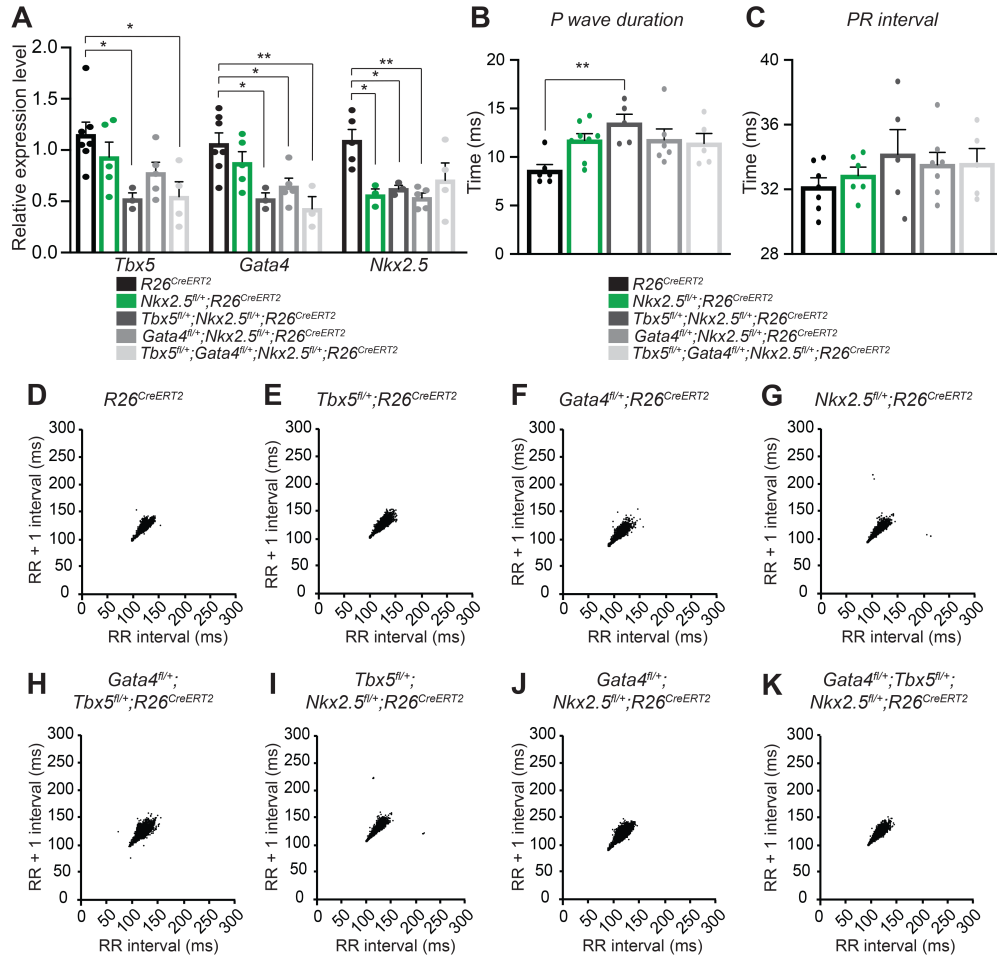
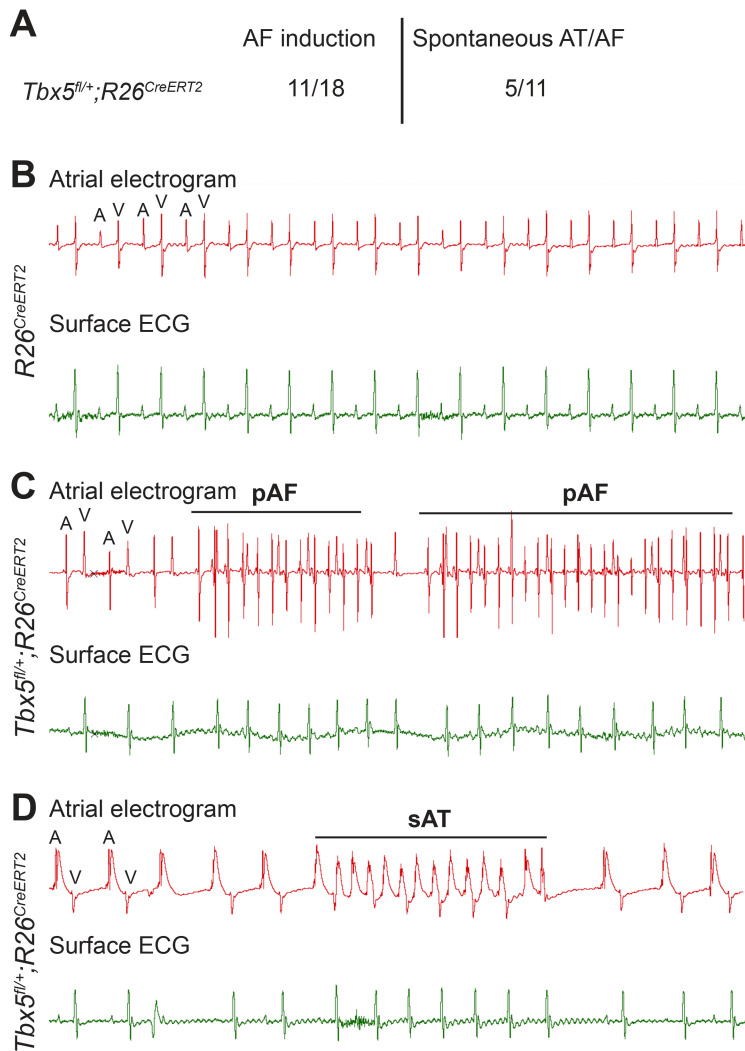


