0.41
miR-125a	0.47	-0.55	-0.10
miR-133b*	0.13	0.02	-0.34
miR-7a	0.18	0.28	-0.71
miR-192	-0.19	0.32	-0.41
miR-140	-0.03	-0.23	-0.02
miR-191	-0.29	0.27	-0.28
miR-30a	-0.11	0.41	-0.60
miR-328	-0.09	-0.14	-0.09

miR-30d	-0.27	0.46	-0.52
miR-30e	-0.11	0.42	-0.63
Let-7g	0.25	-0.08	-0.51
miR-377	-0.54	-0.67	0.84
miR-130a	-0.27	-0.03	-0.13
Let-7c	-0.05	-0.29	-0.12
Let-7i	0.00	-0.21	-0.27
miR-99b	-0.11	-0.03	-0.35
Let-7e	0.06	-0.09	-0.49
miR-29c	0.26	-0.12	-0.67
let-7a	-0.24	-0.22	-0.11
miR-467a	0.36	-0.87	-0.11
miR-29a	-0.14	0.01	-0.51
miR-155	-0.69	0.23	-0.18
miR-17	0.36	-0.48	-0.57
miR-143	0.03	-0.25	-0.47
miR-206	0.13	-0.18	-0.66
miR-186	0.34	-0.53	-0.57
miR-15a	0.42	-0.44	-0.77
miR-151	-0.14	-0.46	-0.20
miR-374b	0.40	-0.36	-0.83
miR-1a	-0.24	-0.26	-0.30
miR-345	-0.19	0.08	-0.71
miR-378a	-0.22	-0.15	-0.45
miR-16	-0.29	0.09	-0.63
miR-22	-0.02	-0.30	-0.51
miR-144	-0.19	0.12	-0.79
miR-223	-0.55	0.51	-0.83
miR-190b	-0.57	-0.62	0.30
miR-203	0.20	-0.45	-0.68
Let-7f	-0.16	-0.42	-0.40
miR-20a	0.20	-0.44	-0.75
miR-187*	-0.46	0.10	-0.65
miR-146a	-0.31	-0.09	-0.63
Let-7d	-0.32	-0.25	-0.46
miR-93	0.08	-0.40	-0.71
miR-351	-0.20	-0.07	-0.78
miR-26a	0.12	-0.51	-0.70
miR-122	-0.47	-0.26	-0.37
miR-499	0.16	-0.41	-0.85
miR-190a	0.36	-0.73	-0.73
miR-149	-0.14	-0.44	-0.56
miR-218	0.48	-0.15	-1.55

miR-449b	-0.11	-0.05	-1.07
miR-30c	0.29	-0.43	-1.11
miR-145a	-0.14	-0.55	-0.57
miR-195a	-0.16	-0.56	-0.57
miR-92a	0.18	-0.62	-0.86
miR-101b	0.36	-0.76	-0.92
miR-451a	-0.40	-0.21	-0.76
miR-224	0.50	-0.81	-1.07
miR-101a	0.16	-0.68	-0.90
miR-19a	-0.06	-0.44	-0.96
miR-26b	-0.14	-0.50	-0.93
miR-322	-0.16	-0.82	-0.59
miR-450a	-0.08	-0.77	-0.74
miR-30a*	0.12	-0.59	-1.16
miR-467d	-0.17	-0.83	-0.64
Let-7d*	-0.12	-0.66	-0.92
miR-25	-0.12	-0.63	-0.96
miR-194	-0.20	-0.72	-0.82
miR-181b	-0.09	-0.40	-1.26
miR-30b	0.05	-0.63	-1.17
miR-181d	-0.26	-0.33	-1.17
miR-10b	-0.72	-0.37	-0.70
miR-19b	-0.28	-0.61	-0.98
miR-335	-0.11	-0.68	-1.11
miR-150	-0.10	-1.05	-0.86
miR-30e*	-0.17	-0.67	-1.20
miR-669p	-0.77	-0.35	-1.03
miR-10a	-0.53	-0.89	-0.91
miR-126*	-0.28	-1.17	-0.90
miR-126	-0.09	-1.09	-1.19
miR-145a*	-0.09	-0.86	-1.46
miR-181a	-0.35	-0.79	-1.35
miR-181c	-0.23	-0.64	-1.64
miR-204	1.36	-0.54	-3.57
miR-3102	-0.88	-1.16	-2.22

Table S2. List of PCR primers for cloning and RT-PCR.

