Scn2b Deletion in Mice Results in Ventricular and Atrial Arrhythmias

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**Background**—Mutations in SCN2B, encoding voltage-gated sodium channel β2-subunits, are associated with human cardiac arrhythmias, including atrial fibrillation and Brugada syndrome. Because of this, we propose that β2-subunits play critical roles in the establishment or maintenance of normal cardiac electric activity in vivo.

**Methods and Results**—To understand the pathophysiological roles of β2 in the heart, we investigated the cardiac phenotype of Scn2b null mice. We observed reduced sodium and potassium current densities in ventricular myocytes, as well as conduction slowing in the right ventricular outflow tract region. Functional reentry, resulting from the interplay between slowed conduction, prolonged repolarization, and increased incidence of premature ventricular complexes, was found to underlie the mechanism of spontaneous polymorphic ventricular tachycardia. Scn5a transcript levels were similar in Scn2b null and wild-type ventricles, as were levels of Na1.5 protein, suggesting that similar to the previous work in neurons, the major function of β2-subunits in the ventricle is to chaperone voltage-gated sodium channel α-subunits to the plasma membrane. Interestingly, Scn2b deletion resulted in region-specific effects in the heart. Scn2b null atria had normal levels of sodium current density compared with wild type. Scn2b null hearts were more susceptible to atrial fibrillation, had increased levels of fibrosis, and higher repolarization dispersion than wild-type littermates.

**Conclusions**—Genetic deletion of Scn2b in mice results in ventricular and atrial arrhythmias, consistent with reported SCN2B mutations in human patients. *(Circ Arrhythm Electrophysiol. 2016;9:e003923. DOI: 10.1161/CIRCEP.116.003923.)*

**Key Words:** action potentials  ■  atrial fibrillation  ■  fibrosis  ■  potassium channels  ■  sodium channels

Voltage-gated sodium channels (VGSCs) are responsible for the upstroke of the cardiac action potential (AP) and are required for impulse propagation in the heart. They are composed of 1 pore-forming α-subunit and 2 nonpore-forming β-subunits. Although α-subunits are sufficient for conduction, β-subunits regulate sodium current (I\textsubscript{Na}) by modulating kinetics, voltage dependence, and channel cell surface expression. In addition, some VGSC β-subunits regulate potassium currents (I\textsubscript{K}) and function as cell adhesion molecules that are substrates for sequential cleavage by BACE-1 (beta-site amyloid precursor protein enzyme 1) and γ-secretase. Mutations in the genes encoding β-subunits are implicated in human disease, including cardiac arrhythmia. Mutations in SCN2B, encoding β2, are associated with atrial fibrillation (AF) and Brugada syndrome (BrS) in humans, suggesting that β2 is critical in establishing or maintaining normal cardiac electric activity. β2 is covalently linked to α, including the major cardiac VGSC, Na\textsubscript{1.5}, via disulfide bonds. β2 enhances the trafficking of neuronal VGSC α-subunits to the plasma membrane and modulates the voltage dependence of channel gating. Consistent with the channel trafficking role of β2, Scn2b null neurons have reduced VGSC cell surface expression with no change in total cellular protein levels. Heterologous coexpression of Na\textsubscript{1.5} with a SCN2B patient mutation linked to arrhythmia resulted in reduced I\textsubscript{Na} density compared with Na\textsubscript{1.5} alone, suggesting defective cell surface VGSC trafficking. However, the role of β2 in heart has not been explored in vivo. Here, we test the hypothesis that β2 plays critical roles in cardiac excitability via regulation of cell surface VGSC levels and I\textsubscript{Na} density. We report changes in I\textsubscript{Na} and, unexpectedly, I\textsubscript{K} density and ventricular and atrial arrhythmias in Scn2b null mice. These novel results advance our understanding of the physiological and pathophysiological roles of β2 in the heart.
WHAT IS KNOWN

- Voltage-gated sodium channel β2-subunits are cell adhesion molecules expressed in both brain and heart. In neurons, β2-subunits are known to function as chaperone sodium channel pore-forming α-subunits to the plasma membrane. In addition, generation of C-terminal β2 fragments by sequential cleavage of β2-subunits by BACE-1, and γ-secretase has been shown to regulate sodium channel α-subunit gene transcription in neurons.