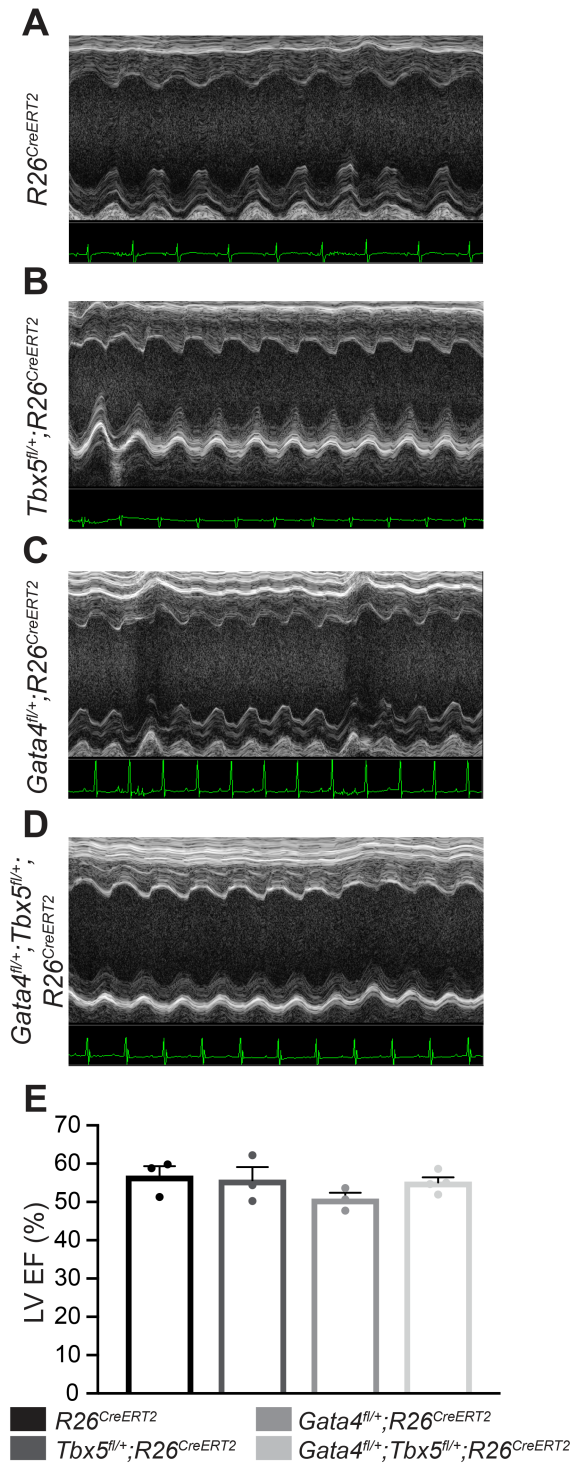
## Supplementary Material



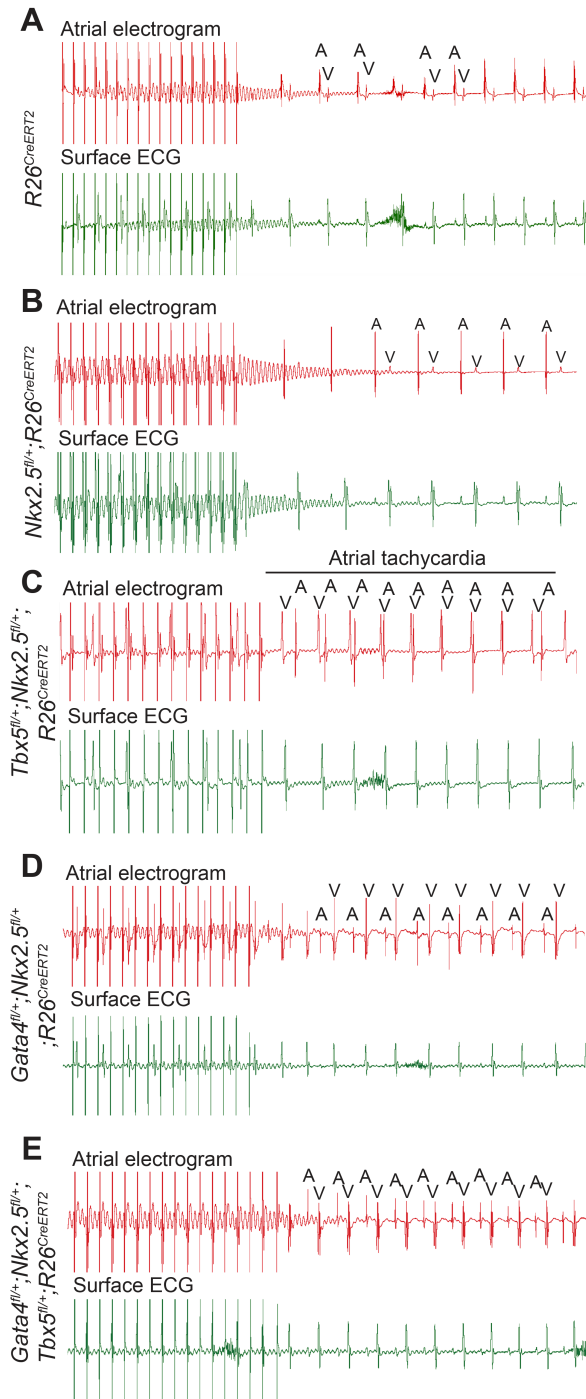
**Supplemental Figure 1. Combined *Gata4* (or *Tbx5*) and *Nkx2-5* haploinsufficiency does not affect adult atrial rhythm.** (A) Relative transcript expression by qPCR in the left atrium of *Nkx2.5* heterozygotes, *Tbx5/Nkx2.5* and *Gata4/Nkx2.5* compound heterozygotes and *Gata4/Tbx5/Nkx2.5* triple heterozygotes 2 weeks after TM treatment. Data are represented as means  $\pm$  SEM normalized to GAPDH and relative to *R26<sup>CreERT2</sup>* mice (set as 1) ( $n=5-7$  *R26<sup>CreERT2</sup>*,  $n=3-4$  *Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=3-4$  *Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=4$  *Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* and  $n=4$  *Tbx5<sup>fl/+</sup>;Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*). Experiments were performed in technical duplicates. *P* value was determined by one-way ANOVA followed by post-hoc Tukey test; \**P* < 0.05 was considered significant. (B, C) *P*-wave duration and PR interval calculated from ambulatory telemetry ECG recordings from *R26<sup>CreERT2</sup>* ( $n=12$ ), *Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=8$ ), *Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=5$ ), *Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=4$ ) and *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=5$ ) mice. *P* values were determined ANOVA followed by post-hoc Tukey test. (D-G) Representative Poincaré plot of RR interval (RR<sub>n</sub>) against the subsequent beat (RR<sub>n</sub> + 1) ( $n=7$  for *R26<sup>CreERT2</sup>*,  $n=5$  *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=6$  *Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=6$  *Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=7$  *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=5$  *Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*,  $n=6$  *Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* and  $n=5$  *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice).



