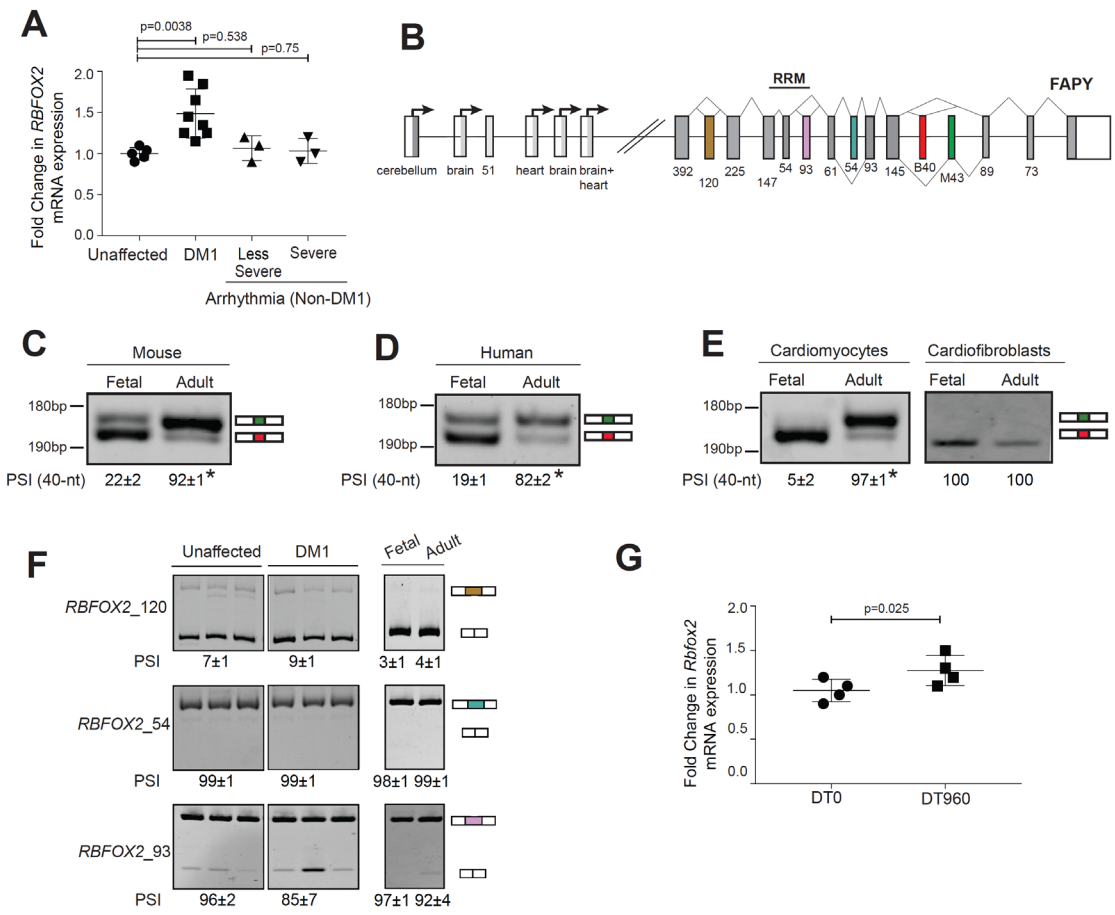
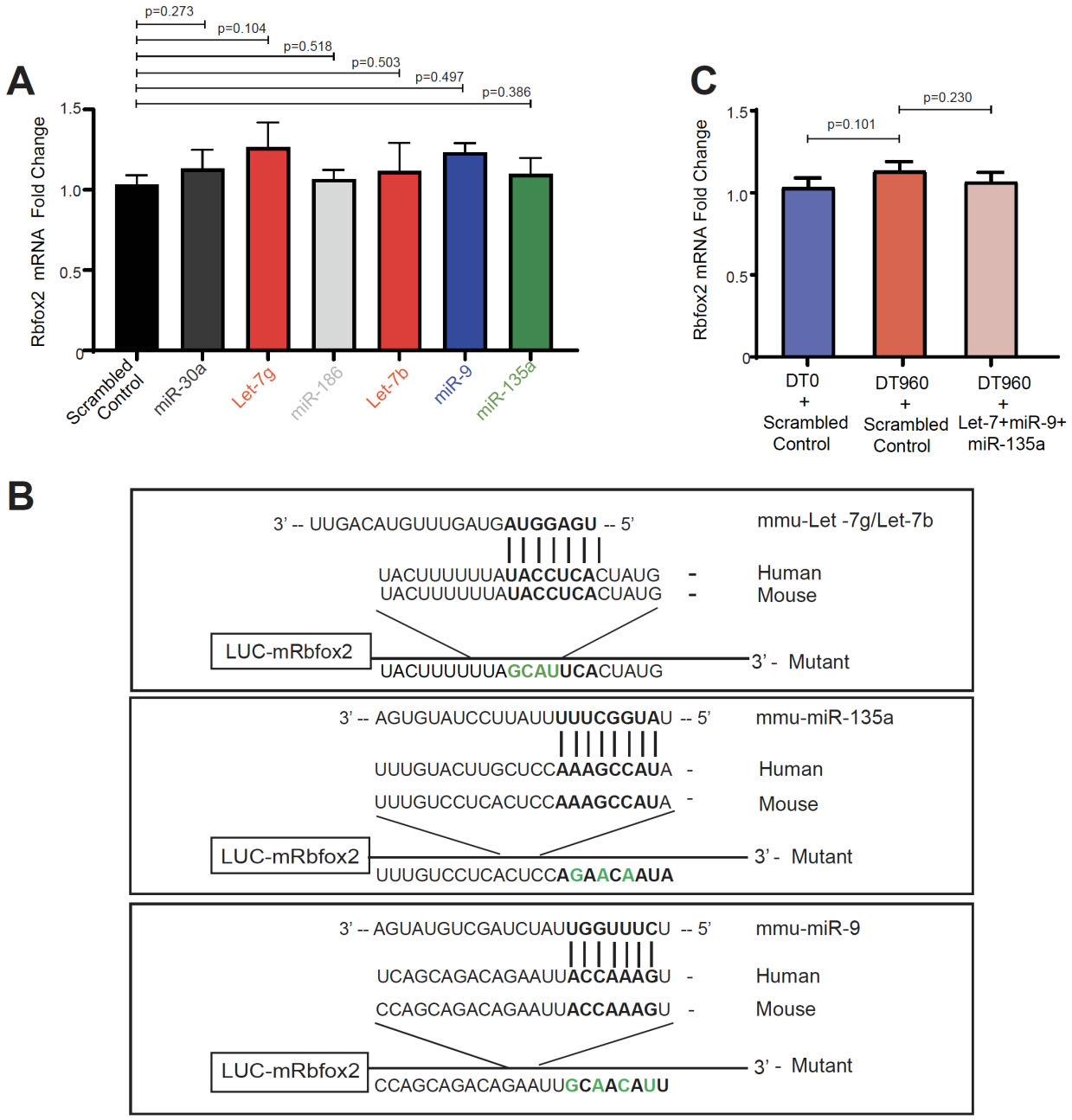
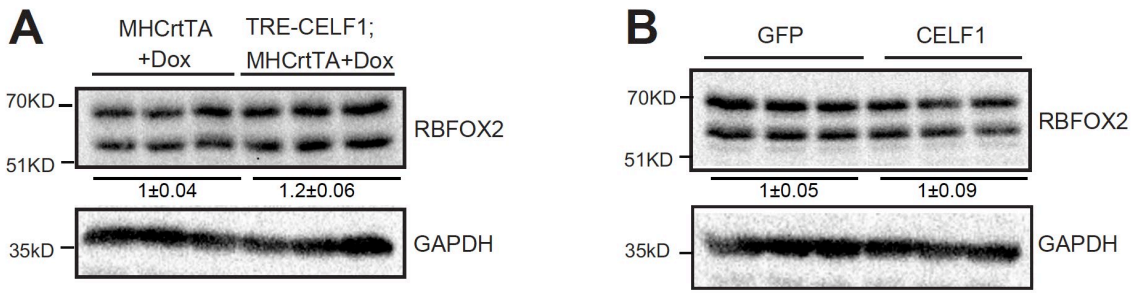


