SUPPLEMENTAL INFORMATION

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Molecular Cloning and DNA Constructs
For minigene reporter, mouse tail genomic DNA was used as template. Oligos containing ApaI and BglII digestion sites were used to amplify Mef2a and Mef2d exon α2 with the flanking introns. Oligos containing mutant and deletion RBFox1 binding site were used to generate mutant minigene reporters in PCR reaction. After amplification, the PCR products were purified through QIAquick Gel Extraction Kit (Qiagen) according to manufacturer's instruction. The purified PCR products were digested and ligated into linearized pDUP33 minigene vector. For RBFox1 overexpression construct, oligos containing KpnI and XhoI digestion sites were used to amplify muscle specific RBFox1. The digested PCR product was subsequently ligated into linearized pShuttle-CMV vector. Primers used for real-time PCR analysis and constructs cloning see Table S3.

Mouse generation:
For RBFox1-transgenic mouse: The cardiac specific tetracycline-inducible mice overexpressing RBFox1 was generated using a mouse muscle specific RBFox1 cDNA inserted into the inducible α-myosin heavy chain promoter expression vector. The RBFox1(C57BL/6 background) mice were crossed with tTA mice to produce wild-type, tTA, RBFox1 and RBFox1/tTA mice. The wildtype and single transgenic mice were used as control (non-TG) and RBFox1/tTA mice were used as RBFox1-TG. The Non-TG and RBFox1-TG mice were fed with food containing 0.625g/kg Doxycycline obtained from Harlan (Teklad Global Rodent Diet). Mice were switched to regular diet to induce RBFox1 expression for at least 2 weeks before cardiac function or TAC procedure was measured or operated. The RBFox1-cardiac specific knockout mouse (CKO): B6.129S2-$^{Rbfox1tm1.1Dblk}$/J mouse line was purchased from The Jackson Laboratory(1). These mice were crossed with Nkx2.5-Cre mice to generate RBFox1-CKO mice(2). All animals in this study were handled in accordance with the *Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health*.

Histological Analysis:
WGA Staining: Cardiomyocytes surface area and myocyte cross-section area were quantified by staining cell surface border with Wheat germ Agglutinin (WGA). Cardiomyocytes and heart histology sections were fixed and blocked with 5%BSA/PBS at room temperature for 1hr before stained with WGA solution (Invitrogen W-11261) at room temperature for 1 hour. The cells were visualized under a Nikon fluorescent microscope and area of interest was quantified using NIS-Elements AR Analysis program. **Masson-Trichrome Staining**: Heart histology slides were rehydrated and stained using Masson-Trichrome staining kit (Sigma-Aldrich HT-15) according to manufacture's protocol. Fibrosis was visualized under microscope under bright field.

Echocardiogram Analysis for Mouse Heart Function:
Mice were imaged non-invasively for heart function and morphology by echocardiography prior and follow TAC surgery on weekly basis. The mice were anesthetized and maintained with 2% isofluorane in 95% oxygen. A Vevo 2100 (Visual Sonics) echocardiography system with a 30mHz scanhead was used to acquire the data. A parasternal long axis and short axis view were recorded. The short axis view was used to generate M-mode images for analysis of ejection fraction and fraction shortening.

Protein synthesis analysis:
Protein synthesis rate was analyzed through puromycin incorporation assay. Cells are cultured under regular media and treated with PE alone or in combination with RBFox1 adenovirus. Cells were labeled with 1uM puromycin for 30 min at 37 degree before harvesting for Western Blot analysis. Western Blot

was performed using anti-puromycin antibody (Millipore MABE343) and puromycin signal is quantified by densitometry.

Myocardial Samples from non-failing and failing human hearts

The failing heart samples (n=4) were obtained from the left ventricular (LV) anterior wall during heart transplantation or implantation of an LV assist device as described(3). The non-failing heart samples (NF) (n=4) were obtained from the LV free wall and procured from National Disease Research Interchange (NDRI) and University of Pennsylvania. NF heart donors had no history of macroscopic or laboratory signs of cardiac diseases. The tissue collection was approved by the UCLA Institutional Review Board #11-001053 and #12-000207.

Pressure-overload in mouse

Left ventricle tissues were collected from male C57BL/6 mice at indicated time points post trans-aortic constriction (TAC) procedure (HF) and their corresponding Sham controls as described(4). Doppler velocity measurement of right and left carotid arteries were obtained from TAC treated mice to confirm the consistency of the surgery procedure. The heart failure status of the TAC treated animals was established based on a significant increase in heart weight and a significant reduction in ejection fractions measured by echocardiography.

In RBFox1-TG TAC surgery, both male and female were included in the study. In RBFox1-cKO TAC surgery and cardiac function analysis, both male and female were included in the study. The control mice for RBFox1-cKO include three different genotype: Nkx2.5-Cre (-)/ RBFox1-$^{(flox/flox)}$; Nkx2.5-Cre (+)/RBFox1 $^{(+/+)}$ and Nkx2.5-Cre (-)/RBFox1 $^{(+/flox)}$

RNA-Seq

A total of 5ug RNA per sample was processed via poly-A selection and fragmentation. The first-strand cDNA was generated using random primer based reverse transcription and subsequently used for generating second-strand cDNA using RNase H and DNA polymerase. Sequence adapters were ligated using the Illumina Paired-End sample prep kit. Fragments of ~200bp were isolated by gel electrophoresis, amplified by 15 cycles of PCR and sequenced on the Illumina Genome Analyzer II in the paired-end sequencing protocol as described(4).

Western Blot

20ug of mouse heart protein was used for each sample and 10ug of cell lysates from neonatal rat ventricle cardiomyocytes was used for each sample. Western Blot was performed using NuPAGE SDS-PAGE Gel System (Invitrogen) according to the manufacturer's instruction. Anti-RBFox1 antibody was generated as described(5). Anti-RBFox2 antibody was purchased from Abcom (ab51361)**.** Anti-HDAC2 antibody was purchased from Cell Signaling. Anti-Actin antibody was purchased from Santa Cruz Biotechnology (SC-1616). Antibody used for RNA Polymerase II ChIP-Seq is purchased from Abcam (ab5095).

Minigene Reporter for RNA Splicing

The mouse Mef2a and Mef2d -Exonα2 minigene reporter was constructed based on pDUP33 as template(6) which contains splicing donor exons and mouse Mef2a/d Exon α2 and its surrounding intron sequences obtained from mouse genomic DNA. The mutant Mef2a/d minigene reporters were subsequently generated by mutating the putative RBFox1 binding motif in the Mef2a/d intron sequence or deleting the RBFox1 binding motif as indicated in Figure S5.

CLIP

CLIP assay was performed using a kit according to manufacture's protocol with minor modification (Millipore 17-700). Briefly, C2C12 cells were mock infected or infected with DN-RBFox1 and RBFox1 adenovirus. 48hr post infection, cells were UV cross-linked and followed by immune-precipitation with RBFox1 or IgG antibody. After immune-precipitation, protein was digested using proteinase K and

cDNA library was generated from the cross-linked RNA. Different primers targeting Mef2a, Mef2c, Mef2d intron region with or without RBFox1 binding motif were used for RT-PCR analysis as described(7).

Zebrafish Injection

Wild-type zebrafish embryo was injected at one-two cell stage with either morpholino (GENE TOOLS, LLC) or mRNA at indicated concentration/amount. Standard control morpholino is purchased from GENE TOOLS (CCTCTTACCTCAGTTACAATTTATA).Zebrafish phenotype was analyzed at 48hpf under stereo microscope as previously described(8).

Rasl-SEQ

Rasl-seq was performed according to previously described(9). Briefly, Rasl-seq was performed to profile large number of mRNA isoforms using pooled pairs of oligonucleiotides each flanked by a universal primer to target specific splice junctions in the spliced mRNAs. The current Rasl-seq protocol is designed to detect a total of 3885 alternative splicing events in mouse genome. Total RNA from 25 mouse hearts including control, RBFox1-TG and RBFox1-CKO mice under baseline and post-pressure overload induced stress was isolated and subject to rasl-seq analysis. Differentially expressed exons were identified from normalized reads and analyzed by clustering or comparison for overlapping exons among different groups.

Real-time PCR

1ug RNA was used for first-strand cDNA synthesis using Random Primer (Invitrogen) and SuperScriptII Reverse Transcriptase (Invitrogen) according to manufacturer's instruction. Real-time PCR was performed using IQ SYBR Green Supermix (Bio-Rad) with CFX-96 Real-time PCR Detection System (Bio-Rad).

Cell Culture

Neonatal rat ventricular myocytes (NRVM) were prepared from 2 day old Sprague Dawley rat by enzymatic digestion with collagenase (Worthington) and pancreatin (Sgima) in 1xADS buffer at 37^{o}C as described previously(10). NRVM were cultured in serum-free DMEM supplemented with 100U/mL Pen/Strep (Invitrogen) and 5% ITS (w/v) (BD Biosciences). HEK293 cells (ATCC: CRL-1573) and C2C12 cells (ATCC: CRL-1772) were cultured in DMEM with 10% fetal bovine serum and 5% Pen/Strep (Invitrogen). Lipofectamine 2000 (Invitrogen) was used to transfect DNA and siRNA according to manufacturer 's recommendations as previously described(10).

Statistical Analysis

Data are expressed as mean ± STDEV. For comparison between two groups, differences were analyzed by Student's t-test. For multiple groups' comparison, differences were analyzed by one-way ANOVA. p values ≤ 0.05 were considered as significant.

References Cited in Supplemental Methods:

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5. Tang ZZ, Zheng S, Nikolic J, and Black DL. Developmental control of CaV1.2 L-type calcium channel splicing by Fox proteins. *Mol Cell Biol.* 2009;29(17):4757-65.
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7. Ule J, Jensen K, Mele A, and Darnell RB. CLIP: a method for identifying protein-RNA interaction sites in living cells. *Methods.* 2005;37(4):376-86.
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9. Li H, Qiu J, and Fu XD. RASL-seq for massively parallel and quantitative analysis of gene expression. *Curr Protoc Mol Biol.* 2012;Chapter 4(Unit 4 13 1-9.
10. Ota A, Zhang J, Ping P, Han J, and Wang Y. Specific regulation of noncanonical p38alpha activation by Hsp90-Cdc37 chaperone complex in cardiomyocyte. *Circ Res.* 2010;106(8):1404-12.

Supplement Table 1

Alternative Splicing Events Candidate Genes

Gene ID	Exon	Splicing Event in Failing Heart
Map3k7	chr4:32081849:+_chr4:32081929:+	Exon Skipping
Ccne1	chr7:38884266:-_chr7:38884377:-	Exon Skipping
Bach2	chr4:32504410:+_chr4:32504558:+	Exon Skipping
Lyst	chr13:13773559:+_chr13:13773789:+	Exon Skipping
Ptprd	chr4:75781449:-_chr4:75781460:-	Exon Skipping
Cmya5	chr13:93854500:-_chr13:93854595:-	Exon Skipping
Slc7a1	chr5:149159729:-_chr5:149159887:-	Exon Inclusion
Ppp3cb	chr14:21322469:-_chr14:21322498:-	Exon Inclusion
Palm2	chr4:57899161:+_chr4:57899199:+	Exon Skipping
Pdlim5	chr3:141969248:-_chr3:141969262:-	Exon Skipping
Slmap	chr14:27284687:-_chr14:27284758:-	Exon Skipping
Synpo2l	chr14:21484966:-_chr14:21485480:-	Exon Skipping
Cugbp2	chr2:6470878:-_chr2:6471021:-	Exon Skipping
Rbpms	chr8:34899864:-_chr8:34899967:-	Exon Skipping
Prpf39	chr12:66156421:+_chr12:66156520:+	Exon Inclusion
Map2k7	chr8:4240688:+_chr8:4240735:+	Exon Skipping
Map3k3	chr11:105985100:+_chr11:105985220:+	Exon Skipping
Mbnl2	chr14:120803860:+_chr14:120803954:+	Exon Inclusion
Mff	chr1:82738393:+_chr1:82738551:+	Exon Skipping
Slc27a1	chr8:74093241:+_chr8:74093347:+	Exon Skipping
Camk2d	chr3:126505855:+_chr3:126505887:+	Exon Inclusion
Camk2g	chr14:21565014:-_chr14:21565082:-	Exon Inclusion
Mef2a	chr7:74412953:-_chr7:74413090:-	Mutually Exclusive
Mef2d	chr3:87962143:+_chr3:87962277:+	Mutually Exclusive
Mtus1	chr8:42135339:-_chr8:42135470:-	Exon Skipping
Smtn	chr11:3420755:-_chr11:3420919:-	Exon Skipping
Myo7a	chr7:105221630:-_chr7:105221756:-	Exon Skipping
Deaf1	chr7:148510341:-_chr7:148510515:-	Exon Skipping
Mef2a	chr7:74413155:-_chr7:74413286:-	Mutually Exclusive
Mef2d	chr3:87961956:+_chr3:87962093:+	Mutually Exclusive
Mef2c	chr13:83764965:+_chr13:83765102:+	Mutually Exclusive
Mef2c	chr13:83764760:+_chr13:837649032:+	Mutually Exclusive