**Supplemental Figure 2. Adult *Tbx5* heterozygotes have spontaneous atrial arrhythmias under anesthesia.** (A) Pacing induction by intra-atrial pacing of *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice showing episodes of spontaneous AF or AT. 5 of 11 (45%) *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice developed spontaneous atrial arrhythmias (B-D) Intracardiac atrial electrogram recordings and corresponding surface ECG of *R26<sup>CreERT2</sup>* (B), and *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice (C, D) with paroxysmal AF (C) or spontaneous atrial tachycardia (D) 2 weeks post-TM treatment. Paroxysmal AF was observed in 3 of 11 and *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice while spontaneous AT occurred in 2 of 11 *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice. A, atrial electrical signal; AF, atrial fibrillation; AT, atrial tachycardia; pAF, paroxysmal AF; sAT, spontaneous atrial tachycardia; V, far field ventricular electrical signal.



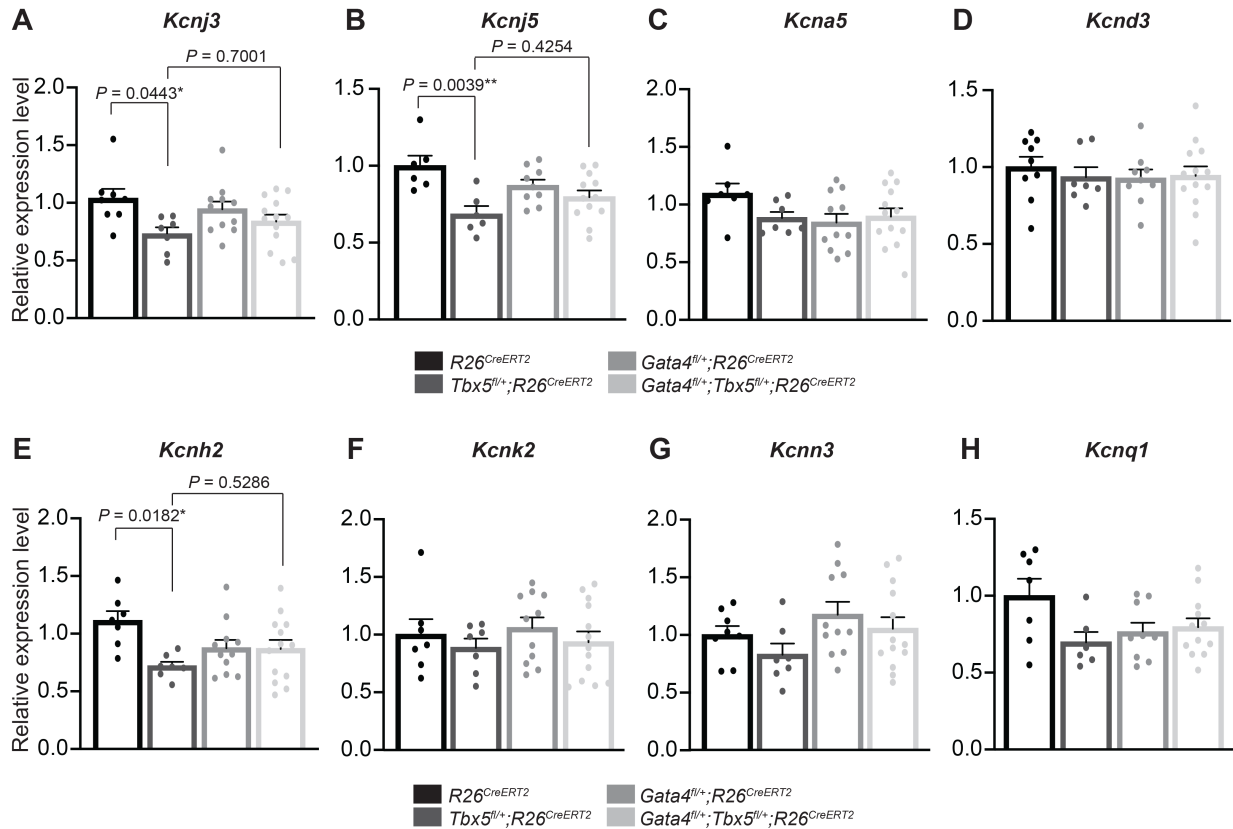
**Supplemental Figure 3. Left ventricular function of  $Tbx5^{fl/+};R26^{CreERT2}$  adult mice is not changed.** (A-D) M-mode echocardiography from  $R26^{CreERT2}$  (A),  $Tbx5^{fl/+};R26^{CreERT2}$  (B),  $Gata4^{fl/+};R26^{CreERT2}$  (C) and  $Tbx5^{fl/+};Gata4^{fl/+};R26^{CreERT2}$  (D) mice 2 weeks post-TM treatment. Surface ECGs are represented at the bottom.  $N = 3-4$  mice per genotype. (E) Left ventricular ejection fraction (LVEF) was calculated from the M-mode images.  $P$  values were calculated from ANOVA followed by post-hoc Tukey test.