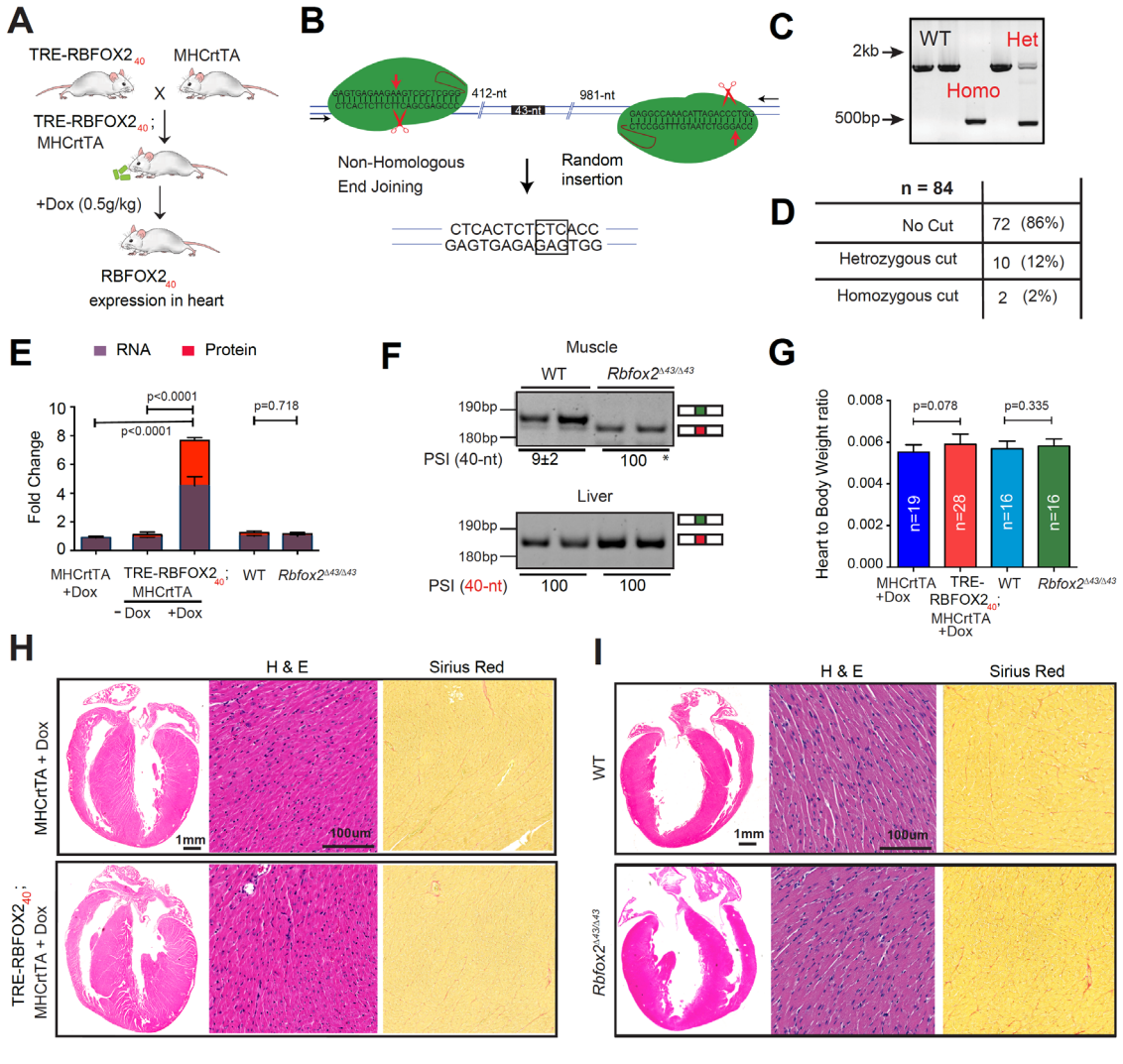
Supplementary Figure 1. *RBFOX2* mRNA expression and splicing analysis in human and mouse hearts. Related to Figure 1. (A) Quantification of *RBFOX2* mRNA levels in unaffected (n=8), DM1 (n=9), and arrhythmic non-DM1 (Severe arrhythmia n=3, Less severe arrhythmia n=3) human heart samples. **(B)** Schematic of *Rbfox2* gene structure. Multiple promoters produce distinct first exons in specific tissues. The RNA recognition motif (RRM) is encoded by the 54-nt and 93-nt exons. The 93-nt exon in the RRM is skipped in some cases to generate a dominant negative isoform. Mutually exclusive exons M43 (muscle-expressed 43-nt exon) and B40 (brain-expressed 40-nt exon) are alternatively spliced to generate the muscle and non-muscle RBFOX2 protein isoforms. Black boxes and white boxes represent translated and untranslated sequences respectively. RT-PCR analysis monitoring the inclusion of *RBFOX2* 40-nt and 43-nt exons in fetal and adult **(C)** mice hearts (n=4), **(D)** human hearts (n=3), and **(E)** freshly isolated mouse cardiomyocytes and cardiofibroblasts (n=3). PSI: Percent Spliced In. **(F)** RT-PCR analysis monitoring the inclusion of *RBFOX2* 54-nt, 120-nt and 93-nt exons between unaffected (n=6), and DM1 (n=9) as well as fetal (n=3) and adult (n=3) human heart samples. **(G)** Quantification of *Rbfox2* mRNA levels in HL-1 cells transfected with DT0 or DT960 plasmids for 48h. All data are mean ± s.d., and p-values were derived from a parametric t-test (two-sided, unpaired), with Welch's correction. **P*<0.05.