- Mutations in the gene encoding sodium channel β2-subunits, SCN2B, are associated with atrial fibrillation and Brugada syndrome in human patients. However, the function of β2 in heart has not been studied.

WHAT THE STUDY ADDS

- Scn2b deletion results in changes in reduced sodium current density and, unexpectedly, reduced potassium current density in ventricular myocytes, with subsequent slowing of conduction in the right ventricular outflow tract region.

- Slowed conduction and prolonged repolarization in the right ventricular outflow tract interact with premature ventricular complexes to initiate functional reentry, increasing the susceptibility of Scn2b null hearts to ventricular arrhythmia.

- In contrast to the ventricle, atrial sodium current density and biophysical properties are not different between genotypes, demonstrating region-specific functionality of β2 in the heart.

- Scn2b null mice are more susceptible to atrial fibrillation and have increased atrial fibrosis compared with wild type. Increased functional and anatomic heterogeneity in the atrium because of repolarization dispersion and fibrosis may contribute to the mechanism of atrial arrhythmia in Scn2b null hearts.

Methods

Myocyte isolation, electrophysiological recording, quantitative reverse transcriptase–polymerase chain reaction (qRT-PCR), Western blot, super-resolution patch clamp, optical mapping, intracardiac recording, and assessment of fibrosis are described in the Data Supplement.

Animals

This study was performed in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and with approval from the University of Michigan Institutional Animal Care and Use Committee. Male and female Scn2b null and wild-type (WT) mice,8 bred at ages indicated in each method in the Data Supplement.

Cell Isolation

WT and Scn2b null cardiac myocytes were acutely isolated.10

Single-Cell Electrophysiology

Voltage clamp $I_{Na}$ and $I_K$ recordings were performed at room temperature. Current clamp recordings were acquired at 37°C. Cells with a diastolic membrane potential more negative than −65 mV were used for analysis.

qRT-PCR

qRT-PCR was performed as described.11

Western Blotting

Western blots were performed as described.11

Optical Mapping

Ventricular Mapping

Hearts were perfused through the aorta with warm, oxygenated Tyrode solution followed by 7 μM Blebbistatin (Cayman Chemical)12,13 and 100 μM Di-4-ANEPPS (Life Technologies) to uncouple excitation–contraction and to stain the heart, respectively. High-resolution optical mapping was performed, imaging at 800 frames per second. Optical movies were recorded at baseline and at progressively shorter cycle lengths.

Atrial Mapping

The atrium was excised, and a camera was positioned to face the posterior walls of both atria. Arrhythmias were induced by applying burst pacing at 66.67 Hz for 5 or 10 s before and after carbachol administration.

Intracardiac Recording

An octapolar catheter (Science) was inserted through the jugular vein and advanced into the right atrium (RA) and ventricle. Programmed electric stimulation was assessed to determine the baseline sinus node recovery time, atrial refractory period, atrioventricular nodal effective refractory period, and ventricular refractory period. Arrhythmia inducibility was assessed by the application of 12 to 18 atrial bursts of pacing, before and after intraperitoneal carbachol (50 ng/g) administration. AF was defined as the occurrence of rapid and fragmented atrial electrograms (lack of regular P waves) with irregular AV nodal conduction and ventricular rhythm, all lasting at least 1 s.

Assessment of Fibrosis

Left atrial and RA posterior walls from both sexes and genotypes were collected and analyzed.