**Supplemental Figure 4. Combined *Gata4* (or *Tbx5*) and *Nkx2.5* haploinsufficiency does not increase susceptibility to AF. (A-F)** Intracardiac atrial electrogram recordings and corresponding surface ECG of  $R26^{CreERT2}$  ( $n=9$ ),  $Nkx2.5^{fl/+}; R26^{CreERT2}$  ( $n=8$ ),  $Tbx5^{fl/+}; Nkx2.5^{fl/+}; R26^{CreERT2}$  ( $n=8$ ),  $Gata4^{fl/+}; Nkx2.5^{fl/+}; R26^{CreERT2}$  ( $n=12$ )  $Gata4^{fl/+}; Tbx5^{fl/+}; Nkx2.5^{fl/+}; R26^{CreERT2}$  ( $n=6$ ) mice. A, atrial electrical signal; V, far field ventricular electrical signal. (F) Pacing induction by intra-atrial pacing of mice in A-H. *P* values were determined by Fisher's exact test; \**P* < 0.05 and \*\**P* < 0.01.

**F**

	AF induction
$R26^{CreERT2}$	0/9
$Nkx2.5^{fl/+}; R26^{CreERT2}$	1/8
$Tbx5^{fl/+}; Nkx2.5^{fl/+}; R26^{CreERT2}$	3/8
$Gata4^{fl/+}; Nkx2.5^{fl/+}; R26^{CreERT2}$	1/12
$Gata4^{fl/+}; Nkx2.5^{fl/+}; Tbx5^{fl/+}; R26^{CreERT2}$	0/6



**Supplemental Figure 5. Decreased expression of potassium channels in *Tbx5* heterozygotes is not rescued by *Gata4* haploinsufficiency.** (A-H) Relative transcript expression by qPCR of known AF potassium genes from left atrium of *R26<sup>CreERT2</sup>* ( $n=6$ ) *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=5$ ), *Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=7$ ) and *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* ( $n=9$ ) mice 2 weeks after TM treatment. *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice showed a 20-30% reduction in *Kcnj3*, *Kcnj5* and *Kcnh2* gene expression, and was not normalized in *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice. Data are normalized to GAPDH and relative to *R26<sup>CreERT2</sup>*. *P* values were analyzed with one-way ANOVA followed by post-hoc Tukey test.