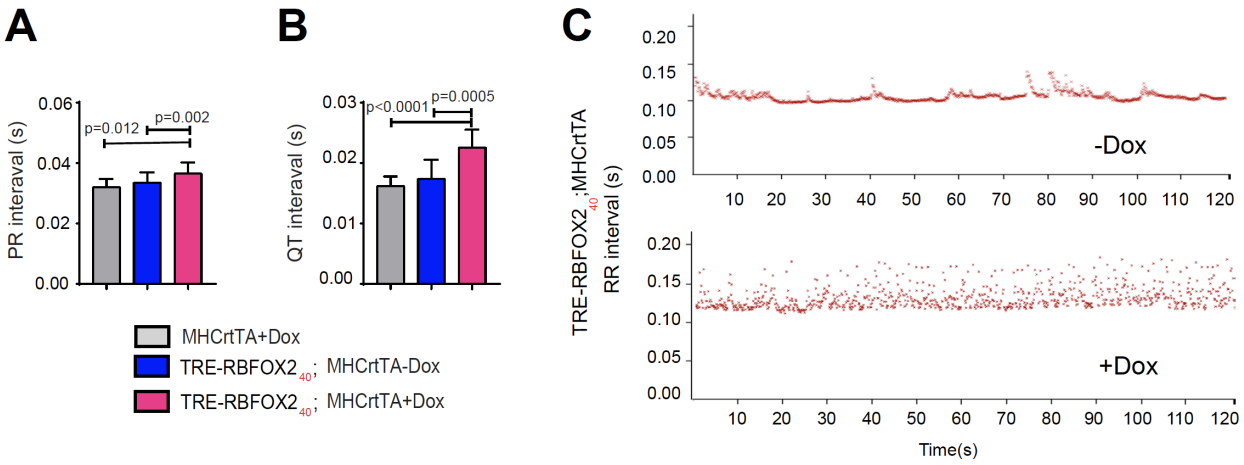
Supplementary Figure 2. miRNA regulation of *Rbfox2* in DM1 cardiac cultures. Related to Figure 2. (A) qRT-PCR analysis of *Rbfox2* mRNA expression in HL-1 cells following treatment with scrambled control or indicated miRNA mimics. n=4 independent transfections. **(B)** Putative Let-7, miR-9 and miR-135a seed sequences in the 3′-UTRs of human and mouse *RBFOX2* transcripts. Specific mutations introduced into luciferase reporter constructs are shown in green. **(C)** qRT-PCR analysis of *Rbfox2* mRNA expression in HL-1 cells after co-transfection with DT0 or DT960 plasmids, and scrambled control or a cocktail of indicated miRNA mimics. n=4 independent transfections. All data are mean ± s.d., and p-values were derived from one-way ANOVA plus Dunnett's post-hoc test.