Supplemental Table 1. List of alternatively spliced candidate genes in pressure-overload induced mouse failing heart

Supplemental Table 2

Sham

Position	Kmer	Conservation	Enrichment	Known?
Upln 1st				
	CGGCG	7.21E-005	6.14E-004	
	GCGGC	1.90E-003	2.31E-002	
	CCGCC	2.74E-003	1.34E-002	
Upln 2nd				
	TTTTT	1.23E-004	8.78E-014	FESS;TIA
	TGATA	2.30E-003	8.59E-003	
	CACGT	6.01E-003	1.09E-002	
Exon				
	TACCA	1.75E-005	2.07E-004	
	TGTGT	3.32E-004	8.79E-003	CELF;
	AAGGA	6.39E-004	6.46E-004	RESE;
Dnln 1st				
	TGCAT	3.26E-008	3.33E-005	RBFox;
	GCATG	1.32E-007	1.36E-006	RBFox;
	CTGCT	1.48E-004	1.94E-002	
Dnln 2nd				
	ACATA	5.60E-004	9.89E-004	
	TGTGT	7.49E-004	3.93E-005	CELF;
	CGCGG	7.68E-004	6.12E-003	

Heart Failure

Position	Kmer	Conservation	Enrichment	Known?
Upln 1st				
	CACAC	9.25E-004	7.74E-005	hnRNPL
	CGTGG	5.89E-003	1.25E-003	
	GAGCA	8.03E-003	1.14E-002	
Upln 2nd				
	CTAAC	2.62E-004	9.62E-003	ASF;
	ACGCA	2.27E-003	3.00E-002	RESE;
	TATTT	2.84E-003	5.03E-003	
Exon				
	AAGGA	8.95E-007	6.94E-004	RESE;
	AGAAG	9.22E-007	1.26E-004	RESE;
	CTGGA	4.10E-006	7.57E-005	RESE;
Dnln 1st				
	CGCAC	2.67E-006	7.85E-004	
	GCGCA	5.02E-005	9.42E-003	
	CAGAA	5.39E-004	1.08E-003	RESE;
Dnln 2nd				
	GCATG	5.48E-005	1.70E-002	RBFox;
	AAACA	5.01E-004	6.81E-006	RESE;
	TTTAA	4.91E-003	9.38E-003	

Supplemental Table 2:Enrichment of RNA splicing factor binding motifs for alternatively spliced exons in cardiac transcriptome

Summary of enriched binding motifs among exons differentially included in normal or failing heart transcriptome identified through RNA-seq [4]. Using *de novo* motif discovery, 5-mers that are both evolutionarily conserved and highly enriched among the exons in the flanking introns and exonic regions. Five regions for each exon are analyzed and annotated: upstream intron first 250 nt (UpIn 1st), upstream intron last 250nt (UpIn 2nd), downstream intron first 250nt (DnIn 1st), downstream intron last 250nt (DnIn 2nd). RBFox binding motifs are highlighted in red.

Supplemental Table 3: List of RT-PCR primers.

Primer	Sequence 5'-3'
Pabpc4 RT-F	GGCTGTGGCTCCATACAAGT
Pabpc4 RT-R	TTGCAACTTGGTTCTTGGTG
Prpf39 RT-F	CGAAATTGAAAATGGGACTCA
Prpf39 RT-R	ATGCCTCACTCCTTCAATGC
Prpf40b RT-F	GTCAGAGACCACCAGCCATT
Prpf40b RT-R	CTCCCACACGGATTGCTTAT
Ptb1 RT-F	TCCAAGCTCACCAGTCTCAA
Ptb1 RT-R	AAGGCTCCATGGACATTAGG
Rbm4 RT-F	GCCTCGACAACACAGAGTTT
Rbm4 RT-R	GCACCACGAAGGGTGTATTC
Rbm4b RT-F	GCCAAGTTTGAGGAGTACGG
Rbm4b RT-R	GAGGGTTCAGTCCGCAATTA
Rbm45 RT-F	CACGAGTCAGCAGCAGTTTC
Rbm45 RT-R	AATCTTCCCGTGCAGAGTTG
Rbpms RT-F	GCAGTTCTGCTGAGGCTGT
Rbpms RT-R	CGATCTTGGCTTTACTGTTGC
Rnps1 RT-F	CACCTCTCCAAAGGCTATGC
Rnps1 RT-R	AGAGCGAGACCTCCTACGC
Rrp1 RT-F	AGAGCTGCTAACCACCGAGA
Rrp1 RT-R	GCTTCACCCTCTCCACTCTG
Sf1 RT-F	CAGCACAACCCAAATGGAC
Sf1 RT-R	CATGGAGGAAGAGGACCAGA
SC-35 Exp RT-F	GAGTCATTCTGCTGACAGC
SC-35 Exp RT-R	ATCATCAGCTAGATGTGCTC
HnrnpA1 Exp RT-F	TGGAAGCAA TTTTGGAGGTGG
HnrnpA1 Exp RT-R	GGTTCCGTGGTTTAGCAAAGT
HnrnpA2 Exp RT-F	AAGAAATGCAGGAAGTCCAAAGT
HnrnpA2 Exp RT-R	CTC CTCCATAACCAGGGCTAC
PTB Exp RT-F	AGCAGAGACTACAC TCGACCT
PTB Exp RT-R	GCTCCTGCATACGGAGAGG
9G8 Exp RT-F	ATCGCTATAGCCGACGAAGA
9G8 Exp RT-R	CGAGGAGATGCTGATCTTGA
ASF Exp RT-F	CACTGGTGTCGTGGAGTTTG
ASF Exp RT-R	GGCTTCTGCTACGACTACGG
CELF4 Exp RT-F	CAGCCCCCTTCACATAGAAA
CELF4 Exp RT-R	AGTGCACTCCTCGATGTTCC
Fox1 Exp RT-F	GTGGTTATGCTGCGTACCG
Fox1 Exp RT-R	GGAGCAAGTGTGTGGTGGTA
Fox2 Exp RT-F	AACCAGGAGCCAACAACAAC

Fox2 Exp RT-R	ACTCCCGTAGAGGGTCAGGT
HnrnpC Exp RT-F	CCTCCTCCTCCTCCTATTGC
HnrnpC Exp RT-R	TTGGAAGAAGATCCCCTTTG
Hnrnpl Exp RT-F	AAGAGGCAGGCACTGGTAGA
Hnrnpl Exp RT-R	CTCCGGGAGTCATCAGAGTC
MBNL1 Exp RT-F	CGTGCCAATGTTTTCAGTTG
MBNL1 Exp RT-R	GGCAAGATCTCTGCTGGAAC
Nova1 Exp RT-F	TGAGAGGGTTTGCTTGATCC
Nova1 Exp RT-R	CGATCAGGATTAACGGTGGT
Sam68 Exp RT-F	TTTGTGGGGAAGATTCTTGG
Sam68 Exp RT-R	GGGGGTCCAAAGACTTCAAT
Sf1 Exp RT-F	CCCTAACCCTGAGGACAGGT
Sf1 Exp RT-R	GGGTTGAGAGCAACCATCTC
SRp20 Exp RT-F	TGGAACTGTCGAATGGTGAA
SRp20 Exp RT-R1	GACGCTGAAAGGGCTAGTTG
Tia1 Exp RT-F	TTTGTGGAGTTCCATGAGCA
Tia1 Exp RT-R	GGTTGTTGCCCAATTCACTT
TIAL1 Exp RT-F	GTTGGGGATTTGAGTCCAGA
TIAL1 Exp RT-R	GGACTTTCCAGTTGCCATGT
TRA2A Exp RT-F	GCAGAAGGCATACTGGAAGC
TRA2A Exp RT-R	AAAAGCAAATCCACGTGACC
TRA2B Exp RT-F	TACTCGAAGGCGTCATGTTG
TRA2B Exp RT-R	TAGCTTCCTTGGCAT
CUGBP1 Exp RT-F	GTTCCAAGGACCTGGTCTGA
CUGBP1 Exp RT-R	CCAGGGAGGACCTTCATGTT
CUGBP2 Exp RT-F	GCCTCAAGTGCAGCTTTTCT
Cugbp1 RT-F	GCAGCTCAGAACACACCAAG
Cugbp1 RT-R	TTCCATGGTACTCCCAGTGC
Cugbp2 RT-F	ACCCAGGCCTACTCAGGAAT
Cugbp2 RT-R	AGGTTTGCTGTCGTTTTTGG
Camk2g E RT-F	AGTTCCAGCGTGCACCTAAT
Camk2g E RT-R	TTGTTGATGGCTTCGATCAG
Cmya5 E RT-F	AAATGGACAAGGCACTGGAC
Cmya5 E RT-R	CTCATCATACTGCGCCAAAA
Map2k7 E RT-F	CTTGGATATCAGCCCACAGC
Map2k7 E RT-R	CGGTGTGAACAAGGTTGATG
Map3k3 E RT-F	CTCCATCCTGTTGAAAAACCA
Map3k3 E RT-R	GTGCTGTTGTCGCTCAGGTA
Mef2a E RT-F	GAGCCTCATGAAAGCAGGAC
Mef2a E RT-R	CGAGTGAACTCCCTGGGTTA
Mff E RT-F	GCAGTTGGCAGGCTAAAAAG