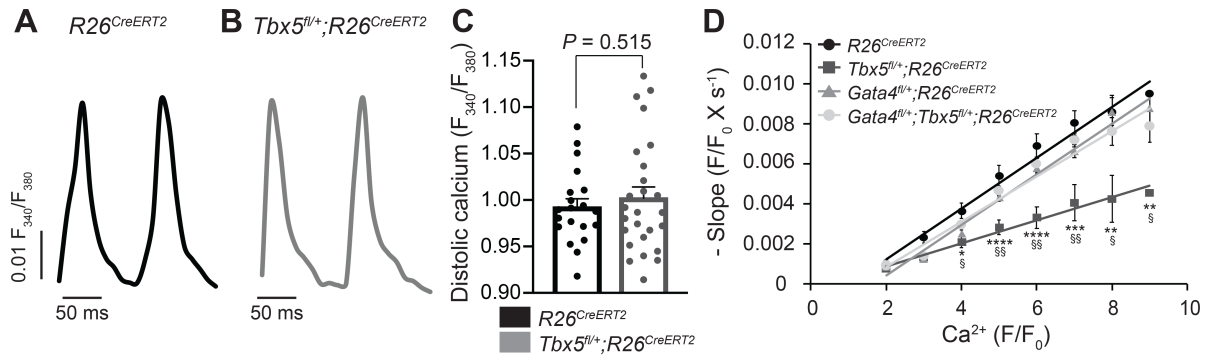
TBX5

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TTCACAGACAGACCACCAGAGGCTAGGCAGCGATCGTGGTGGCTTTTCTTCCCCTTTATAGTTTAGTGT  
ATTTGAAACAGAAATTTTTTTTCCAAAAACCTGGTGTGGGGAAAGTGTACCCTGTGAACTGTGCCCTCC  
TBX5  
GGCTGCCTGCCAACAGTTTGGTTTCTGTTGGTGTCATCCACAGCGAGCCCAGTCGCCCTGCACGGCC  
TBX5  
AAGGTGTCCTGTGAAATATATAGACCAGGGTCAGCTCGGCTCACAGGGTCCGATGGCCTCAGCAGACCTT  
GATA4      GATA4  
TCCGGGGGGGTGAGGGGTGGCGCACTGCCATGGGGCAATTATCTCCAGGAGATAGGGACGATGGACAAGCA  
TBX5  
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AAGGAATTTCACTCCTCCCTCCCTCCAGCCCGTCATCGTCGTTTTCTCTATCCCCTGAACAGAAATAT  
GATA4  
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GATGGGTGGGGGCTTGGGGAGGGGGTCAGGAGCGGGTGGTTAAGCTGTGGCGAGTGATTAGGGGAGCTGG  
CTGCCATCTCAGGCAGGTGAGGCTTAAGTGTGGACAGCCCCAGTGCCTCCTGGTACTCTGTAAAGAGGC  
GATA4  
ACCGAGGACCCAGGGAGGGAACATTTATCAGGGAATGCTCTTCTCTGGGAAAATGTGAACCAAACCAGA  
GGGGTCAAGCCTCAAGGATTAGCTCAGACAATGGGGCCTACTAACACAGGACACCAGCTTCTTTCCCA  
GTCTCTGCCTCCCTGGAACCTCAGTCTTCAGGGCTTCCCAGAGGCTGCGCAGTTTGAACCTCTGGGGCTT  
TAATGGCTCGCACCTACCTACCTACCTACCTACCTACCTACCTACCTACCTACCGATCTACCT

**Supplemental Figure 6. Nucleotide sequence of the 5'-upstream region of the mouse *Atp2a2* gene.** *Atp2a2* enhancer located 19Kb upstream of the start site (mm9 Chr5: 122970476-122971591) with GATA and TBX canonical binding sites shown in blue and green.

AGAGGGTCCCAAAGGAACACATCCTATCGCCGGAGCAAGACGAGCAGCTGCTAGGTTGACGGGACAATCA  
 TCACATACCTTCCCTTGTCCCTTTCCTCCCGTCTCCAGGAGCTCACACGTGCTTCTCTTTACCCAGCAG  
 CAAGACACTATTCTTTACATTATTAGCATCCTGAGTTTCTGGACCTCCCTGTTGGAGACAACAGGACAAC  
 AGCACCAGCTGATTTCTAAGGCCATTTCTTGATTCAGTGTGGACTTTGTCAGCTACAATGGGAAGCCAG  
