Supplementary Data

A Tbx5-Scn5a Molecular Network Modulates Function of the Adult Murine Cardiac Conduction System

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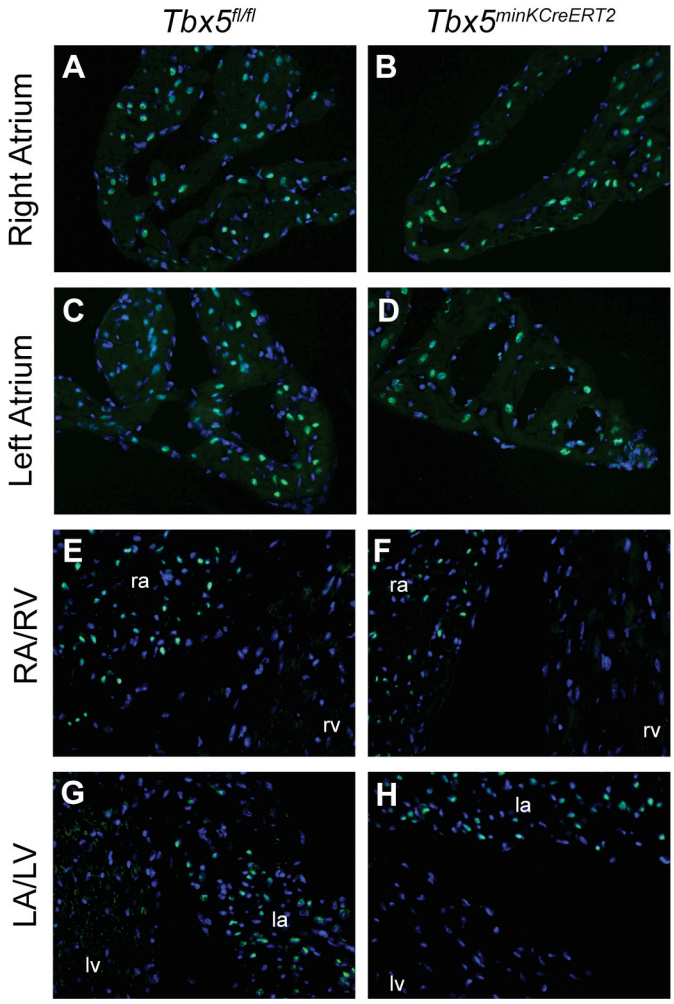
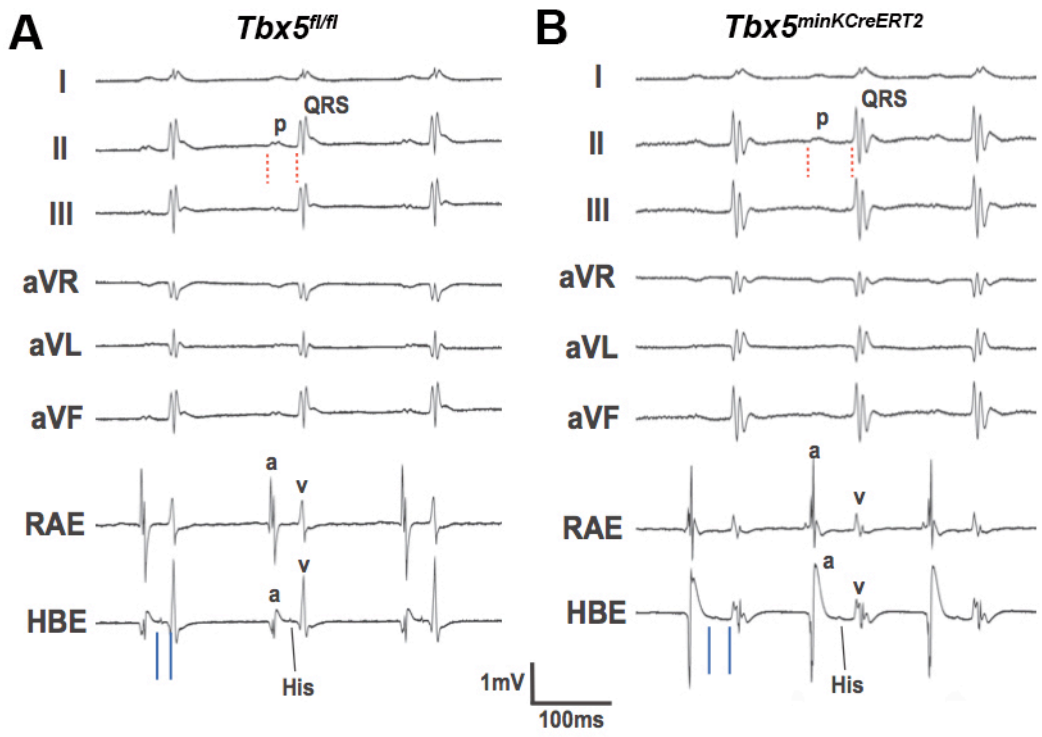
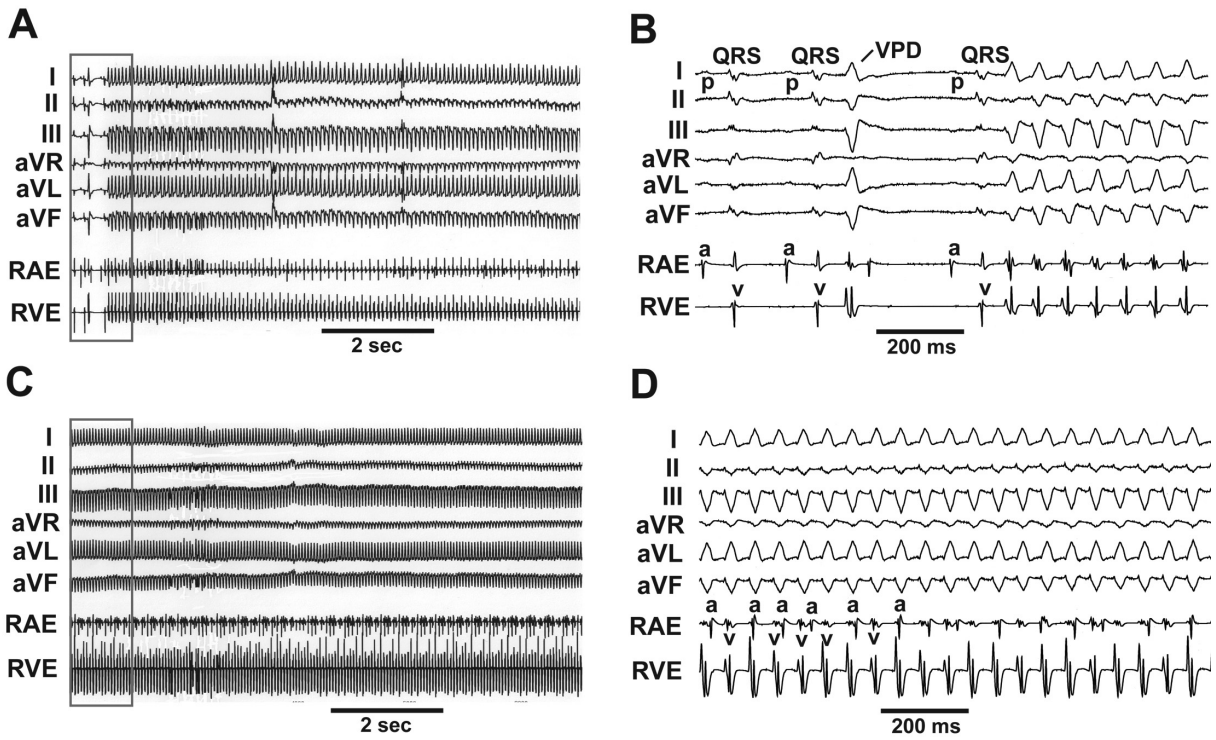
Supplementary Methods