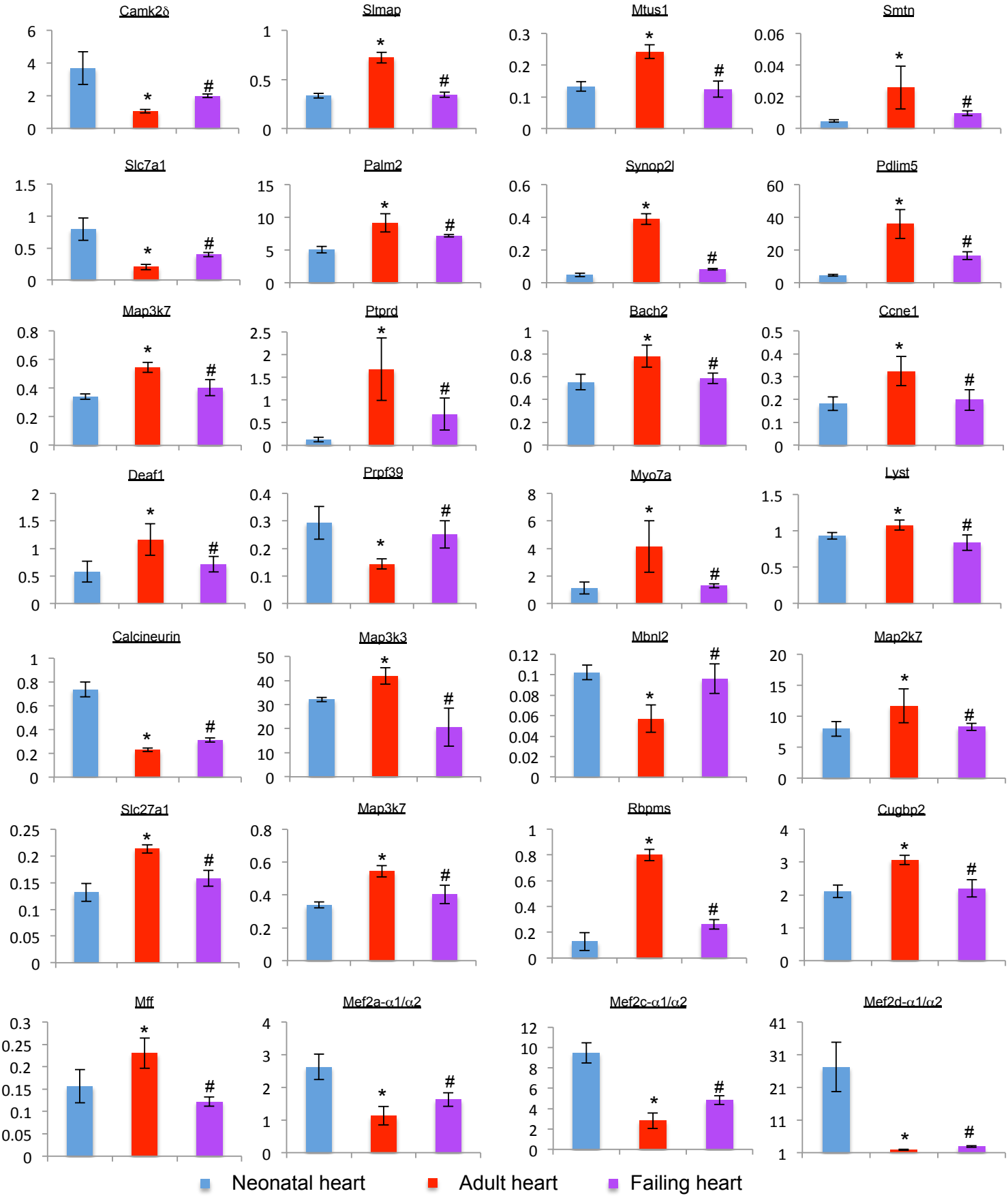
Mff E RT-R	CTGACGTTGTCATGATGAGGAT
Slc25a5 E RT-F	ACCCGTCTAGCAGCTGATGT
Slc25a5 E RT-R	AAAAGCCTTGCTCCCTTCAT
A2bp1 cDNA F1	CTCAGGCCTCCACTAGTGATGAATTGTGAAAGAG AGCAGCT
A2bp1 cDNA F2	CTCAGGCCTCCACTAGTCATGTTGGCGTCGCAAGG AGTCC
A2bp1 cDNA R	CTCAGGCCTCCTCTAGAGATATGGAGCAAAACGG TTGTATCC
zA2bp1 nu RT-F	ACAGTGACAGTTACGGACGAGTTT
zA2bp1 cy RT-F	TGAAATTTCTTGTTTACGGACGAGT
zA2bp1 RT-R	TAATATGGCGCGAAACGACT
zA2bp1l RT-F	ATCCAGCCACATATCGAGTG
zA2bp1l RT-R	ACTGGCCATTGTTCCAACTC
muA2bp1 F Flag	TAATCTCGAGATGGACTACAAGGACGACGATGAC AAGTTGGCGTCGCAAGGAGTCCT
Fox-1 Neu F	GTTTACCAGGATGGATTTTATGGTG
Fox-1 53R	GGTGTTACAAGAAATTTCATCTGTTG
Fox-1 76R	AAACTCGTCCGTAACTGTCACTGTAGGCAG
Fox-1 Mus F	AAGAGCCAGTGTATGGCAATAAA

Supplemental Table 4

Ensembl Gene ID	Gene Symbol	foldChange	pval
ENSRNOG00000013973	Lcn2	171.4865558	0
ENSRNOG00000017414	IRF7	53.1643994	1.67E-304
ENSRNOG00000031743	GBP2	38.52187197	1.27E-259
ENSRNOG00000032240	gbp5	62.02930695	9.18E-245
ENSRNOG00000037198	usp18	64.91098284	3.95E-240
ENSRNOG00000022839	Ifit3	48.10842372	3.27E-233
ENSRNOG00000026415	col14a1	0.067074976	5.37E-229
ENSRNOG00000022637	NULL	12.21657861	1.50E-216
ENSRNOG00000001963	MX2	113.8352924	1.17E-212
ENSRNOG00000011971	C1s	24.12130876	2.41E-205
ENSRNOG00000001187	OASL	35.64755332	1.55E-190
ENSRNOG00000006151	Reg3b	14.20878678	5.39E-181
ENSRNOG00000014786	CCNE1	14.76338029	2.67E-177
ENSRNOG00000000419	CFB	21.20260821	3.34E-168
ENSRNOG00000000239	CCL7	90.64799258	1.68E-161
ENSRNOG00000022256	CXCL10	57.21871978	1.33E-150
ENSRNOG00000029195	Uba7	28.08079946	1.12E-143
ENSRNOG00000018257	HPX	106.9956782	1.94E-141
ENSRNOG00000019648	Col6a3	0.107641632	8.38E-138
ENSRNOG00000009822	TLR2	13.23376594	1.41E-135

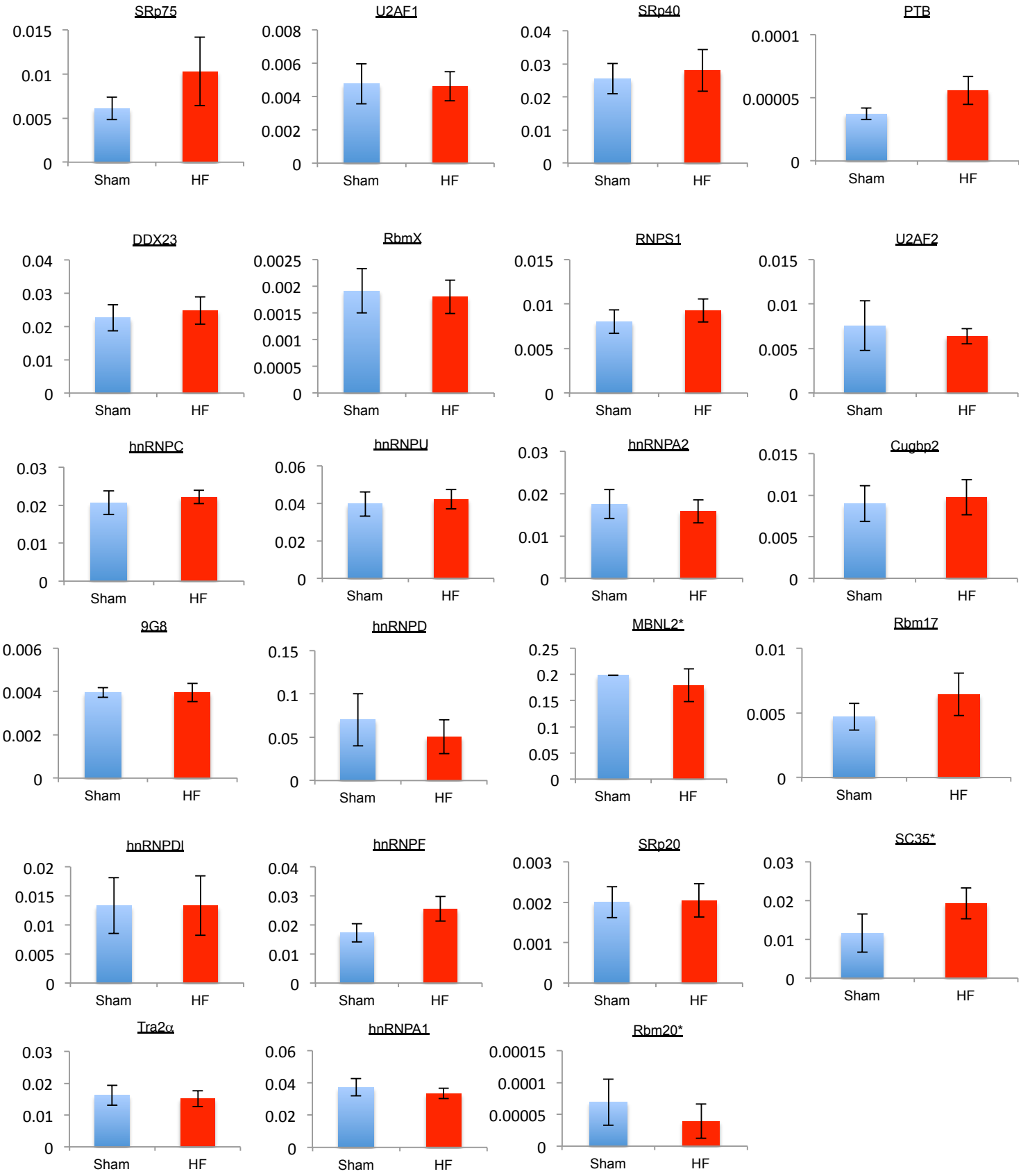
Supplemental Table 4: Top20 significant changed genes in RBFox1 expressed neonatal cardiomycytes
List of top 20 significantly changed genes in Figure 1J

Figure S1


Camk2δ
Slmap
Mtus1
Smtn
Slc7a1
Palm2
Synop2l
Pdlim5
Map3k7
Ptprd
Bach2
Ccne1
Deaf1
Prpf39
Myo7a
Lyst
Calcineurin
Map3k3
Mbnl2
Map2k7
Slc27a1
Map3k7
Rbpms
Cugbp2
Mff
Mef2a-α1/α2
Mef2c-α1/α2
Mef2d-α1/α2
Neonatal heart
Adult heart
Failing heart

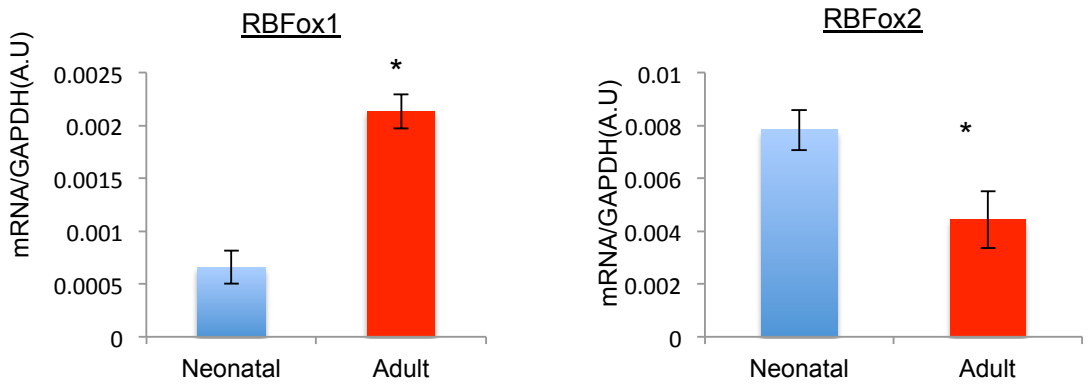
Supplemental Figure S1: Relative expression levels of 32 splicing events were measured by real-time qPCR using exon specific primers in Sham and TAC induced failing mouse hearts. The RNA samples were obtained from left ventricles of mouse P1 neonatal hearts (Neonatal), 5 month old normal adult hearts (Adult) and age-matched pressure-overload induced failing hearts (Failing Heart) as indicated. (n=3 for each group). *, $p<0.05$ between healthy adult and neonatal hearts, #, $p<0.05$ between healthy adult and failing hearts. Significant differences between groups were determined by student t-test .