 GAAGTGACACCGAGGGTCATTCATACCAGTAAGACCCATCATGAGGGAAAACCTTATACTGGTATTTGGT  
 CCATCACAATGGCCAAAATACGTGGCATGAACAACCTAAAAATAAACTATTGGCTTCTAGATCCAGAG  
 GTTTCAGTGTAGGACAGCAGGGCAGGCAGAGAGGAGGCTTGGTCCAGAGCAACAGTAGTCTGTAGCAA  
 AGTTTGCTCATGTGGTAGGAGACTAGGAAACAGAGAACGGGGCCAGAACTCAGGGCTGAGTATTACCTTT  
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 TGCCATCATCCAGGGAACAATCATTTAAAGCATAAGCCTATGGGGACATTTTAGATCCAAACCACGATAC  
 TCCTTTTCCCTCCCCCTCCATGCATCTCCTGTGTCTGCATACATACAGAACCAAGTTCTGGACACTG  
 GATA4  
 AGAATGCCCAAGTCTGAGACCTCCGTGGAAGATAAAGTTGGGATTACAGCTATTTTCTTAATTTCTTA  
 TCCATTGGGAATCTGGTTTGGGGTTCATTTGAATAGGACCTAGGAAGTAGATATGGGATGACAGACTC  
 TBX5  
 CCTCCTTTGAAAGCACTCACAAGTCAATAAAGAATAGAGATCAGCAACTCCATGGCAGTGAGGTGTGTGC  
 TTAATCTAGTGATATCCAGAGAGGCTGTGCCCTGCCACATACCAGAGCCCTGCAGAACAAGACCCACAGG  
 GATA4  
 ACTTTGCTGATAAGGACAAGAGGCTTAGCAGTGACAAATCGTGACGCACATCATTTATCTTCATGACCCCA  
 TGCTGCAAAGCAGCTCTACTACACTGAAGGAACTTACTGTAACTGTACTGGTTGCATTTGTCACTG  
 AGAAGCCCCGTGGGATACCCTGGGCTCCTGTGTTTCCCTACTAGGCAATGAAGATCATGCCTTCTCCA  
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 CCTCCTGCCCCAGTTACTCTCAGATCTACCATCCAGGAGAAGAGGGCTCTGTCTGCTCCCTGGGCACTGG  
 CTATGCTGGAGGGTCAGGAGGCAAAAGGAGTCTGCAAACATCTAGAAAGCATCAGAGAAAACCACTACTGG  
 GTTTTGCTTGTCTACAGGGCAGTGGTGGTCACGGAAAATGAAAAATTAATTAGGAGAACATGACTGCTG  
 GATTTCCCTTTATTCTTGAACCAAATCAAATCAATACAGTTCTCAGATCCTGCCAATAAAAGGCAAAGACC  
 GATA4  
 TAATTGTGCCTGACTTGGGAAGTCCCAAGAAACAGAACCACAAGATATCTAGGGCAGGACAGAGATAAGG  
 CAATCCTAAGAGCCCAGTGACCAGCTTGGCTAGCTGGAATAGCAAACCTTTATACTCAGTGAGAGACCCTG  
 TBX5  
 ACCTCCCCACCCCTCCGTACACCAATTAAAAACAAAAAAGAAGTGGATGGCTATGAGGATGACATCGT  
 GGTTAAGCCTAAGTGTGAAGACTAAAGCTTGGAGCCTTCCCTTGGAAAGATCCAGAGCATATCCTTGAG