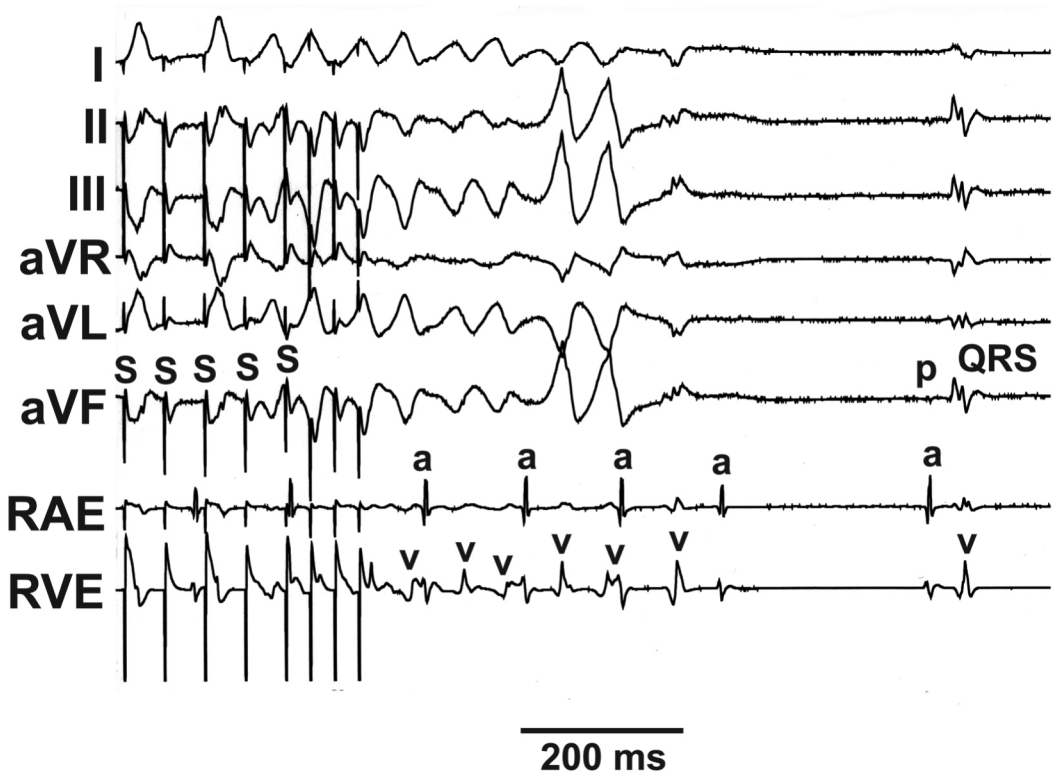
Supplementary Figure 1: Tbx5 expression is maintained in the atria of *Tbx5*^{minKCreERT2} mice. Tbx5$^{fl/fl}$ and *Tbx5*^{minKCreERT2} mice were administered tamoxifen at 6-7 weeks of age and Tbx5 expression was evaluated by immunofluouresence 4-5 weeks later. *Tbx5*$^{fl/fl}$ mice (A, C, E, G) and *Tbx5*^{minKCreET2} (B, D, F, H) demonstrated Tbx5 expression throughout the right atrium (A-B; E-F), left atrium (C-D; G-H), but not the right or left ventricles (E-H). ra: right atrium, rv: right ventricle, la: left atrium, lv: left ventricle. Nuclei are stained with DAPI (blue). Original magnification in all panels was 40x.