Supplementary Figure 3. Effects of CELF1 overexpression on RBFOX2 protein levels in mouse heart and HL-1 cells. Related to Figure 3. Immunoblot analysis of RBFOX2 protein in **(A)** hearts of *tet*-inducible, heart-specific CELF1 bitransgenics (TRE-CELF1; MHCrtTA) and littermate control (MHCrtTA) mice induced with 6g/kg Dox for ten days (n=3 mice for each genotype) as well as in **(B)** HL-1 cells following infection with GFP or CELF1 expressing adenoviruses (n=3 independent transfections). All data are mean ± s.d., and p-values were derived from a parametric t-test (two-sided, unpaired), with Welch's correction.



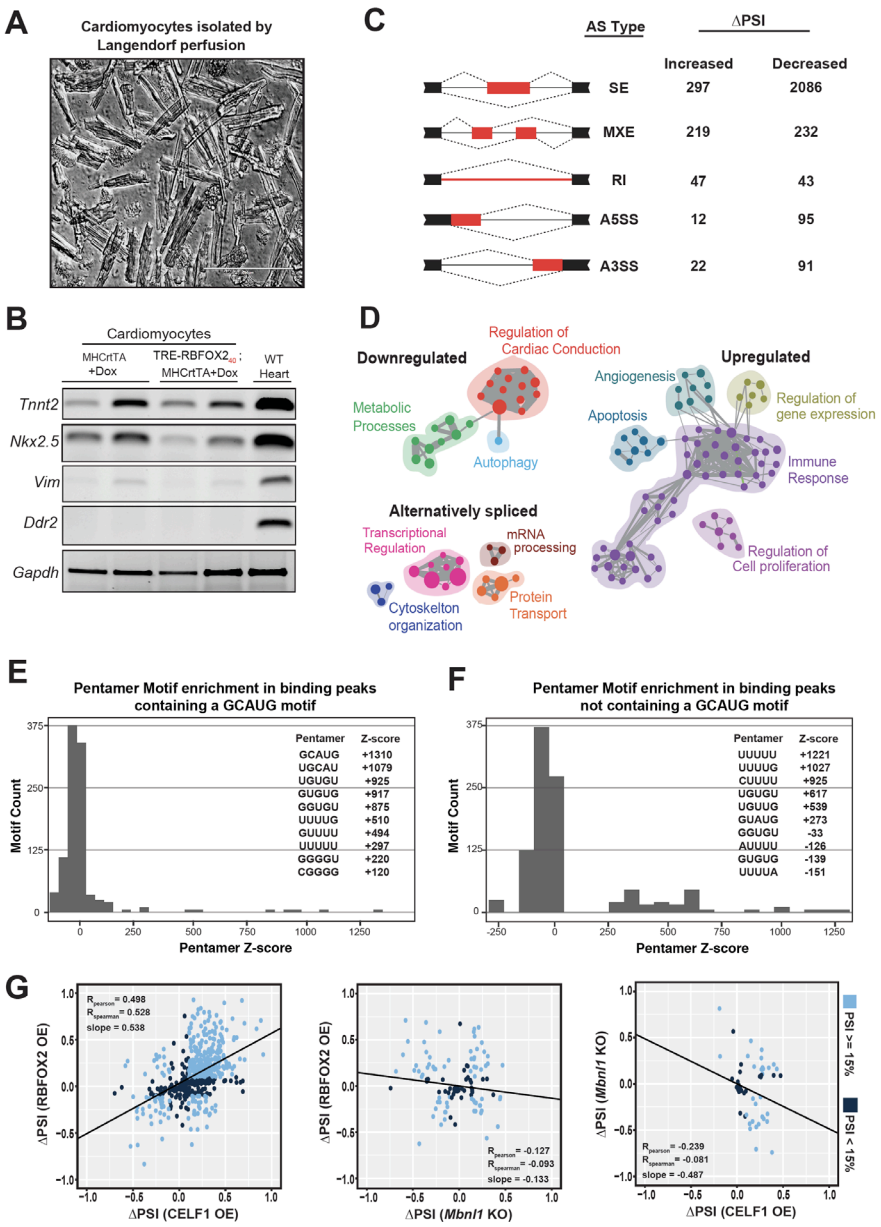
Supplementary Figure 4. Generation of TRE-RBFOX2$_{40}$;MHCrtTA bitransgenic and *Rbfox2*$^{\Delta 43/\Delta 43}$ mice. Related to Figure 4. (A) Schematic of TRE-RBFOX2$_{40}$; MHCrtTA bitransgenic mice to inducibly express FLAG-tagged RBFOX2$_{40}$ protein isoform in the heart. **(B)** Schematic representation of CRISPR/Cas9 approach to generate *Rbfox2*$^{\Delta 43/\Delta 43}$ mice. Relevant genomic sequence with guide RNAs targeting the intronic regions flanking 43-nt exon are shown. **(C)** Genotyping results showing successful generation of *Rbfox2*$^{\Delta 43/\Delta 43}$ mice with 1.4kb of deletion spanning the 43-nt exon. **(D)** 12% of the screened founders were heterozygous and 2% were homozygous for the targeted deletion. **(E)** *Rbfox2* mRNA and protein expression in the hearts of hemizygous MHCrtTA, and TRE-RBFOX2$_{40}$; MHCrtTA bitransgenic mice fed 0.5g/kg Dox containing chow for 3 days, as well as of wildtype (WT) and *Rbfox2*$^{\Delta 43/\Delta 43}$ mice. n=5 for each genotype. **(F)** RT-PCR analysis of *Rbfox2* 43-nt and 40-nt exons in the muscle and liver tissues from WT and *Rbfox2*$^{\Delta 43/\Delta 43}$ mice. PSI: Percent Spliced In; n=4-6 mice for each genotype. **(G)** Heart-to-body weight ratios, Representative **(H)** H&E, and **(I)** Sirius Red staining of hearts from indicated genotypes. All data are mean ± s.d., and p-values were derived from a parametric t-test (two-sided, unpaired), with Welch's correction.