Primers for analyzing Jph2 transcripts	
E1-E2-F	5'-GCTATGGCACGGAGACCTAC-3'
E1-E2-R	5'-CTTGTCGTTCTTCCACTCGC-3'
E2-E3-F	5'-GCGAGTGGAAGAACGACAAG-3'
E2-E3-R	5'-CTGGCTGGTAGAAGTCTGGAG-3'
E3-E4-F	5'-CTCCAGACTTCTACCAGCCAG-3'
E3-E4-R	5'-GCTCCTGCTCATCCTCCAAG-3'
E4-E5-F	5'-CTTGGAGGATGAGCAGGAGC-3'
E4-E5-R	5'-CATACAGATGAGGACGGTGTTG-3'
5U-E1-F	5'-AGTTGTCAGGAGCGAGGATG-3'
5U-E1-R	5'-GTAGGTCTCCGTGCCATAGC-3'
5'UTR-F	5'-CCAGCGGCTGCTTCAGG-3'
5'UTR-R	5'-AGGAAGCCGACCGAAGAGA-3'
Primers for luciferase reporter cloning	
m*Jph2*-WT-FL-F	5'-CCGCTCGAGCCCTCATCTACCAGTGCCAG-3'
m*Jph2*-WT-FL-R	5'-ATAGTTTAGCGGCCGCTGCAGTTGAACCTGTGAGC-3'
m*Jph2*-WT-Core-F	5'-TCGAGCACACTGGCTGCTCTCAGCACTGCCGGC-3'
m*Jph2*-WT-Core-R	5'-GGCCGCCGGCAGTGCTGAGAGCAGCCAGTGTGC-3'
m*Jph2*-SM1-F	5'-GGCAGGACCTGTCTTTCTGGTCACGGTAGCAGGTGTCCCAGGAAG-3'
m*Jph2*-SM1-R	5'-CTTCCTGGGACACCTGCTACCGTGACCAGAAAGACAGGTCCTGCC-3'
m*Jph2*-SM2-F	5'-ACTGGCTGCTCTCAGAGTGCGCGGGGCTTGGTGGCTT-3'
m*Jph2*-SM2-R	5'-AAGCCACCAAGCCCCGCGCACTCTGAGAGCAGCCAGT-3'
h*JPH2*-WT-FL-F	5'-CCGCTCGAGCCGTCGCTTACCAGA-3'
h*JPH2*-WT-FL-R	5'-TAGTTTAGCGGCCGCCCGTGAGCAGATGC-3'
h*JPH2*-WT-Core-R	5'-ATAGTTTAGCGGCCGCCACAGGCACCGTGGGA-3'
h*JPH2*-SM1-F	5'-GGACATGAGGACCCGTCTTTTTGGTCACGGTGGCAGGGTGGCCAAAGAGG-3'
h*JPH2*-SM1-R	5'-CCTCTTTGGCCACCCTGCCACCGTGACCAAAAAGACGGGTCCTCATGTCC-3'
Primers for RT-PCR	
miR-34a-5p-F	5'-TGGCAGTGTCTTAGCTGGTTG-3'
miR-221-3p-F	5'-AGCTACATTGTCTGCTGGG-3'
miR-222-3p-F	5'-AGCTACATCTGGCTACTGGG-3'
miR-124-3p-F	5'-TAAGGCACGCGGTGAATGC-3'
Universal-miR-R	From miScript SYBR Green PCR Kit (Qiagen, 218073)
U6-F	5'-CTCGCTTCGGCAGCACA-3'
U6-R	5'-AACGCTTCACGAAT TTGCGT-3'
RBFox2-RT-F	5'-AACCAGGAGCCAACAACAAC-3
RBFox2-RT-R	5'-ACTCCCGTAGAGGGTCAGGT-3'
Jph2-RT-F	5'-AGAGGCAGCAGAACAGGC-3'
Jph2-RT-R	5'-CTCCAGCAAGCTCTCAGAG-3'
Anf-RT-F	5'-AGGCAGTCGATTCTGCTTGA-3'
Anf-RT-R	5'-CGTGATAGATGAAGGCAGGAAG-3'
Bnp-RT-F	5'-TAGCCAGTCTCCAGAGCAATTC-3'
Bnp-RT-R	5'-TTGGTCCTTCAAGAGCTGTCTC-3'
Cav3-RT-F	5'-GCGACCCCAAGAACATCAAT-3'
Cav3-RT-R	5'-GGAGATACAGGCGAACAGGA-3'
Primers for alternative splicing qPCR	
Pdlim5-E3-E9-F	5'-GGCTCCTTGAATATGACTCTACA-3'
Pdlim5-E3-E9-R	5'-GTCCTCAGTGTCCTCAATCAG-3'
Mef2a-SP2-F	5'-GTGGTGGCAGTCTTGGAATG-3'
Mef2a-SP2-R	5'-AGAGGTTGAGTGGCTTGAGAA-3'
Ldb3-4593-F	5'-CGGAAGATGAGGCTGATGAGT-3'
Ldb3-4593-R	5'-CGGATGCTGGCAGTGGTTA-3'