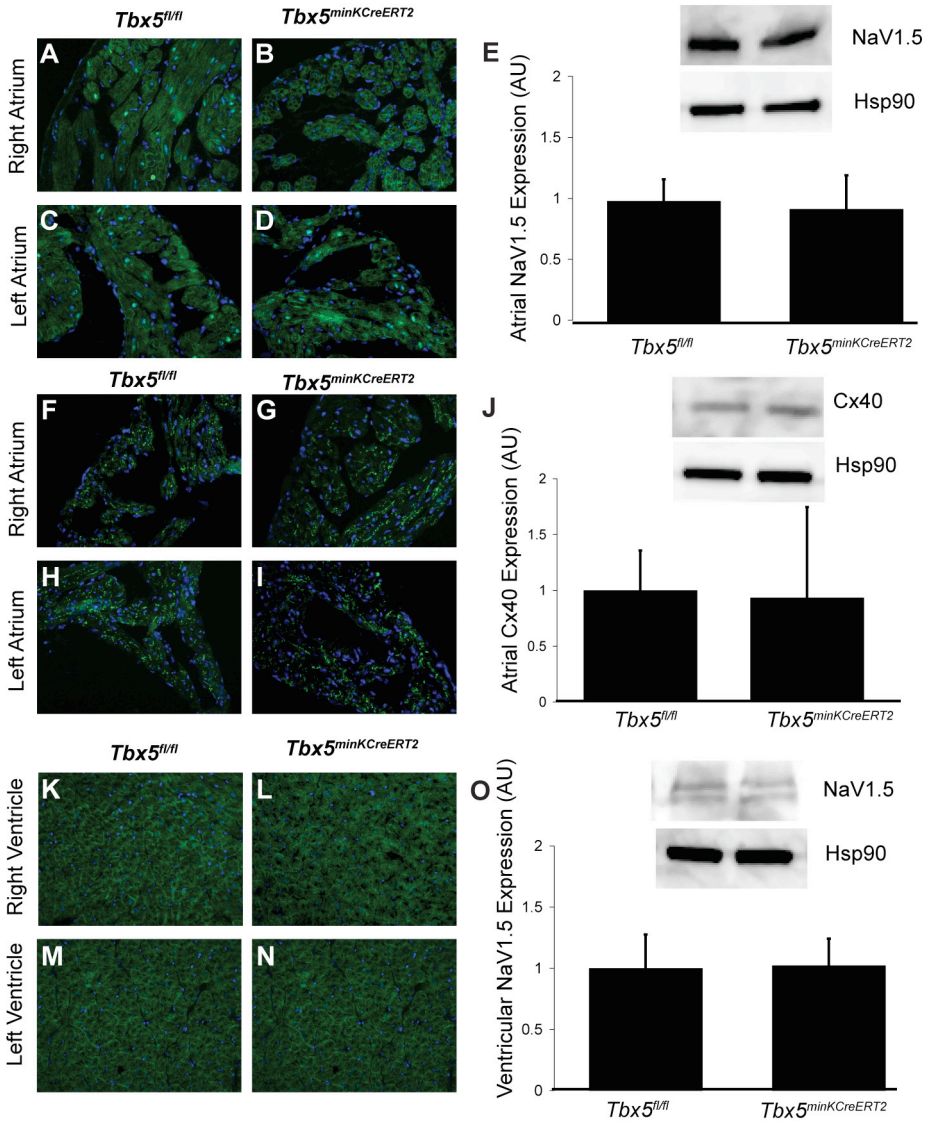
Supplementary Figure 2: Conduction slowing following removal of Tbx5 from the VCS. Representative 6 lead surface ECG and simultaneous intracardiac recordings from the right atria (RAE) and His bundle (HBE) of *Tbx5*$^{fl/fl}$ (a) and *Tbx5*^{minKCreERT2} littermates (b) administered tamoxifen at 6-7 weeks of age and studied 4-5 weeks after tamoxifen administration. *Tbx5*^{minKCreERT2} animals demonstrate prolonged PR, QRS, A-H, H_d, and HV intervals.