**Supplemental Figure 7. Nucleotide sequence of the 5'-upstream region of the mouse *Ryr2* gene.** *Ryr2* enhancer located 24Kb upstream of the start site (mm9 Chr13: 12223550-12226563) with GATA and TBX canonical binding sites shown in blue and green.



**Supplemental Figure 8. Diastolic calcium levels are unaltered in *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*.** (A, B) Representative diastolic calcium traces after Fura-2 AM staining. (C)  $[Ca]_i$ , determined by dividing fluorescence emission following 340 nm excitation by 380 nm excitation, was unchanged in *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* compared to littermate controls ( $n = 3$  mice per genotype). (D) SERCA activity was decreased at all levels of calcium *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* compared to littermate controls and normalized in *Gata4/Tbx5* compound heterozygotes.  $P$  values were calculated using an ANOVA followed by post-hoc Tukey test. (\* *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* versus *R26<sup>CreERT2</sup>* mice; § *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* versus *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* mice).



### Supplementary Tables

Genotype	Parameters		
	P-wave (ms)	PR interval (ms)	QRS (ms)
<i>R26<sup>CreERT2</sup></i>	10.14 ± 1.07	32.15 ± 0.57	11.90 ± 0.25
<i>Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup></i>	14.19 ± 0.66**	35.02 ± 1.15**	12.04 ± 0.32
<i>Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup></i>	11.59 ± 0.75	31.64 ± 0.93	11.81 ± 0.29
<i>Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup></i>	11.66 ± 0.72	33.80 ± 1.31	11.76 ± 0.26
<i>Gata4/Tbx5</i>	13.23 ± 0.82	32.84 ± 1.49	12.51 ± 0.31
<i>Tbx5/Nkx2.5</i>	13.48 ± 1.03	33.59 ± 1.22	12.31 ± 0.98
<i>Gata4/Nkx2.5</i>	11.78 ± 1.22	32.85 ± 0.57	12.45 ± 0.21
<i>Gata4/Tbx5/Nkx2.5</i>	10.40 ± 1.15	34.16 ± 1.70	12.51 ± 0.38

\*\**P*<0.01 compared to *R26<sup>CreERT2</sup>*.

*Gata4/Tbx5*, *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*

*Tbx5/Nkx2.5*, *Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*

*Gata4/Nkx2.5*, *Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*

*Gata4/Tbx5/Nkx2.5*, *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*

**Supplemental Table 1. Conscious ambulatory telemetry parameters.** Conscious ambulatory telemetry ECG parameters obtained from *Tbx5*, *Gata4* and *Nkx2.5* adult heterozygotes, *Gata4/Tbx5*, *Tbx5/Nkx2.5* and *Gata4/Nkx2.5* compound heterozygotes and *Gata4/Tbx5/Nkx2.5* triple heterozygotes 2 weeks after receiving TM. Values are mean ± standard error the mean obtained from *R26<sup>CreERT2</sup>* (*n*=12), *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=5), *Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=6), *Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=8), *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=8), *Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=5), *Gata4<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=4) and *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;Nkx2.5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (*n*=5) mice.