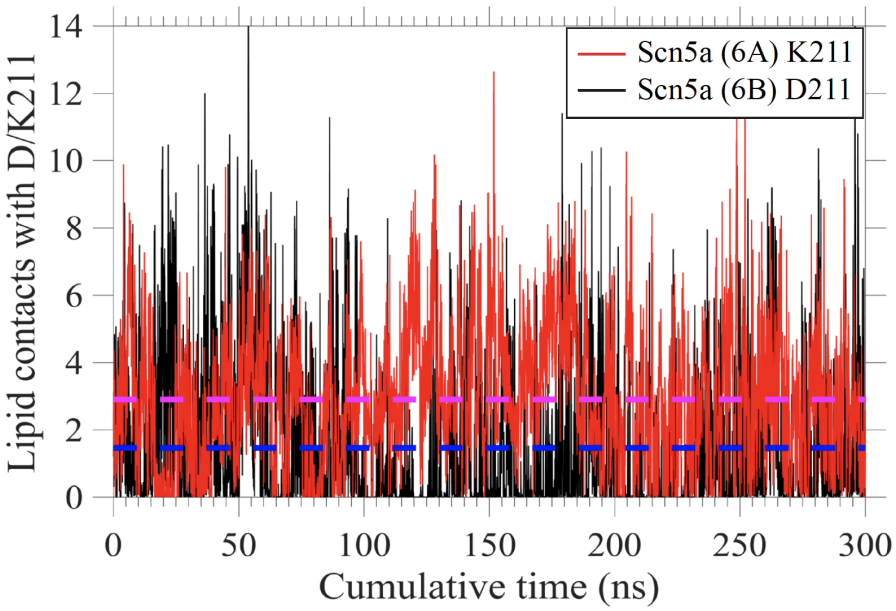
D

Parameter	MHCrtTA +Dox	TRE-RBFOX2$_{40}$; MHCrtTA+Dox	WT	*Rbfox2*$^{\Delta 43/\Delta 43}$
Heart Rate(BPM)	360.21	336.84	358.81	365.5
Diameter;s (mm)	2.78	3.05	1.72	2.00
Diameter;d (mm)	4.19	3.97	3.38	3.50
Volume;s (ul)	29.43	37.13	10.15	14.42
Volume;d (ul)	78.78	69.71	47.5	51.4
Stroke Volume (ul)	49.34	32.57 *	37.34	36.9
Ejection Fraction (%)	62.44	46.15 *	80.42	73.61
Fractional Shortening (%)	33.51	23.01 *	49.81	43.24
Cardiac Output (mL/min)	17.71	11.44	13.34	13.69