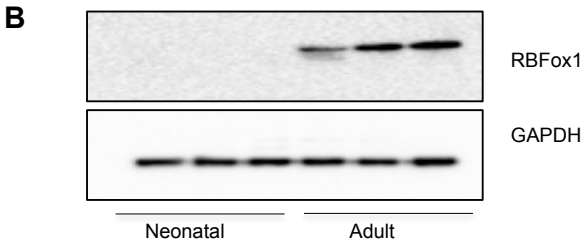
Figure S2

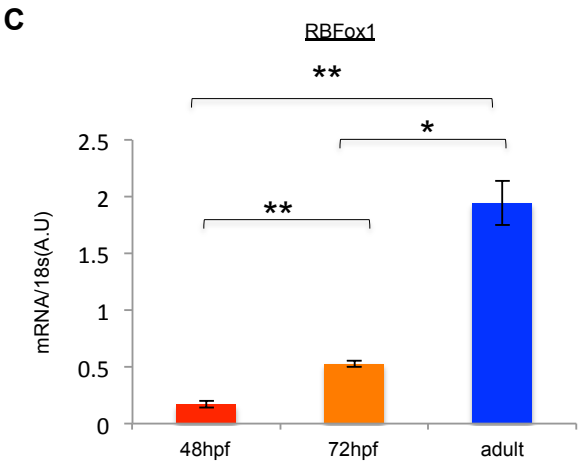

SRp75
0.015
0.01
0.005
0
Sham
HF
U2AF1
0.008
0.006
0.004
0.002
0
Sham
HF
SRp40
0.04
0.03
0.02
0.01
0
Sham
HF
PTB
0.0001
0.00005
0
Sham
HF
DDX23
0.04
0.03
0.02
0.01
0
Sham
HF
RbmX
0.0025
0.002
0.0015
0.001
0.0005
0
Sham
HF
RNPS1
0.015
0.01
0.005
0
Sham
HF
U2AF2
0.015
0.01
0.005
0
Sham
HF
hnRNPC
0.03
0.02
0.01
0
Sham
HF
hnRNPU
0.06
0.04
0.02
0
Sham
HF
hnRNPA2
0.03
0.02
0.01
0
Sham
HF
Cugbp2
0.015
0.01
0.005
0
Sham
HF
9G8
0.006
0.004
0.002
0
Sham
HF
hnRNPD
0.15
0.1
0.05
0
Sham
HF
MBNL2*
0.25
0.2
0.15
0.1
0.05
0
Sham
HF
Rbm17
0.01
0.005
0
Sham
HF
hnRNPDl
0.02
0.015
0.01
0.005
0
Sham
HF
hnRNPF
0.04
0.03
0.02
0.01
0
Sham
HF
SRp20
0.003
0.002
0.001
0
Sham
HF
SC35*
0.03
0.02
0.01
0
Sham
HF
Tra2α
0.03
0.02
0.01
0
Sham
HF
hnRNPA1
0.06
0.04
0.02
0
Sham
HF
Rbm20*
0.00015
0.0001
0.00005
0
Sham
HF

Supplemental Figure S2: Expression profile of splicing regulators in pressure-overload induced failing hearts. Known splicing regulators expression profile is determined using real-time PCR in adult 8 weeks post-TAC or Sham operated hearts. N=3 each sample. * indicates the splicing regulators with known function in hearts. Significant differences between groups were determined by student t-test

Figure S3

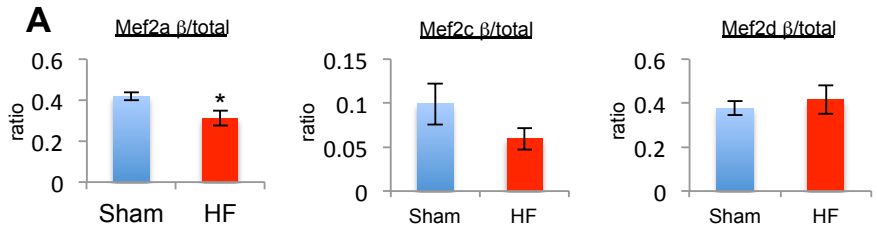

RBFox1
mRNA/GAPDH(A.U)
0.0025
0.002
0.0015
0.001
0.0005
0
*
Neonatal
Adult
RBFox2
mRNA/GAPDH(A.U)
0.01
0.008
0.006
0.004
0.002
0
*
Neonatal
Adult

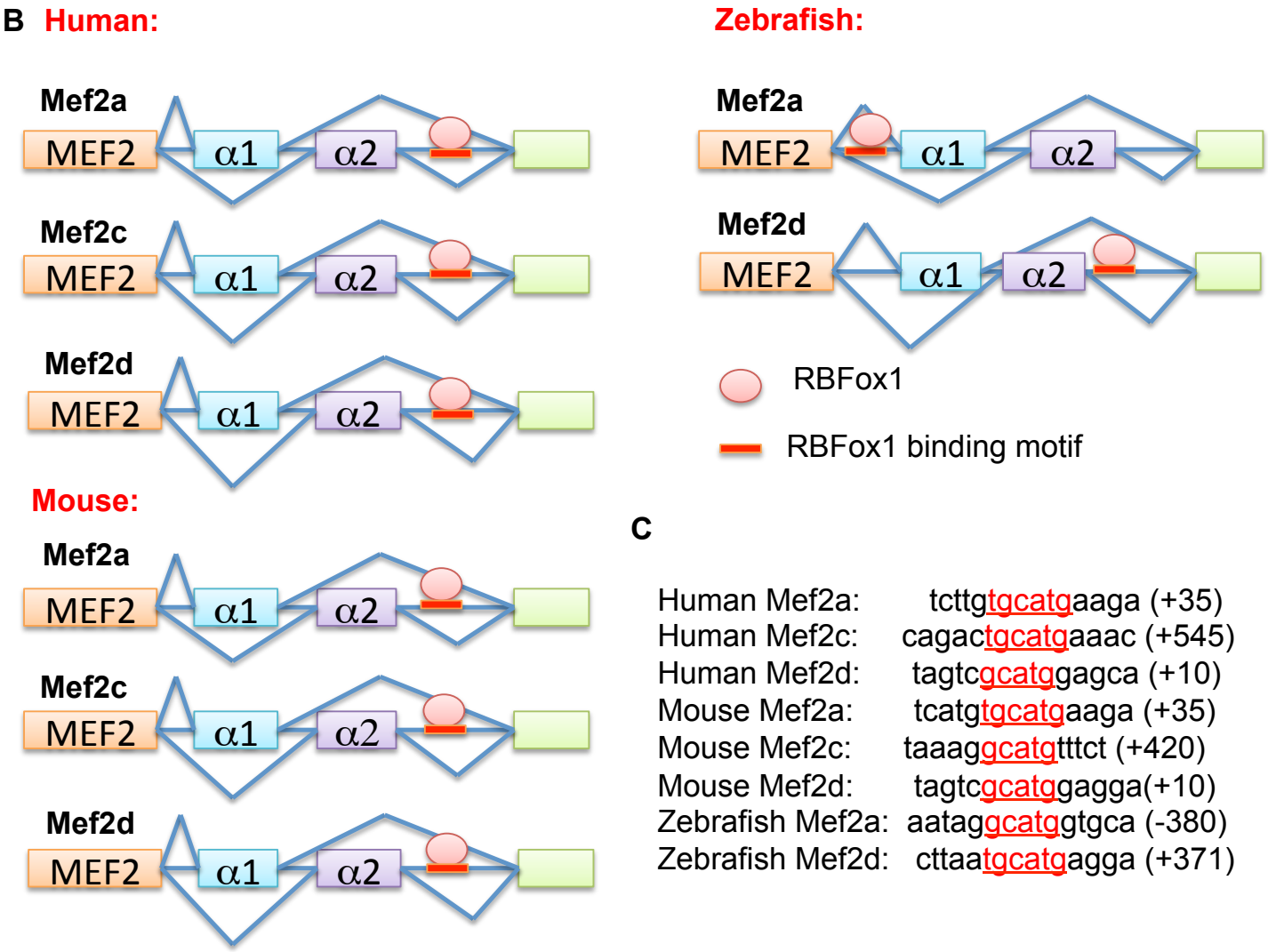

B
RBFox1
GAPDH
Neonatal
Adult


C
RBFox1
**
*
**
mRNA/18s(A.U)
2.5
2
1.5
1
0.5
0
48hpf
72hpf
adult

Supplemental Figure S3: RBFox1 expression is significantly induced during cardiac development. A. RBFox1 and RBFox2 expression was determined by real-time PCR in post-natal day 1 mouse hearts and compared with 2 month old adult mouse hearts. N=3 each sample, *,$p<0.05$. **B.** RBFox1 expression level is determined by Western Blot analysis in post-natal day 1 neonatal hearts comparing to 8 weeks old adult male mouse hearts. N=3 each sample. **C**. RBFox1 expression level is determined by real-time PCR during different zebrafish cardiac developmental stages. Zebrafish embryos hearts were isolated at indicated developmental stages and RNA was extracted from 30 pooled hearts as one sample. Significant differences between groups were determined by student t-test.

Figure S4



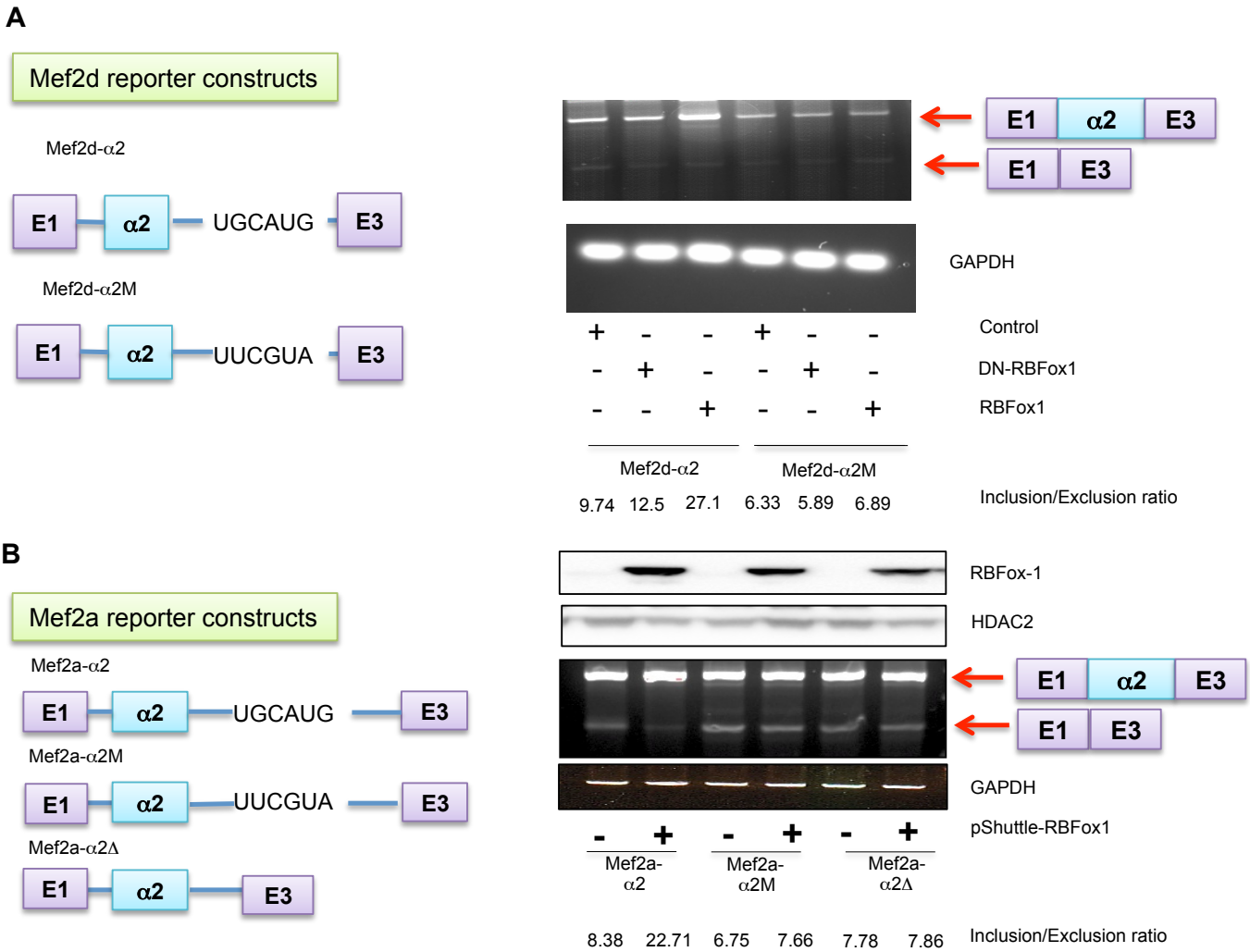


C

Human Mef2a: tcttgtgcatgaaga (+35)
Human Mef2c: cagactgcatgaaac (+545)
Human Mef2d: tagtcgcatggagca (+10)
Mouse Mef2a: tcatgtgcatgaaga (+35)
Mouse Mef2c: taaaggcatgtttct (+420)
Mouse Mef2d: tagtcgcatggagga(+10)
Zebrafish Mef2a: aataggcatggtgca (-380)
Zebrafish Mef2d: cttaatgcatgagga (+371)

Supplemental Figure S4: Alternative splicing profile of MEF2 hearts. **A.** Mef2 family member b exon expression level was quantified by real-time PCR in Sham and failing mouse hearts. N=3 each sample. **B.** Schematic view for the MEF2 mutually exclusive spliced exon and conserved RBFox1 binding motif in MEF2 genes in human, mouse and zebrafish. **C.** Sequence containing RBFox1 binding motif in MEF2. Number represents location from α2 exon. *: Number in zebrafish Mef2a represents location from α1 exon. Significant differences between groups were determined by student t-test (**A**).