Genotype	Parameters				
	$dV/dt_{max}$ (mV/ms)	RMP (mV)	APA (ms)	APD50 (ms)	APD90 (ms)
<i>R26<sup>CreERT2</sup></i>	221.52 ± 23.29	-76.3 ± 2.55	133.89 ± 2.49	9.46 ± 1.32	71.33 ± 11.24
<i>Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup></i>	157.44 ± 14.15	-77.15 ± 1.46	128.27 ± 4.57	13.33 ± 2.24	172.44 ± 26.08**†††
<i>Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup></i>	147.73 ± 19.88	-77.53 ± 1.55	121.51 ± 5.22*	10.08 ± 2.42	66.04 ± 13.28
<i>Gata4/Tbx5</i>	170.41 ± 18.85	-79.50 ± 0.21	136.01 ± 5.17	9.90 ± 1.67	68.22 ± 14.15

**Supplemental Table 2. Action potential parameters of adult-specific *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>*, *Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup>* and *Gata4/Tbx5* compound haploinsufficiency.** Single cell electrophysiology parameters obtained from *Tbx5* and *Gata4* adult heterozygotes as well as *Gata4/Tbx5* compound heterozygotes and *Gata4/Tbx5/Nkx2.5* triple heterozygotes 2 weeks after receiving TM. Values are mean ± standard error the mean obtained from *R26<sup>CreERT2</sup>* (n=9), *Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (n=14), *Gata4<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (n=10) and *Gata4<sup>fl/+</sup>;Tbx5<sup>fl/+</sup>;R26<sup>CreERT2</sup>* (n=13). APA, Action potential amplitude; APD50, action potential at 50% repolarization; APD90, Action potential at 90% repolarization; RMP, resting membrane potential

Gene	Primers	
	Forward	Reverse
<i>Tbx5</i>	GGCATGGAAGGAATCAAGGT	CTAGGAAACATTCTCCTCCCTGC
<i>Gata4</i>	AAACGGAAGCCCAAGAACCTGAAT	GAGCTGGCCTGCGATGTCTAGGTG
<i>Nkx2.5</i>	ACATTTTACCCGGGAGCC	GGCTTTGTCCAGCTCCAC
<i>Ryr2</i>	CAAATCCTTCTGCTGCCAAG	CGAGGATGAGATCCAGTTCC
<i>Atp2a2</i>	CTGGTGATATAGTGGAATTGCTG	GGTCAGGGACAGGGTCAGTA
<i>Sn</i>	CTGAGGTCCTTGGTAGCCTG	GGTGTGTCAGGCATTGTGAG
<i>NCX</i>	TTCTCATACTCCTCGTCATCG	TTGAGGACACCTGTGGAGTG
<i>CamK2B</i>	ACCCTCTACTTTCTCTCCTCC	ACTTTGGTGTCTTCGTCCTC
<i>Pln</i>	TTATGCCAGGACGGCAAAG	CACTGTGACGATCACCGAAG
<i>Cacna1c</i>	CTACAGAAACCCATGTGAGCAT	CAGCCACGTTGTCAGTGTTG
<i>Kcnj3</i>	GCTGGCAACTACACTCCCTG	AACATGCAGCCGATGAGGAA
<i>Kcnj5</i>	TGTAAGAGCTCCGTGCTTGG	TGTGGAGATGTCTCGTGCTC
<i>Kcna5</i>	AAAATTGGAGACGATGACGG	ATGAGGCCCATCACTGTAGG
<i>Kcnd3</i>	GGGTGGCAGGCAGGTTAGA	CCTGCTGCTCCCGTCGTA
<i>Kcnh2</i>	ATGGCTCAGATCCAGGCAGTTA	CAAGGAGAGCGGTCAGGTAATG
<i>Kcnk2</i>	TGGCTACGGGTGATCTCTAAG	GCTGGAACTTGTCGTAGATCTC
<i>Kcnn3</i>	CAAGAATGCCGCCGCCAATGTC	CCAGGCTGCCAATCTGCTTTTC
<i>Kcnq1</i>	GAGGATAGGAGGCCAGACCA	AAGTACTGCATGCGCCTGAT

**Supplemental Table 3. Quantitative PCR primers used.**