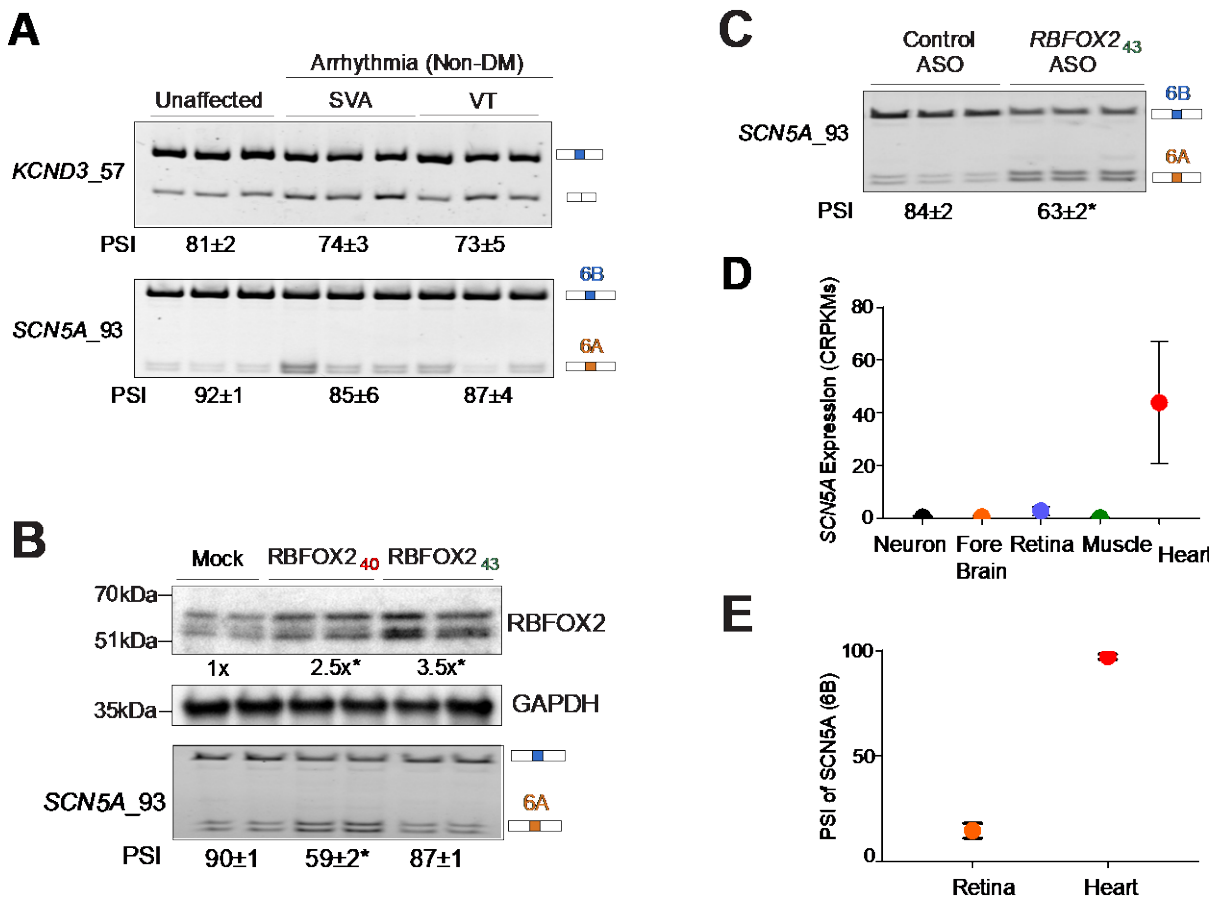
Supplementary Figure 5. Cardiac function tests of TRE-RBFOX2$_{40}$; MHCrtTA bitransgenic and *Rbfox2*$^{\Delta 43/\Delta 43}$ mice. Related to Figure 4. Surface ECG analysis showing **(A)** PR, and **(B)** QT intervals of MHCrtTA mice (n=10) fed 0.5g/kg Dox containing chow for 9 days, and the TRE-RBFOX2$_{40}$; MHCrtTA bitransgenic mice (n=14) 48h before and 9 days after 0.5g/kg Dox administration. **(C)** Variation in RR interval from ECG analysis in TRE-RBFOX2$_{40}$; MHCrtTA bitransgenic mice 48h before and 9 days after 0.5g/kg Dox administration (n=4). **(D)** Echocardiographic analysis of MHCrtTA (n=10), and TRE-RBFOX2$_{40}$; MHCrtTA bitransgenics induced with 0.5g/kg Dox for 9 days (n=14), as well as wildtype (WT, n=6) and *Rbfox2*$^{\Delta 43/\Delta 43}$ (n=8) mice. All data are mean ± s.d., and p-values were derived from a parametric t-test (two-sided, unpaired for **A, B, D** and paired for **C**), with Welch's correction.



Supplementary Figure 6. RNA-seq analysis of cardiomyocytes isolated from Dox induced TRE-RBFOX2$_{40}$; MHCrtTA mice. Related to Figure 5. (A) Photograph of isolated adult ventricular cardiomyocytes from TRE-RBFOX2$_{40}$; MHCrtTA bitransgenics induced with 0.5g/kg Dox for 3 days. **(B)** RT-PCR analysis of cardiomyocyte (*Tnnt2*, *Nkx2.5*) and cardiofibroblast (*Vim*, *Ddr2*) markers in the indicated samples. **(C)** Classification of splicing event types that change significantly after RBFOX2$_{40}$ overexpression. ΔPSI: Difference in Percent Spliced In. **(D)** Gene ontology analysis of differentially expressed and spliced genes following RBFOX2$_{40}$ overexpression. Distribution of z-scores of 1024 pentameric motifs in sequences **(E)** with and **(F)** without GCAUG motif near RBFOX2$_{40}$-regulated exons in cardiomycoytes. Top 10 enriched motifs with relevant z-scores are listed. **(G)** Scatter plots of pairwise comparisons for ΔPSIs following RBFOX2$_{40}$ overexpression (OE) in cardiomyocytes, and CELF1 overexpression and *Mbnl1*$^{\Delta E3/\Delta E3}$ in mouse hearts.



Supplementary Figure 7. Lipid contact frequency within the bilayer for K211 of SCN5A (6A) isoform in comparison to D211 in SCN5A (6B) isoform. Related to Figure 6. Lipid contacts were calculated between heavy atoms of lipid molecules and the sidechain carboxylate (Asp) or ammonium (Lys) groups. The magenta and blue horizontal lines show the average lipid contacts for SCN5A (6A) isoform and SCN5A (6B) isoform respectively. All three independent simulations for each isoform were concatenated into one single trajectory for the analysis. The average contact counts between K211 and lipid molecules was 2.9 which was double than that of D211.