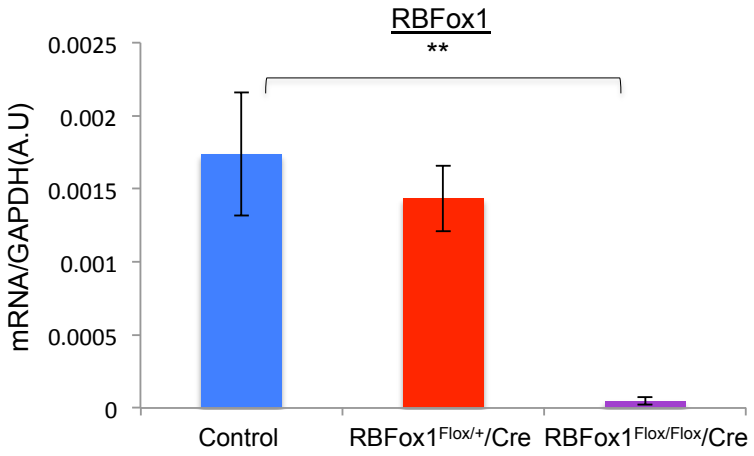
Figure S5


A
Mef2d reporter constructs
Mef2d-α2
E1
α2
UGCAUG
E3
Mef2d-α2M
E1
α2
UUCGUA
E3
E1
α2
E3
E1
E3
GAPDH
+ - - + - -
- + - - + -
- - + - - +
Control
DN-RBFox1
RBFox1
Mef2d-α2
Mef2d-α2M
9.74 12.5 27.1 6.33 5.89 6.89
Inclusion/Exclusion ratio
B
Mef2a reporter constructs
Mef2a-α2
E1
α2
UGCAUG
E3
Mef2a-α2M
E1
α2
UUCGUA
E3
Mef2a-α2Δ
E1
α2
E3
RBFox-1
HDAC2
E1
α2
E3
E1
E3
GAPDH
pShuttle-RBFox1
- + - + - +
Mef2a-α2
Mef2a-α2M
Mef2a-α2Δ
8.38 22.71 6.75 7.66 7.78 7.86
Inclusion/Exclusion ratio
C
Control
Ad-DN-RBFox1
Ad-RBFox1
UV crosslink
IP RNA-Protein Complex
Proteinase K Digestion
RT-PCR
D
RNA-IP
5%input
Mef2a In5
Mef2a In6
IgG
RBFox1
- + - + - +
+ - + - + -
Control
DN-RBFox1
RBFox1
Control
DN-RBFox1
RBFox1
RNA-IP
5%input
Mef2c In4
Mef2c In7
IgG
RBFox1
- + - + - +
+ - + - + -
Control
DN-RBFox1
RBFox1
Control
DN-RBFox1
RBFox1

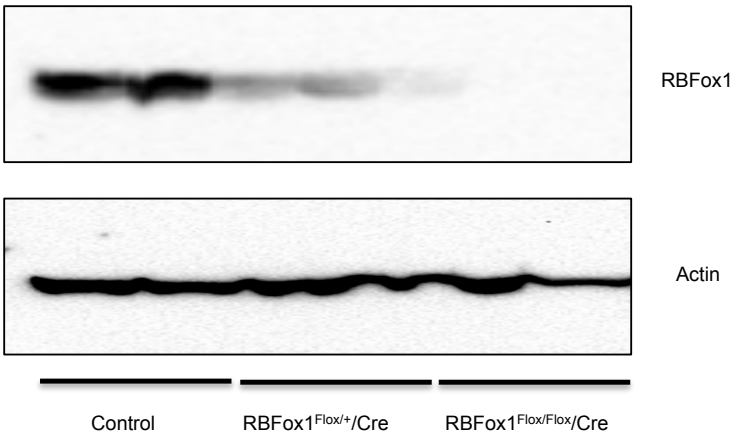
Supplemental Figure S5: MEF2 is a direct downstream target of RBFox1 in NRVM. A. Minigene reporter construct containing mouse Mef2d Exon α2 and adjacent intron fragment indicated as in Figure 1 were used to transfected with NRVM with RBFox1 adenovirus co-injection. 48hr post injection, cells were collected and RNA was extracted for RT-PCR analysis. PCR product was separated on 4% agarose gel, inclusion/exclusion ratio was quantified and listed. **B.**Minigene reporter constructs containing mouse Mef2a Exon α2 and adjacent intron fragment containing putative RBFox1 binding motif (UGCAUG), mutant RBFox1 binding motif (UUCGUA) are illustrated. Reporter construct was transfected into HEK293 cells alone or in combination with RBFox1 expression vector. 48hr post transfection, cells were harvested and semi-quantitative RT-PCR analysis was performed to determine the relative level of transcripts containing exon α2 or excluded exon α2. Upper band represents α2 inclusion splicing variant. Lower band represents α2 exclusion splicing variant. GAPDH was used as internal control. Products were visualized on 4% agarose gel and further quantified based on intensity. RBFox1 expression level is confirmed by Western Blot. Number represents Exon α2 inclusion/exclusion ratio. **C**. Schematic view of the CLIP analysis in C2C12 cells. **D**. C2C12 cells were infected with DN-RBFox1 or RBFox1 adenovirus and compared with mock-infected cells. 48hr post infection, cells were UV cross-linked followed by indicated antibody. Primers were designed at indicated intron region in Mef2a and Mef2c and RT-PCR was performed to confirm the interaction.

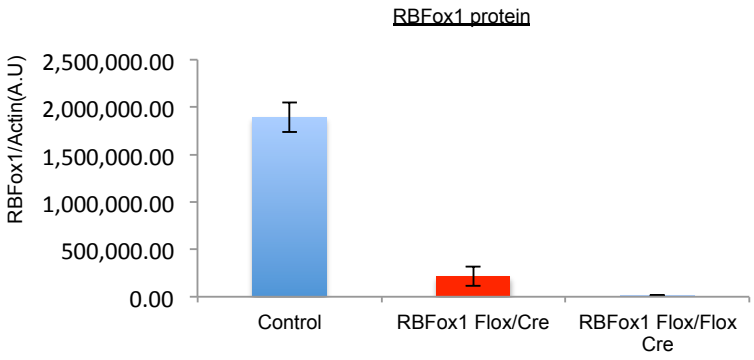
Figure S6

A


RBFox1
**
mRNA/GAPDH(A.U)
0.0025
0.002
0.0015
0.001
0.0005
0
Control
RBFox1Flox/+/Cre
RBFox1Flox/Flox/Cre

B

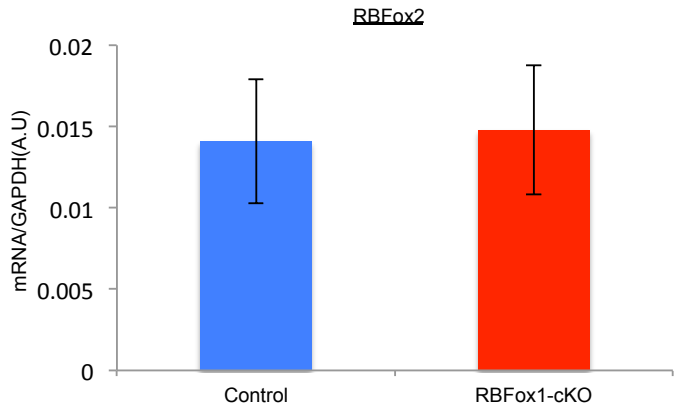

RBFox1
Actin
Control
RBFox1Flox/+/Cre
RBFox1Flox/Flox/Cre


RBFox1 protein
RBFox1/Actin(A.U)
2,500,000.00
2,000,000.00
1,500,000.00
1,000,000.00
500,000.00
0.00
Control
RBFox1 Flox/Cre
RBFox1 Flox/Flox Cre

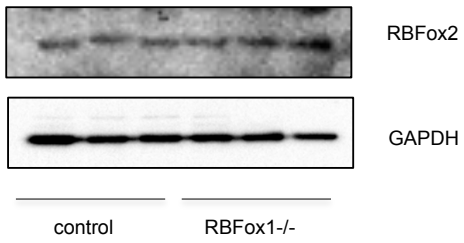
Supplemental Figure S6: RBFox1 is efficiently inactivated in RBFox1-cKO mice. A. Real-time PCR quantification of RBFox1 expression level in Control; RBFox1-$^{Flox/+}$/Cre and RBFox1-$^{Flox/Flox}$/Cre mice hearts. n=3 each sample, *,p<0.05 **B.** Upper: Western blot analysis of RBFox1 protein expression in Control; RBFox1-$^{Flox/+}$/Cre and RBFox1-$^{Flox/Flox}$/Cre mice hearts. n=2 each sample. Lower: Quantification of RBFox1 expression level based on Western shown in upper panel. Significant differences between groups were determined by student t-test (**A**).