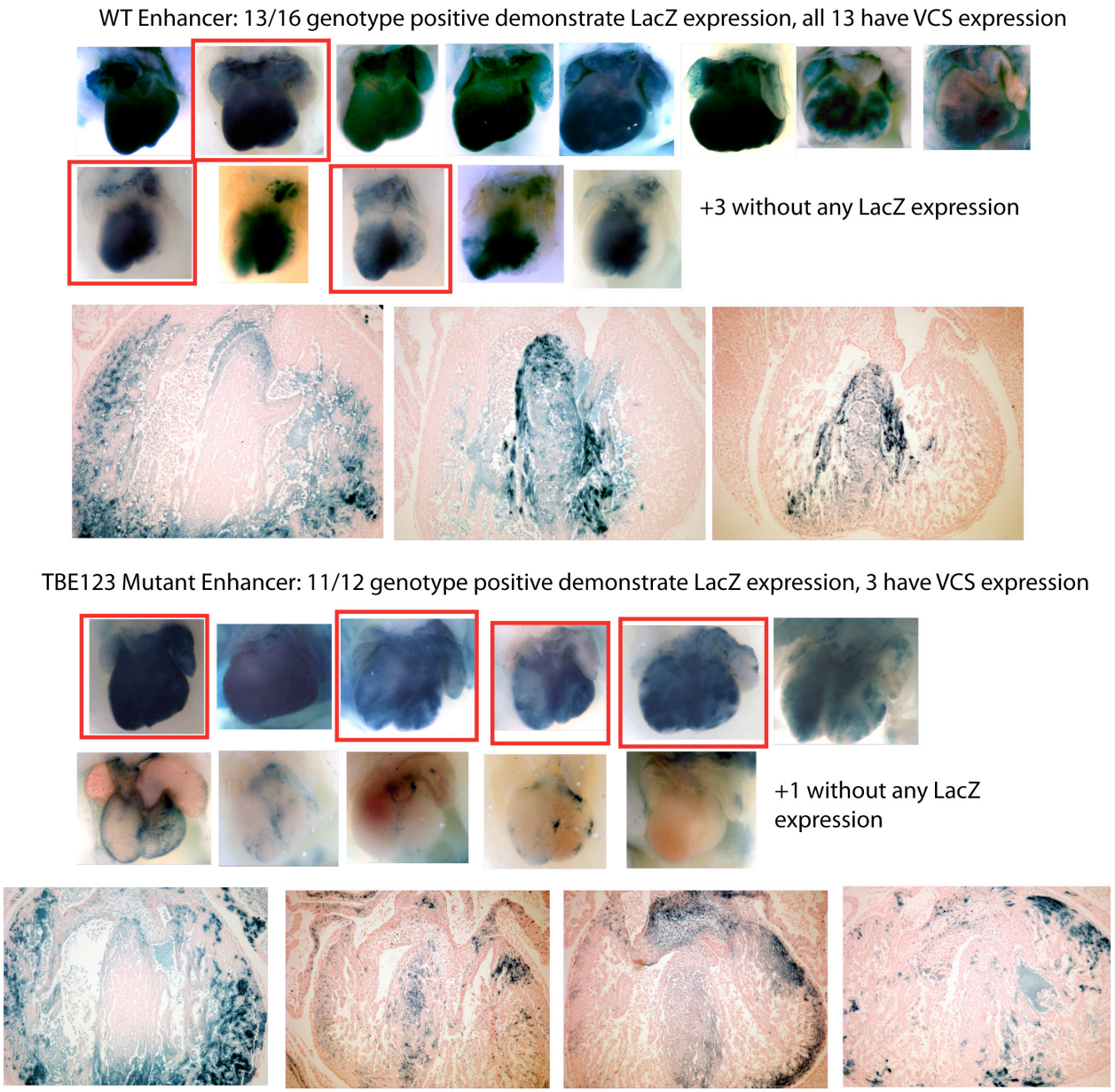
Supplementary Figure 3: *Tbx5*^{minKCreET2} mice have spontaneous, sustained VT. (A) Initiation of spontaneous, monomorphic VT in a mouse with ventricular conduction system-specific deletion of Tbx5. Note the first two depolarizations are consistent with sinus rhythm followed by a ventricular premature depolarization (VPD) with the same morphology as the VT that begins following one more sinus rhythm complex. Shown are surface ECG leads I, II, III. aVR, aVL, aVF along with intracardiac recordings of the right atrial electrogram (RAE) and right ventricular electrogram (RVE). (B) Shown in this panel is initiation of the VT from panel A outlined by the box at a faster speed. Note that at the onset of the VT there does not appear to be VA disassociation but instead there is likely retrograde 1:1 ventricular-atrial conduction with fusion of the atrial and ventricular electrograms. (C) Continuation of the same spontaneous VT shown in panel A that begins approximately 15 seconds after the end of the tracings shown in panel A. (D) Shown in this panel are the electrograms from panel C outlined by the box at a faster speed. At this time point there is now clear evidence of VA disassociation with fewer atrial electrogram (a) present on the RAE tracing compared to the number of ventricular electrograms (v) on the RVE tracing. This rhythm continued for over 2 minutes before the mouse died. P=P-wave; QRS=QRS-complex; a=atrial electrogram; v=ventricular electrogram.