Supplementary Figure 8. Alternative splicing analysis of voltage-gated sodium and potassium channels. Related to Figure 7. (A) RT-PCR analysis comparing the inclusion of 57-nt exon in *KCND3*, and 93-nt mutually exclusive exons (6A and 6B) in *SCN5A* transcripts between unaffected (n=3), and arrhythmic non-DM [SVA: Sustained Ventricular Arrhythmia (n=3); and VT: Ventricular Tachycardia (n=3)] human heart samples. Percent Spliced In (PSI) values are shown below the gel images. **(B)** Immunoblot analysis of RBFOX2 protein levels (top) from HL-1 cells transfected with RBFOX2$_{40}$ or RBFOX2$_{43}$ expression plasmids. Quantification of relative band intensities for RBFOX2 normalized to GAPDH are shown below the gel. RT-PCR analysis of 93-nt mutually exclusive exons (6A and 6B) in *Scn5a* transcripts (bottom) from indicated samples. Percent Spliced In (PSI) values are shown below the gel images. n=3 independent transfections. **(C)**. RT-PCR analysis of 93-nt mutually exclusive exons (6A and 6B) in *Scn5a* transcripts from HL-1 cells treated with control ASO or with an ASO targeting the 5'ss of *Rbfox2* 43-nt exon for 48h. Percent Spliced In (PSI) values are shown below the gel images. n=4 independent transfections. **(D)** Comparison of *SCN5A* expression in different tissues from VastdB database. **(E)** Adult *SCN5A* (6B) isoform expression in human retina vs. heart from VastdB database. All data are mean ± s.d., and p-values were derived from a parametric t-test (two-sided, unpaired), with Welch's correction. *p<0.05.

SUPPLEMENTARY TABLES

Supplementary Table 1. Primer and oligonucleotides sequences used in relevant genotyping, qRT-PCR, RT-PCR and cell culture experiments. This supplemental table is related to Figure 1,2,3,4,7.

Target Gene	Type	Primer Sense	Sequence (5'->3')
MHCrtTA	Genotype	F	CTGGGTTGCGTGTTGGAAGATC
MHCrtTA	Genotype	R	GTGGGAGATCGAGCAGGCCCTCG
3'TRE-*Rbfox2*	Genotype	F	ACAGCCTGCTACTGCAACC
3'TRE-*Rbfox2*	Genotype	R	GCGATGCAATTTCCTCATTT
5'TRE-*Rbfox2*	Genotype	F	AAGTGAAAGTCGAGCTCGGTA
5'TRE-*Rbfox2*	Genotype	R	GTTGTTGTTGGCTCCTGGTT
Rbfox2$^{\Delta 43/\Delta 43}$	Genotype	F	CAAGACACCTTCTTTCTACCTG
Rbfox2$^{\Delta 43/\Delta 43}$	Genotype	R	GCTGGAGCTGTTAAACTGATG
Rbfox2$^{\Delta 43/\Delta 43}$ 43	Genotype	F	ATCCATCACCATGCCTTTGC
Rbfox2$^{\Delta 43/\Delta 43}$ 43	Genotype	R	GGAGCTGGAATGGTTAGTAT
*RBFOX1*_43_40_human	RT-PCR	F	GCACCGTGTACAACACCTTC
*RBFOX1*_43_40_human	RT-PCR	R	ACTGTAGGCAGCGGCAGT
*RBFOX2*_43_40_human	RT-PCR	F	GGTACCTCCAACAGCCATCC
*RBFOX2*_43_40_human	RT-PCR	R	GTGTACACCCTGCCATAA
*Rbfox2*_43_40_mouse	RT-PCR	F	GGTACCTCCAACAGCCATCC
*Rbfox2*_43_40_mouse	RT-PCR	R	GTGTACACCCTGCCGTAA
*RBFOX2*_54_human	RT-PCR	F	CCCTGTCTGCATCAGCACTA
*RBFOX2*_54_human	RT-PCR	R	CAGAAGGTGGAGCACAGACA
*RBFOX2*_120_human	RT-PCR	F	GCCGGCATAGTCTTGAGTGT
*RBFOX2*_120_human	RT-PCR	R	TTCCTTCAGCCATCTGCCTG
*RBFOX2*_93_human	RT-PCR	F	CAGTTTGGCAAAATCCTAGATG
*RBFOX2*_93_human	RT-PCR	R	GGTGTGACCATCTTCTTATTGG
*CACNA1C*_33_ human	RT-PCR	F	AAATCGCCATGAACATCCTC
*CACNA1C*_33_ human	RT-PCR	R	TTGATGAAGGTCCACAGCAG
*Cacna1c*_33_ mouse	RT-PCR	F	GAGCTGCCTCCTCAAAATCG
*Cacna1c*_33_ mouse	RT-PCR	R	AAGAGGCGGAAGAAGGTGAT
*KCND3*_57_ human	RT-PCR	F	CCAGAAGAGGAGCACATGGG
*KCND3*_57_ human	RT-PCR	R	GGGACTTCTTGTGGATGGGT
*Kcnd3*_57_ mouse	RT-PCR	F	GGCAAGACCACCTCACTCAT
*Kcnd3*_57_ mouse	RT-PCR	R	TGGCTGGACAGAGAAGGACT
*SCN5A*_93_ human	RT-PCR	F	CTTCTGCCTGCACGCGTTCAC
*SCN5A*_93_ human	RT-PCR	R	CAGAAGACTGTGAGGACCATC
*Scn5a*_93_ mouse	RT-PCR	F	CTTCTGCCTGCATGCGTTCAC
*Scn5a*_93_ mouse	RT-PCR	R	CAGAAGACAGTAAGGACCATC
Tnnt2	RT-PCR	F	CGGAAGAGTGGGAAGAGACA