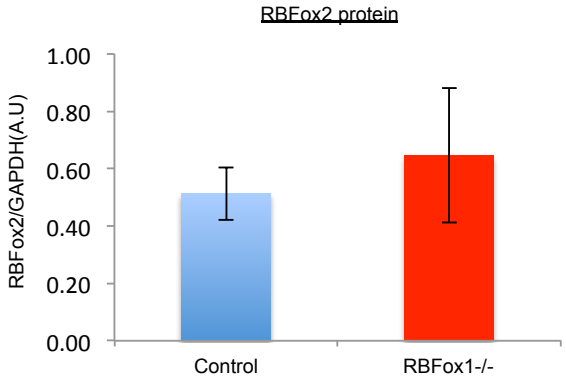
Figure S7

A


RBFox2
0.02
0.015
0.01
0.005
0
mRNA/GAPDH(A.U)
Control
RBFox1-cKO

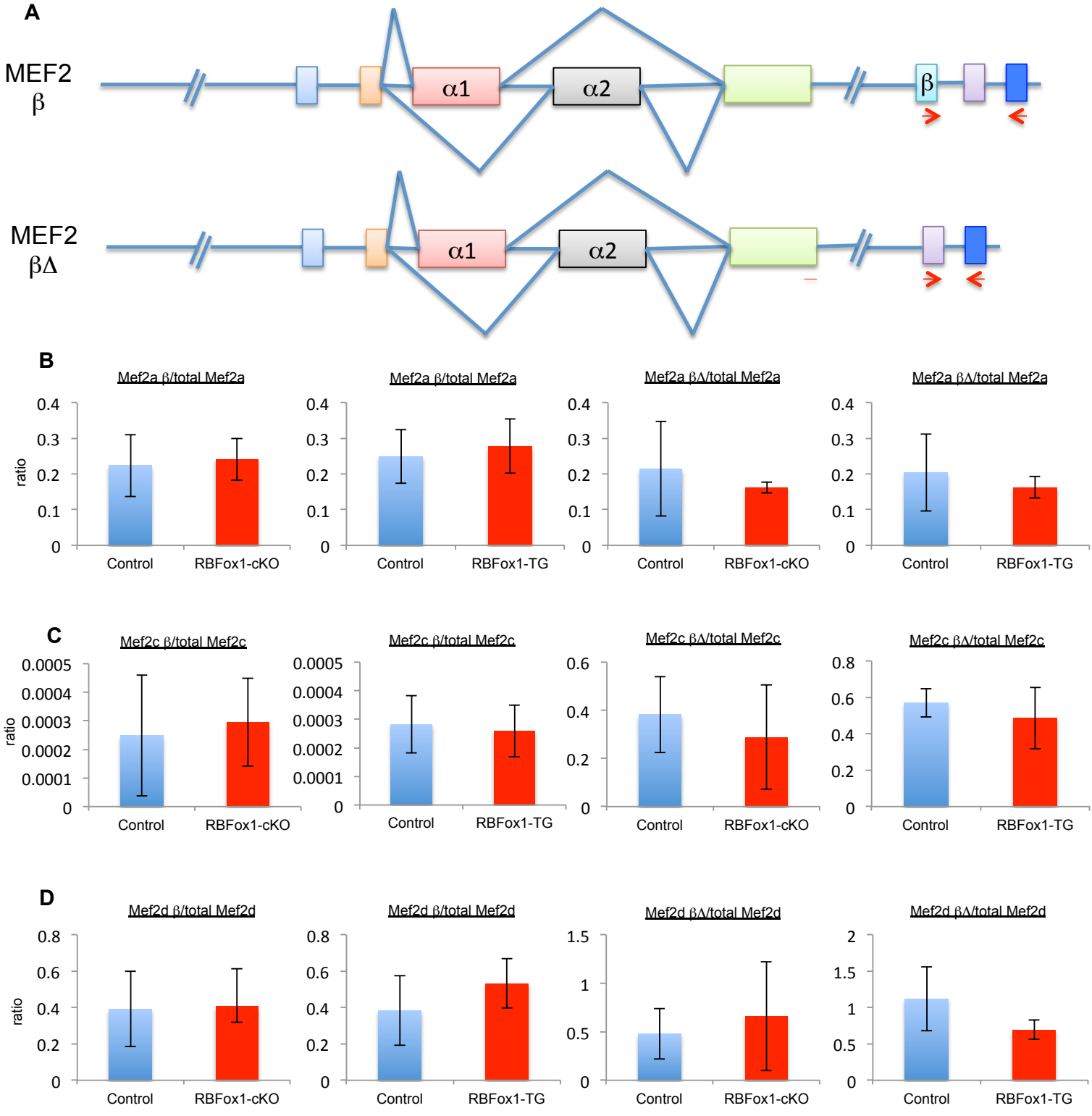
B


RBFox2
GAPDH
control
RBFox1-/-


RBFox2 protein
1.00
0.80
0.60
0.40
0.20
0.00
RBFox2/GAPDH(A.U)
Control
RBFox1-/-

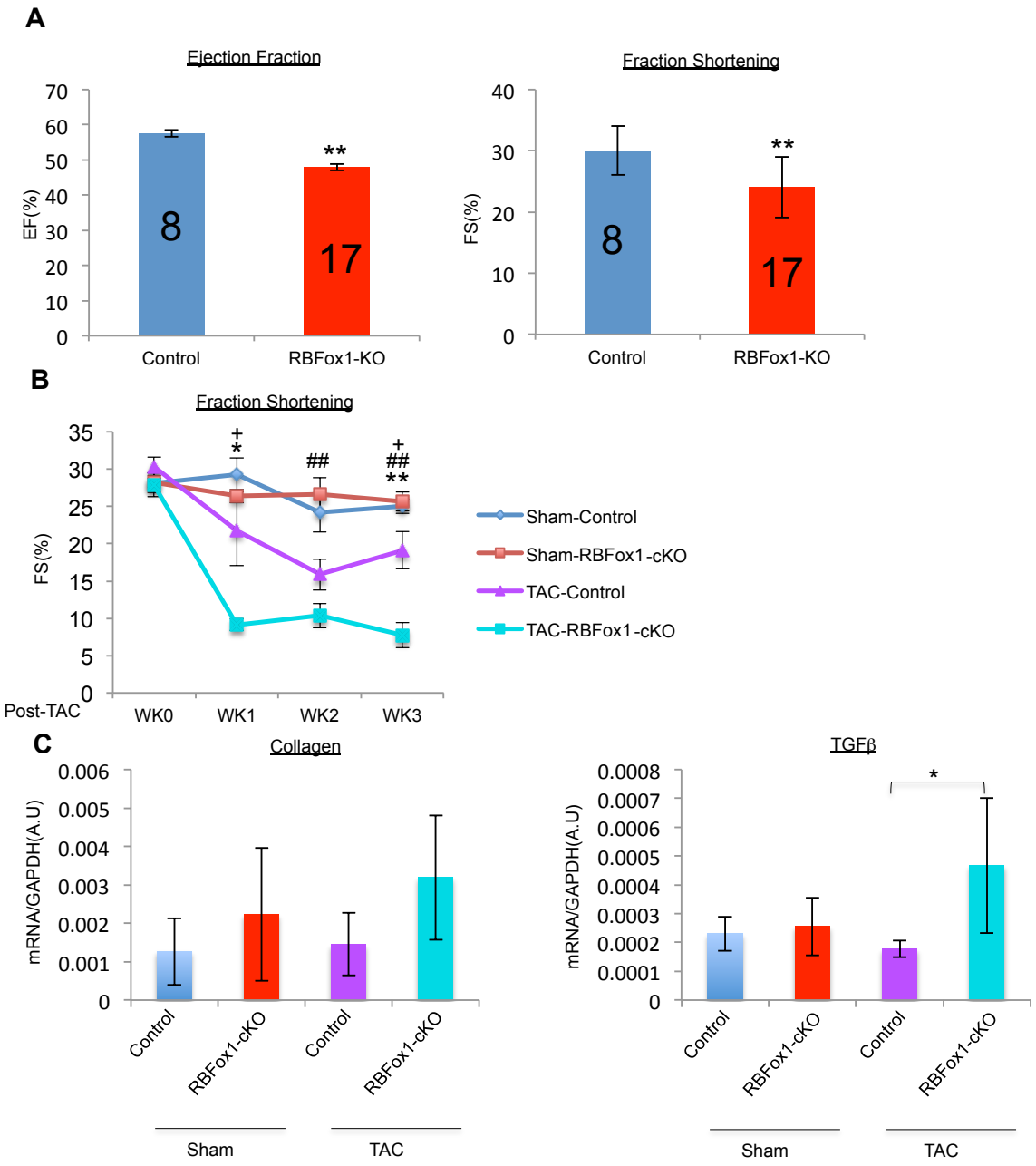
Supplemental Figure S7: RBFox2 expression is unaffected in RBFox1-cKO mouse hearts. *A*. Real-time qPCR quantification of RBFox2 expression level in Control; RBFox1-$^{Flox/+}$/Cre and RBFox1-$^{Flox/Flox}$/Cre mice hearts. **B**. Left: Western blot analysis of RBFox2 expression in control and RBFox1-CKO mouse hearts. GAPDH was used as loading control. N=3 each sample. Right: Quantification of RBFox2 protein expression level based on Western Blot shown on the left.

Figure S8


A
MEF2
β
α1
α2
β
MEF2
βΔ
α1
α2
B
Mef2a β/total Mef2a
Mef2a β/total Mef2a
Mef2a βΔ/total Mef2a
Mef2a βΔ/total Mef2a
ratio
0.4
0.3
0.2
0.1
0
Control
RBFox1-cKO
RBFox1-TG
C
Mef2c β/total Mef2c
Mef2c β/total Mef2c
Mef2c βΔ/total Mef2c
Mef2c βΔ/total Mef2c
ratio
0.0005
0.0004
0.0003
0.0002
0.0001
0
0.6
0.8
Control
RBFox1-cKO
RBFox1-TG
D
Mef2d β/total Mef2d
Mef2d β/total Mef2d
Mef2d βΔ/total Mef2d
Mef2d βΔ/total Mef2d
ratio
0.8
0.6
0.4
0.2
0
1.5
1
0.5
2
Control
RBFox1-cKO
RBFox1-TG

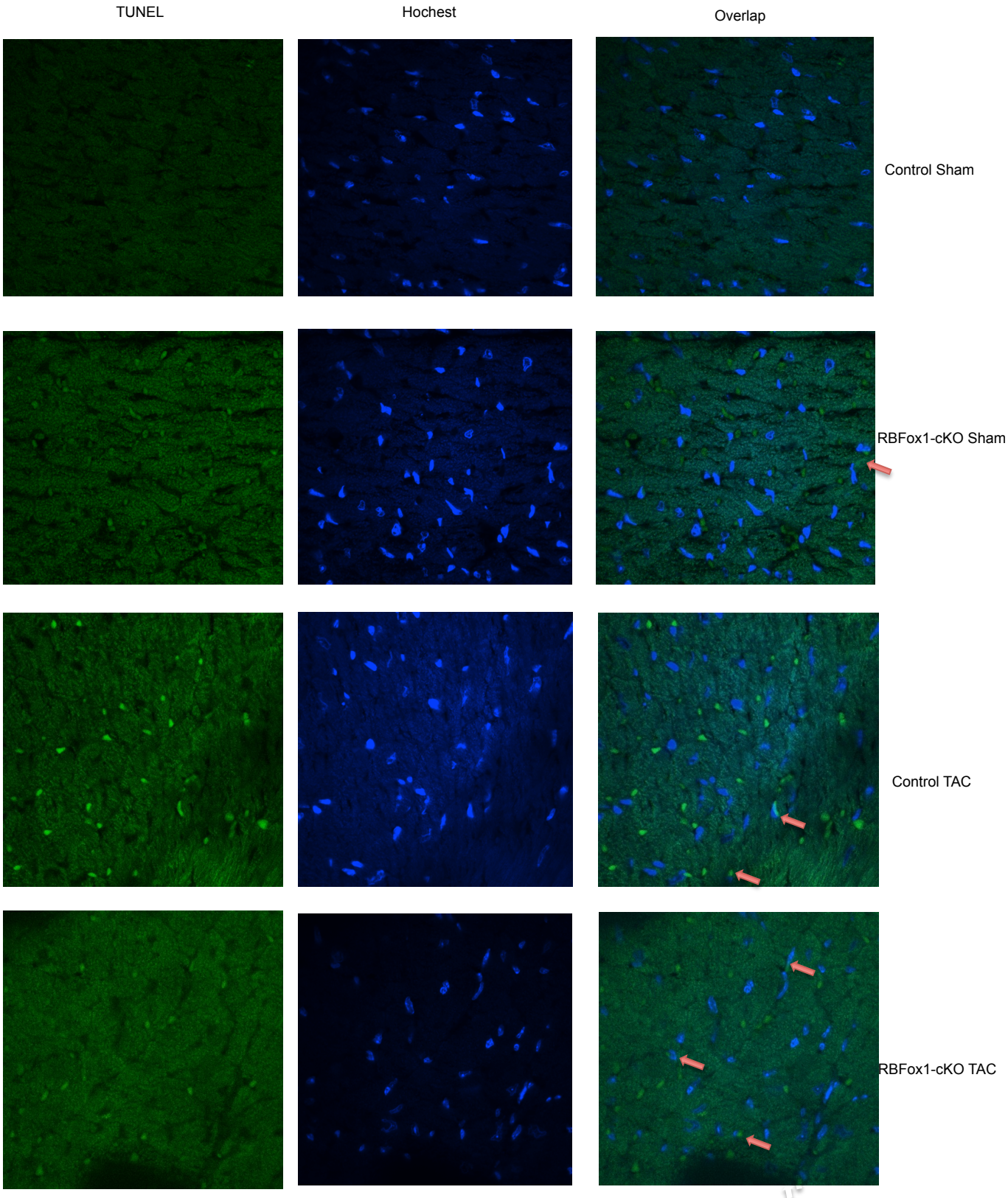
Supplemental Figure S8: MEF2 β exon is not directly regulated by RBFox1. A. Schematic view of MEF2 gene with β exon indicated. Arrow represented primers used for real-time qPCR quantification. **B.** Real-time PCR analysis of ratio for Mef2a β/total Mef2a; Mef2a βΔ/total Mef2a in Control, RBFox1-cKO and RBFox1-TG mouse hearts. n=3 each sample. **C.** Real-time PCR analysis of ratio for Mef2c β/total Mef2c; Mef2c βΔ/total Mef2c in Control, RBFox1-cKO and RBFox1-TG mouse hearts. n=3 each sample. **D.** Real-time PCR analysis of ratio for Mef2d β/total Mef2d; Mef2d βΔ/total Mef2d in Control, RBFox1-cKO and RBFox1-TG mouse hearts. n=3 each sample.