Supplementary Figure 4. ***Tbx5*$^{fl/fl}$ control mice have only induced, non-sustained polymorphic VT.** At the right side of this panel are the last 5 stimuli (S) of a drivetrain applied at a cycle length of 50-ms followed by three extrastimuli coupled at 30-ms intervals. Following delivery of the last extrastimuli non-sustained, polymorphic VT is induced that lasts approximately 420 ms. Note the presence of VA disassociation at the onset of the polymorphic VT with more ventricular electrograms (v) than atrial electrograms (a). Shown are surface ECG leads I, II, III. aVR, aVL, aVF along with intracardiac recordings of the right atrial electrogram (RAE) and right ventricular electrogram (RVE).



Supplementary Figure 5: Cx40 and $Na_v1.5$ expression are not altered outside the VCS in *Tbx5*^{minKCreERT2} mice. Atrial expression of $Na_v1.5$ was examined in the right (A-B) and left (C-D) atria of *Tbx5*$^{fl/fl}$ (A,C) and *Tbx5*^{minKCreERT2} (B,D) mice by immunofluoresence and Western Blotting of whole atria (E). No differences were detected in atrial $Na_v1.5$ expression. Atrial expression of Cx40 was examined in the right (F-G) and left (H-I) atria of *Tbx5*$^{fl/fl}$ and *Tbx5*^{minKCreERT2} mice by immunofluoresence and Western Blotting of whole atria (J). No differences were detected in atrial Cx40 expression. Ventricular expression of $Na_v1.5$ was examined in the right (K-L) and left (M-N) ventricles of *Tbx5*$^{fl/fl}$ (K, M) and *Tbx5*^{minKCreERT2} (L, N) mice by immunofluoresence and Western Blotting of whole ventricles (O). No differences were detected in $Na_v1.5$ expression. Graphs in E, J, O represent mean +/- SD; representative blots for $Na_v1.5$ and Cx40 (*Tbx5*$^{fl/fl}$ on the left, *Tbx5*^{minKCreERT2} on the right) are shown. Hsp90 blots demonstrate equal loading of protein.



Supplementary Figure 6: Evaluation of WT and TBE123 Mutant Enhancers at E13.5. 16 transgenic embryos were genotypically positive for the WT enhancer. 13 (shown) demonstrated LacZ expression. All embryos with LacZ expression demonstrated VCS expression. 12 transgenic embryos were genotypically positive for the TBE123 mutant enhancer. 11 (shown) demonstrated LacZ expression. 5 (bottom row) demonstrated minimal cardiac expression. Of the 6 embryos with significant cardiac expression, 4 were sectioned, and only 1 showed VCS expression; based on this we conclude that a maximum of 3 mutant embryos have LacZ expression in the VCS. Embryos are arranged in descending order of staining intensity. Boxed embryos were sectioned. Sections are shown at 10x original magnification

Supplementary Table 1: EP Intervals Following Atropine Administration

		SCL (ms)	AH (ms)	Hd (ms)	HV (ms)
***Tbx5*$^{fl/fl}$ (n=3)**	**Baseline**	157.3 ± 38.4	27.3 ± 9.7	4.0 ± 1.7	8.3 ± 3.0
	Post-Atropine	141.3 ± 30.9	28.0 ± 9.9	3.7 ± 1.5	9.0 ± 3.2
***Tbx5*^{minKCreERT2} (n=3)**	**Baseline**	130.0 ± 30.6	33.3 ± 5.9	13.7 ± 3.8	26.3 ± 4.8
	Post-Atropine	146.3 ± 11.6	35.7 ± 3.6	11.7 ± 2.5	26.0 ± 2.4

Mean +/- SD; SCL: sinus cycle length, AH: AtrioHisian interval, Hd: His interval duration, HV: Hisioventricular interval.