Tnnt2	RT-PCR	R	TTCCCACGAGTTTTGGAGAC
Nkx2.5	RT-PCR	F	AAGCAACAGCGGTACCTGTC
Nkx2.5	RT-PCR	R	GGGTAGGCGTTGTAGCCATA
Vim	RT-PCR	F	TGAAGGAAGAGATGGCTCGT
Vim	RT-PCR	R	TTGAGTGGGTGTCAACCAGA
Ddr2	RT-PCR	F	CAAGATCATGTCTCGGCTCA
Ddr2	RT-PCR	R	GCCCTGGATCCGGTAGTAAT
*Rbfox2*_mouse	qRT-PCR	F	GGGAAGCCAGGAACTAAAGG
*Rbfox2*_mouse	qRT-PCR	R	TGTGTCCCTAGGCAATGATG
*RBFOX2*_human	qRT-PCR	F	GGTACCTCCAACAGCCATCC
*RBFOX2*_human	qRT-PCR	R	GTGTACACCCTGCCATAA
Celf1 siRNA			CGUUUGGACAGAUUGAAGAtt
*Rbfox2*_3'-UTR	PCR	F	GACTTGAATTCAAGGCCTCAGTG ACGTGAGACCCCTGCAAATGGG
*Rbfox2*_3'-UTR	PCR	R	CGACTCACTATAGTTCTAGATGG TGTTTCTCTCTTTTATTTAAAAAC
Mut Let-7	SDM	F	TTCAAAGAACATAGTGAATGCTA AAAAAGTATTCTCTCTTTTTTGTT TGTTTTCCTCTTC
Mut Let-7	SDM	R	GAAGAGGAAAACAAACAAAAAA GAGAGAATACTTTTTTAGCATTC ACTATGTTCTTTGAA
Mut miR-9	SDM	F	AGCCATCAGCACCAGATCCAATG TTGCAATTCTGTCTGCTGGCTTCT GATC
Mut miR-9	SDM	R	GATCAGAAGCCAGCAGACAGAA TTGCAACATTGGATCTGGTGCTG ATGGCT
Mut miR-135a	SDM	F	CTGGAAGATGGAAATTCCTATTG TTCTGGAGTGAGGACAAACTGCC T
Mut miR-135a	SDM	R	AGGCAGTTTGTCCTCACTCCAGA ACAATAGGAATTTCCATCTTCCA G

SDM: site directed mutagenesis.

Supplementary Table 2. Information for RNA sequencing experiments including mapping rates for individual files. This supplemental table is related to Figure 5.

Sample Name	SRA File ID	Read Length	Total Reads	Mapped Reads	% Mapped
MHCrtTA 3 day Dox Replicate 1	This paper	2X100	99501365	71572733	71.93
MHCrtTA 3 day Dox Replicate 2	This paper	2X100	90376372	65357985	72.32
TRE-RBFOX2$_{40;}$ MHCrtTA 3 day Dox Replicate 1	This paper	2X100	97577814	67507903	69.18
TRE-RBFOX2$_{40;}$ MHCrtTA 3 day Dox Replicate 2	This paper	2X100	90249175	60344770	66.86
Human Control Replicate 1	SRR1971626	2X100	84000000	78961429	94
Human Control Replicate 2	SRR1971627	2X100	170806642	160968260	94.24
Human Control Replicate 3	SRR1971628	2X100	202795863	190446117	93.91
Human DM1 Replicate 1	SRR1971629	2X100	189993197	179173777	94.31
Human DM2 Replicate 2	SRR1971630	2X100	157762296	149276370	94.62
Human DM3 Replicate 3	SRR1971631	2X100	189593674	179657888	94.76
Mbnl1 WT Replicate 1	SRR533627	1X50	13107864	8358911	63.77
Mbnl1 WT Replicate 2	SRR533628-29	1X50	29500486	17858551	60.54
Mbnl1 WT Replicate 3	SRR533630-31	1X50	27981848	16870533	60.29
Mbnl1 WT Replicate 4	SRR533632	1X50	13563047	8110656	59.8
Mbnl1 WT Replicate 5	SRR533633	1X50	9681738	5694707	58.82
Mbnl1$^{\Delta E3/\Delta E3}$ Replicate 1	SRR533622	1X50	14242938	8431840	59.2
Mbnl1$^{\Delta E3/\Delta E3}$ Replicate 2	SRR533623	1X50	13159419	7562838	57.47
Mbnl1$^{\Delta E3/\Delta E3}$ Replicate 3	SRR533624	1X50	13379187	7841675	58.61
Mbnl1$^{\Delta E3/\Delta E3}$ Replicate 4	SRR533625	1X50	10092421	5861325	58.08
Mbnl1$^{\Delta E3/\Delta E3}$ Replicate 5	SRR533626	1X50	16224009	9088998	56.02
TRE-CELF1$_{;}$ MHCrtTA 3 day Replicate 1	SRR1205709	2X35	11583447	9619626	83.05
TRE-CELF1$_{;}$ MHCrtTA 3 day Replicate 2	SRR1205711	2X35	28294445	22464788	79.4
TRE-CELF1$_{;}$ MHCrtTA 3 day Replicate 3	SRR1205712-13	2X35	25072150	12137386	48.41
MHCrtTA 3 day Dox Replicate 1	SRR1205699	2X35	13888713	11462967	82.53
MHCrtTA 3 day Dox Replicate 2	SRR1205700	2X35	12478885	10107181	80.99
MHCrtTA 3 day Dox Replicate 3	SRR1205701	2X35	11335507	9439782	83.28