Figure S9


A
Ejection Fraction
EF(%)
70
60
50
40
30
20
10
0
8
17
**
Control
RBFox1-KO
Fraction Shortening
FS(%)
40
30
20
10
0
8
17
**
Control
RBFox1-KO
B
Fraction Shortening
FS(%)
35
30
25
20
15
10
5
0
+
*
##
+
##
**
Post-TAC
WK0
WK1
WK2
WK3
Sham-Control
Sham-RBFox1-cKO
TAC-Control
TAC-RBFox1-cKO
C
Collagen
mRNA/GAPDH(A.U)
0.006
0.005
0.004
0.003
0.002
0.001
0
Control
RBFox1-cKO
Control
RBFox1-cKO
Sham
TAC
TGFβ
mRNA/GAPDH(A.U)
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
*
Control
RBFox1-cKO
Control
RBFox1-cKO
Sham
TAC

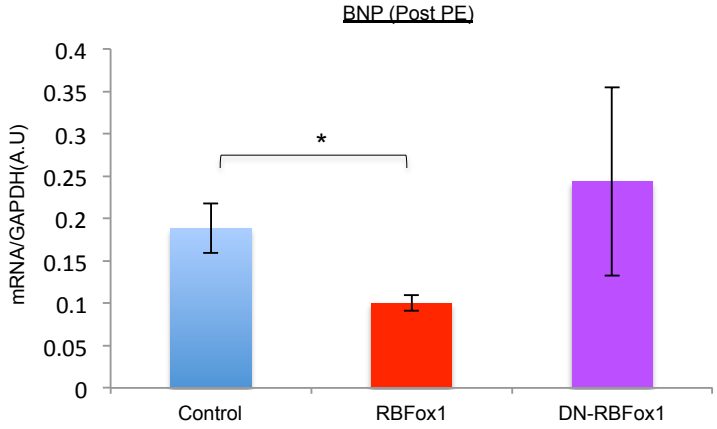
Supplemental Figure S9: RBFox1 is an important splicing regulator mediating mouse cardiac function. A.RBFox1-CKO mice were compared with control mice. Echocardiography was used to determine cardiac Ejection fraction and Fraction shorterning. Control n=8, RBFox1 n=17 (indicated in the bar) **B.** Weekly follow up of fraction shortening for TAC operated control and RBFox1-cKO mice, compared with sham operated littermates**.** Sham-Control (n=6), Sham-RBFox1-cKO (n=6), TAC-Control (n=6), TAC-RBFox1-cKO (n=8) . +,p<0.01 between sham-control and TAC-control;##,p<0.01 between sham-RBFox1-cKO and TAC-RBFox1-cKO; *,p<0.05 between TAC-control and TAC-RBFox1-cKO; **,p<0.01 between TAC-control and TAC-RBFox1 **C.** Real-time qPCR analysis of Collagen III and TGFβ expression level in TAC operated control and RBFox1-cKO mice hearts, compared with sham operated littermates. n=4 each sample, *,p<0.05. Significant differences between groups were determined by student t-test (**A**) or Multi-way Anova (**B-C**).

Figure S10


TUNEL
Hochest
Overlap
Control Sham
RBFox1-cKO Sham
Control TAC
RBFox1-cKO TAC

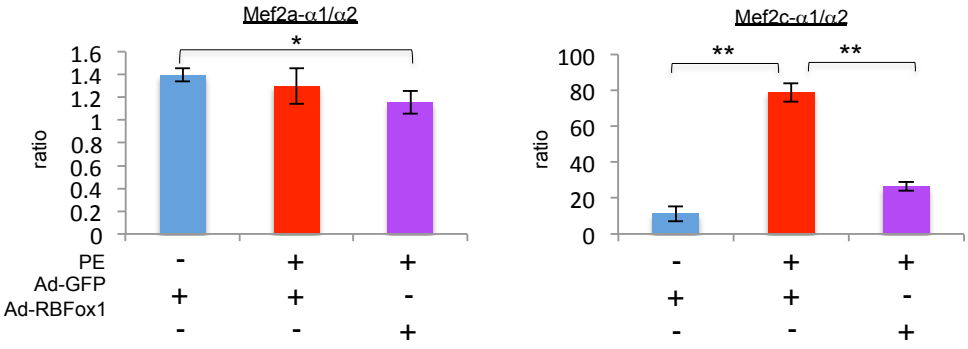
Supplemental Figure S10: Inactivation of RBFox1 potentially caused loss of cardiomyocytes during presure overload stress. TUNEL staining image of control and RBFox1-cKO mice 3 weeks post TAC compared to sham operated controls. Magnification: 60× . Data are representative of at least 3 independent experiments.

Figure S11


BNP (Post PE)
mRNA/GAPDH(A.U)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
*
Control
RBFox1
DN-RBFox1

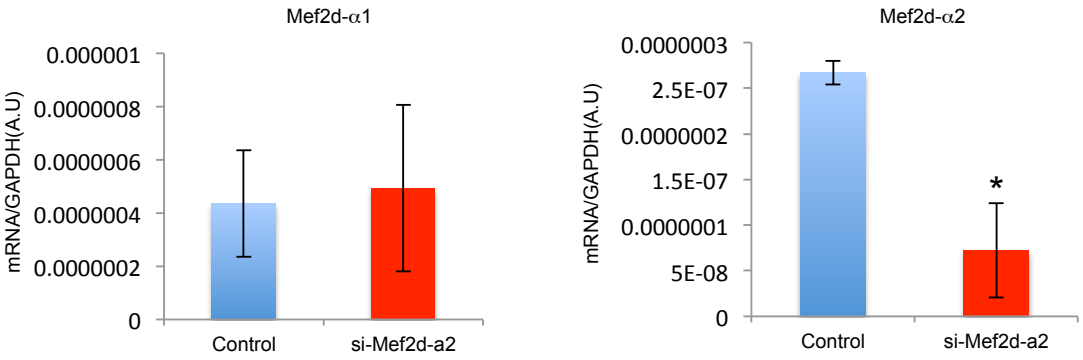
Supplemental Figure S11: RBFox1 splicing regulator activity is important for cardiomyocytes hypertrophy response. Real-time qPCR analysis of BNP expression level in mock infected, RBFox1 or dominant negative RBFox1 (DN-RBFox1) adenovirus infected NRVM post 48hr PE treatment.. n=3 each group, *,$p<0.05$. Significant differences between groups were determined by student t-test.

Figure S12


Mef2a-α1/α2
*
ratio
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
PE
Ad-GFP
Ad-RBFox1
- + +
+ + -
- - +
Mef2c-α1/α2
**
**
ratio
100
80
60
40
20
0
- + +
+ + -
- - +

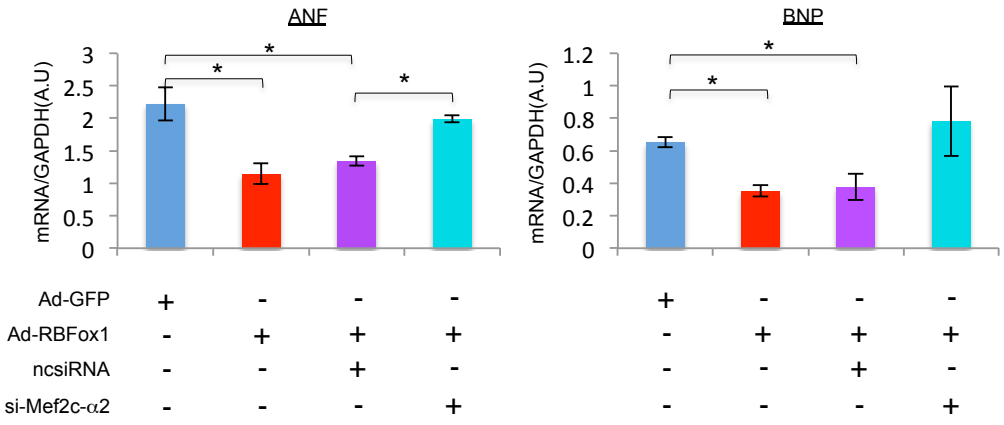
Supplemental Figure S12: Alternative splicing profile of MEF2 is altered upon PE treatment in NRVM. Real-time PCR quantification of Mef2a α1/α2 ratio and Mef2c α1/α2 ratio in control, 48hr PE treated alone or in combination with RBFox1 adenovirus infected cardiomyocytes. n=3 each sample, *,p<0.05. Significant differences between groups were determined by student t-test.

Figure S13


Mef2d-α1
0.000001
0.0000008
0.0000006
0.0000004
0.0000002
0
mRNA/GAPDH(A.U)
Control
si-Mef2d-a2
Mef2d-α2
0.0000003
2.5E-07
0.0000002
1.5E-07
0.0000001
5E-08
0
mRNA/GAPDH(A.U)
*
Control
si-Mef2d-a2

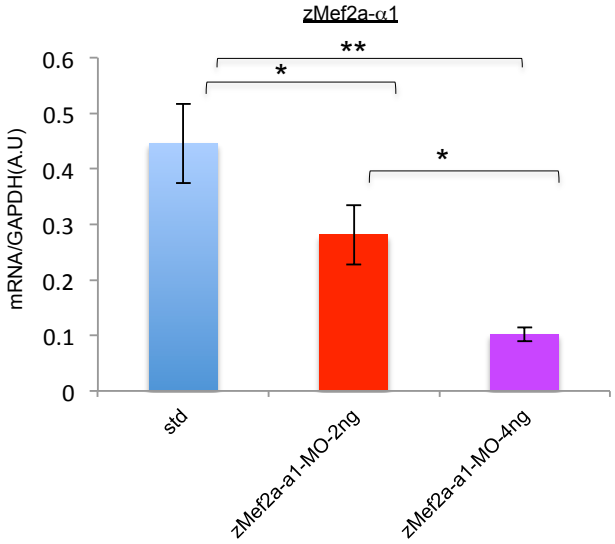
Supplemental Figure S13: Specificity of the siRNA. NRVM was transfected with control or si-Mef2d-α2 for 48hr. Cells were harvested and real-time PCR was performed to determine the expression level of Mef2d-α1 and Mef2d-α2 isoform expression. N=3 each sample. Significant differences between groups were determined by student t-test .

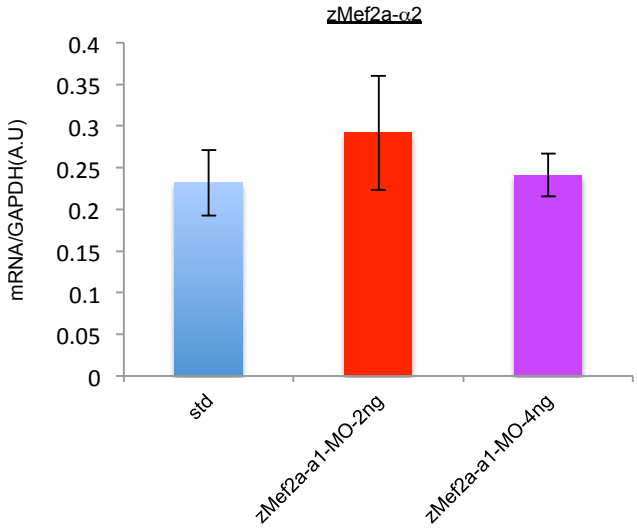
Figure S14

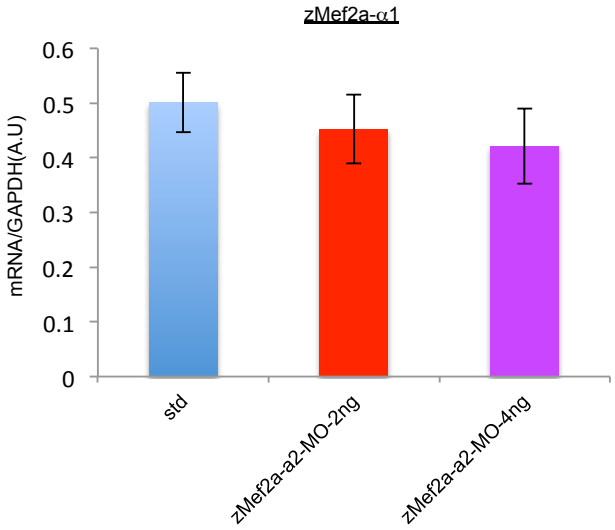

ANF
BNP
mRNA/GAPDH(A.U)
3
2.5
2
1.5
1
0.5
0
1.2
1
0.8
0.6
0.4
0.2
0
*
Ad-GFP + - - - + - - -
Ad-RBFox1 - + + + - + + +
ncsiRNA - - + - - - + -
si-Mef2c-α2 - - - + - - - +

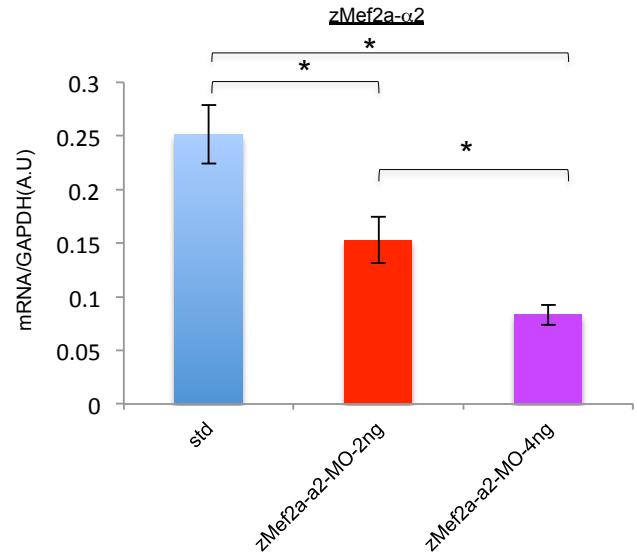
Supplemental Figure S14: Regulatory role of Mef2c α2 transcript in cardiomyocytes hypertrophy. Hypertrophy marker gene expression level in control, PE treated; PE treated with RBFox1 adenovirus infection, PE +RBFox1 adenovirus +nc-siRNA; PE+RBFox1 adenovirus+si-Mef2c-α2. Real-time qPCR is used to determine expression level of ANF and BNP. n=3 each sample. *,$p<0.05$. Significant differences between groups were determined by student t-test.