Supplementary Table 2: Characterization of Second Degree AV Block

	Tbx5^{fl}	*Tbx5*^{minKCreERT2}
	Ambulatory Telemetry	
Dropped beats/ 24 hour recording	5.9±13.99	9.6±15.49
Mice with intermittent Mobitz Type 2 2nd degree AVB	0	7
N	9	10

Mean ± SD; AVB: Atrioventricular block

Supplementary Table 3: Primers used for Cloning

SCN5A enh XhoI Fwd:	ATCTCA CTCGAG AGGCTCTGCAGAGTTCCATCTCT
SCN5A enh BglII Rev:	ATCATC AGATCT CCTCCCAGGCCTGTGAAATGGCCT
TBE1mut Fwd	CTGAGGGCCTCTGAGGAG**AAA**TGAATGAGGGAGGCAGAG
TBE1mut Rev	CTCTGCCTCCCTCATTCA**TTT**CTCCTCAGAGGCCCTCAG
TBE2mut Fwd	GTCCCAGACACCCTCTC**TTT**ACCCCAGCCCTGATAAG
TBE2mut Rev	CTTATCAGGGCTGGGGT**AAA**GAGAGGGTGTCTGGGAC
TBE3mut Fwd	CCAGCCCTGATAAGCAG**AAA**TGAGCCATGGCCGCCTT
TBE3mut Rev	AAGGCGGCCATGGCTCA**TTT**CTGCTTATCAGGGCTGG
HindIII Tbx5cDNA Fwd:	AGCTAG AAGCTT TGCGCACCATGGCCGATACAGAT
NotI Tbx5cDNA Rev:	GAAGAA GCGGCCGC TTAGCTATTCTCACTCCACTCTGGC
mini-TK BglII F	GATCT TTCGCATATTAAGGTGACGCGTGTGGCCTCGA A
mini-TK HindIII R	AGCTT TCGAGGCCACACGCGTCACCTTAATATGCGAA A

Supplementary Methods

Atropine Administration

Atropine was administered to *Tbx5*$^{fl/fl}$ mice and *Tbx5*^{minKCreERT2} littermates during EP studies performed as described in the manuscript. Baseline recordings of surface ECG and intracardiac recordings from the right atrium and His bundle were obtained and repeated 10 minutes after intraperitoneal injection of atropine (20 mg/kg body weight).

Western Blotting

Atria and ventricles were dissected from *Tbx5*$^{fl/fl}$ mice and *Tbx5*^{minKCreERT2} littermates administered tamoxifen at 6-7 weeks of age and studied 4-5 weeks following tamoxifen administration. Tissue was snap frozen in liquid nitrogen, pulverized, and homogenized in RIPA buffer (50 mM Tris-HCL pH 8, 150 mM NaCl, 1% Triton-X, 0.50% sodium deoxycholate, 0.1% SDS, 5 mM EDTA + 1 Roche EDTA-Free complete protease inhibitor tablet/50 mL buffer). Samples were tumbled for 1 hour at 4º C, then centrifuged for 10 minutes at 13,200 x g. Protein concentration was determined using the BCA assay (Pierce) with BSA as a standard. 25 ug of protein was diluted in Laemmli buffer, heated for 10 min at 70º C and subjected to SDS-PAGE on 4-20% TGX gels (Bio-Rad). Proteins were transferred to nitrocellulose membranes, blocked with 5% milk in TBS-T, and incubated with primary antibody overnight at 4º C. Primary antibodies were: rabbit anti-$Na_v1.5$ (Alomone ASC-005, 1:2000), rabbit anti-Cx40 (Zymed 36-4900, 1:500), and rabbit anti Hsp90 (Santa Cruz Biotechnology sc-7947, 1:3000). After rinsing in TBS-T, membranes were incubated in secondary antibody (goat anti-rabbit-HRP, Jackson Immuno-Research, 1:3000 in 2.5% milk), rinsed, and visualized using ECL Prime (Amersham). Quantification was performed using ImageJ.