Figure S15


zMef2a-α1
mRNA/GAPDH(A.U)
0.6
0.5
0.4
0.3
0.2
0.1
0
**
*
*
std
zMef2a-a1-MO-2ng
zMef2a-a1-MO-4ng


zMef2a-α2
mRNA/GAPDH(A.U)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
std
zMef2a-a1-MO-2ng
zMef2a-a1-MO-4ng

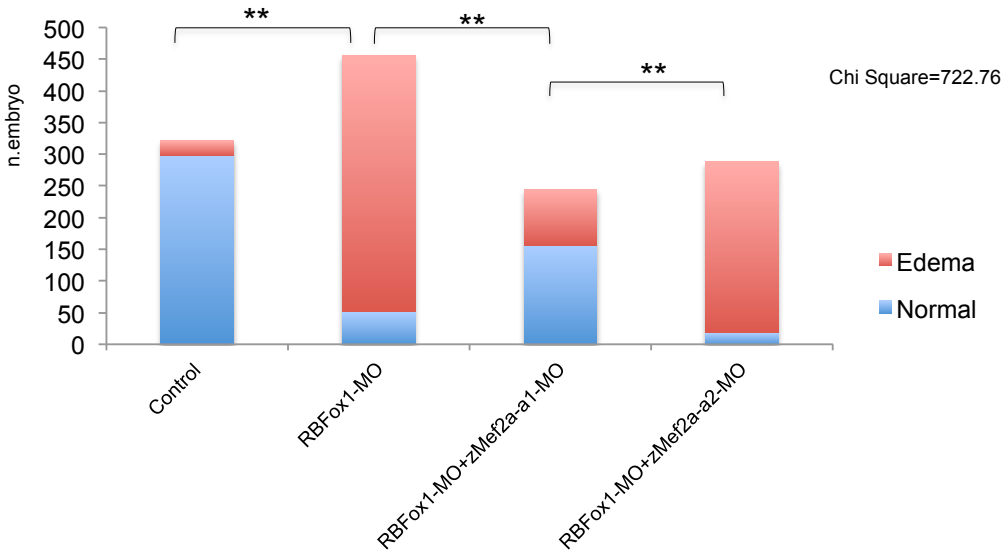

zMef2a-α1
mRNA/GAPDH(A.U)
0.6
0.5
0.4
0.3
0.2
0.1
0
std
zMef2a-a2-MO-2ng
zMef2a-a2-MO-4ng


zMef2a-α2
mRNA/GAPDH(A.U)
0.3
0.25
0.2
0.15
0.1
0.05
0
*
*
*
std
zMef2a-a2-MO-2ng
zMef2a-a2-MO-4ng

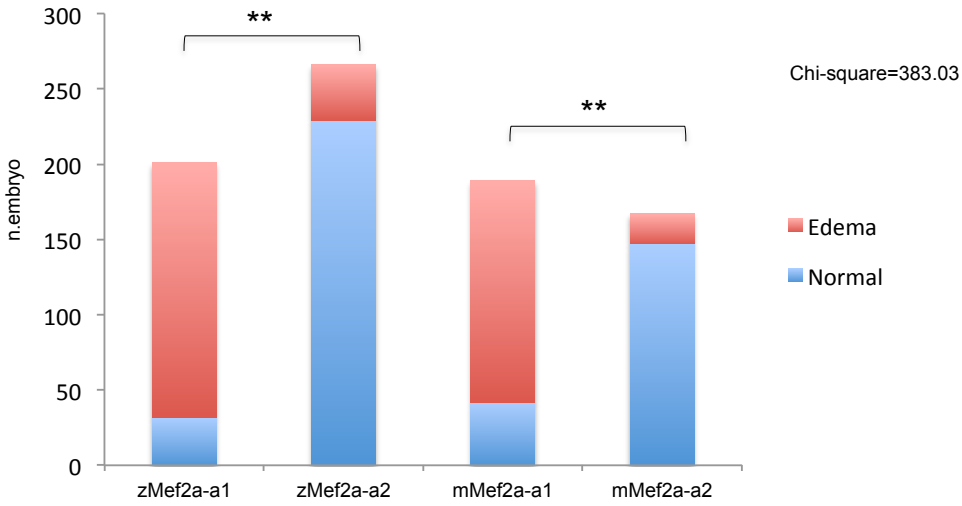
Supplemental Figure S15: Specificity of the morpholino. zMef2a-α1 or zMef2a-α2 mRNA expression level quantified by real-time PCR upon zMef2a-α1/zMef2a-α2 MO injection. N=30 each, *,$p<0.05$. Significant differences between groups were determined by student t-test .

Figure S16

A

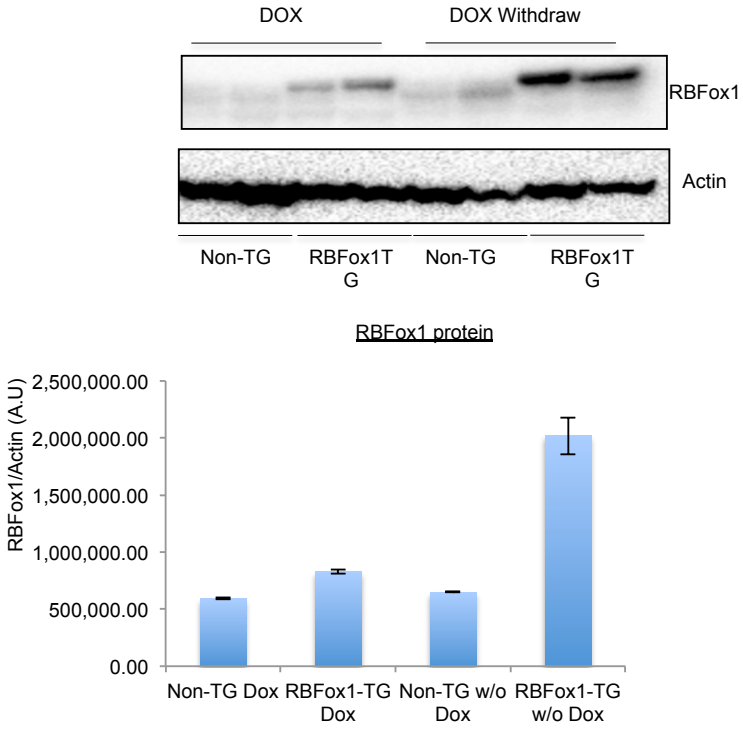


B



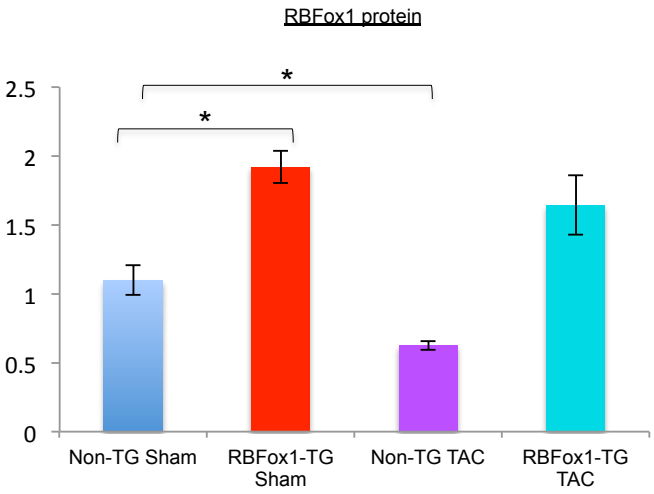
Supplemental Figure S16: Summary of zebrafish phenotypes of the different morphants. Significant differences between groups were determined by Fisher Exact.

Figure S17


DOX
DOX Withdraw
RBFox1
Actin
Non-TG
RBFox1TG
Non-TG
RBFox1TG
RBFox1 protein
RBFox1/Actin (A.U)
2,500,000.00
2,000,000.00
1,500,000.00
1,000,000.00
500,000.00
0.00
Non-TG Dox
RBFox1-TG Dox
Non-TG w/o Dox
RBFox1-TG w/o Dox

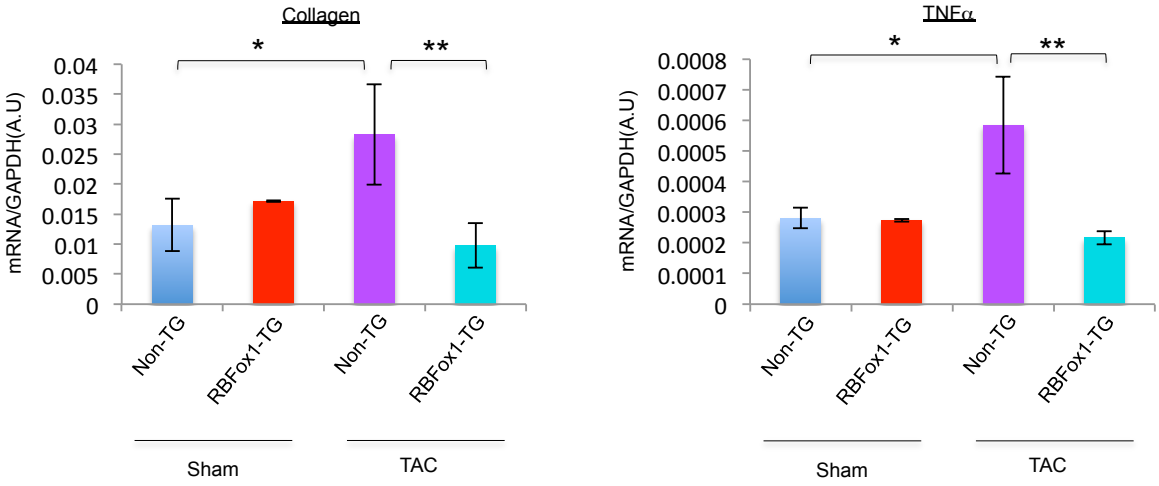
Supplemental Figure S17: RBFox1 expression level is tightly regulated by doxycycline in RBFox1-TG mouse hearts. Upper: Western blot analysis of RBFox1 expression level in mouse fed with Doxycycline containing or regular chow diet in Non-TG and RBFox1-TG mice. Lower: Quantification of RBFox1 protein expression according to Western blot shown in the upper panel.

Figure S18


RBFox1 protein
2.5
2
1.5
1
0.5
0
*
*
Non-TG Sham
RBFox1-TG Sham
Non-TG TAC
RBFox1-TG TAC

Supplemental Figure S18: Quantification of RBFox1 protein expression based on Western Blot shown in Figure 4A. n=3 each sample, *, *p*<0.05.Significant differences between groups were determined by Multi-way Anova.

Figure S19


Collagen
*
**
mRNA/GAPDH(A.U)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Non-TG
RBFox1-TG
Non-TG
RBFox1-TG
Sham
TAC
TNFα
*
**
mRNA/GAPDH(A.U)
0.0008
0.0007
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0
Non-TG
RBFox1-TG
Non-TG
RBFox1-TG
Sham
TAC

Supplemental Figure S19: Restoring RBFox1 expression is sufficient to reduce inflammatory response in intact heart post TAC. Real-time qPCR analysis of Collagen III and TNFa expression level in TAC operated non-TG and RBFox1-TG mouse hearts compared with sham operated littermates. n=4 each sample, *,$p<0.05$. Significant differences between groups were determined by Multi-way Anova.

Figure S20

Total number of samples used in Rasl-Seq in each group

Surgery Group	Number of Samples
WT-Sham	5
WT-TAC	7
RBFox1-TG-Sham	4
RBFox1-TG-TAC	3
RBFox1-CKO-Sham	3
RBFox1-CKO-TAC	3

Total n=25

Supplemental Figure S20: RBFox1 mediate global alternative splicing defects in pathologically stressed heart. Number of samples sequenced for Rasl-seq and corresponding treatment.