Statistics

Statistical significance was evaluated using Mixed Effects Regression analysis to address multiple observations from individual animals, Student t test, Mann–Whitney rank-sum test, or Fisher exact test, as indicated. $P<0.05$ was considered significant.

Results

Scn2b Deletion Results in Decreased $I_{Na}$ Density in Ventricular Myocytes

We measured whole-cell $I_{Na}$ in acutely isolated ventricular and atrial myocytes. Null ventricular myocytes showed a reduction in total $I_{Na}$ density over a voltage range of −50 mV to −20 mV (Figure 1A) and steeper slope (κ) of the activation curve compared with WT, indicating increased sensitivity to changes in voltage (Figure 1B). There were no differences in the voltage dependence of activation or inactivation, kinetics of recovery from inactivation, persistent $I_{Na}$ density, or kinetics of steady-state inactivation between genotypes (Table 1). In contrast to ventricular myocytes, null atrial myocytes showed no changes in $I_{Na}$ or persistent $I_{Na}$ density, voltage dependence of activation, or inactivation between genotypes, suggesting that the role of β2 in heart is region specific (Figure 1C through 1D; Table 2). qRT-PCR (Figure 1E) and Western blotting (Figure 1F) showed no differences in Scn5a mRNA or Na1.5 protein expression.
in the ventricle, respectively, between genotypes, suggesting that changes in $I_{Na}$ density in null ventricle are because of defective VGSC targeting to the plasma membrane, similar to that observed in neurons.8,9

VGSCs are differentially localized to subcellular domains in cardiac myocytes.7,14 We performed super-resolution scanning patch clamp recordings of $I_{Na}$ at the T-tubules and cell crests of ventricular myocytes13 to investigate the changes in the subcellular distribution of VGSCs in response to $Scn2b$ deletion. No differences were apparent between genotypes (Figure I in the Data Supplement), suggesting that, in agreement with immunofluorescence data,14 $I_{Na}$ in null ventricle are most affected at the intercalated discs. Alternatively, it is possible that channel openings at both sites may be less frequent events in the null myocytes.

Scn2b Null Hearts Have Impaired Impulse Propagation in the Right Ventricular Outflow Tract Region

We performed optical mapping of ex vivo. Langendorff perfused hearts to investigate the effects of $Scn2b$ deletion on cardiac conduction (Figure 2). Slowed conduction was observed in the null right ventricular outflow tract (RVOT), but not in the RV region, compared with WT (Figure 2A through 2C). To examine excitability changes in this region more closely, we performed current clamp recordings of APs in RVOT myocytes. AP amplitude, maximal upstroke velocity, and resting membrane potential were not statistically different between genotypes (Figure IIA through IIC in the Data Supplement; WT: $N=8$, $n=10–14$; $Scn2b$ null: $N=5$, $n=11–16$).

Action potential durations (APDs) at early phases ($APD_{30–50}$) were unchanged between genotypes (Figure IIB). In contrast, late-phase APDs were prolonged in null myocytes (Figure IIC; $P<0.05$, null: $n=12$, $N=6$; WT: $n=9$, $N=6$, $MRE$ analysis). We recorded $I_{K,slow}$ density from RVOT myocytes to ask whether alterations in repolarizing currents may underlie the prolongation of late-phase APD in the nulls (Figure 3E). The decay phase of the current was consistent with the prolongation of late-phase APD, we observed a significantly higher incidence of early after depolarizations $\text{APD}_{70–90}$ showed significant prolongation in null myocytes (9/21 cells) compared with WT (1/15 cells; $P<0.05$, null: $n=12$, $N=6$; WT: $n=9$, $N=6$, $MRE$ analysis). No change in atrial $I_{Na}$ density between genotypes ($P=0.07–0.92$) over a voltage range from $−100$ to $5$ mV, null: $n=15$, $N=9$; WT: $n=12$, $N=3$, $MRE$ analysis). $Scn2b$ deletion had no effect on the voltage dependence of activation ($P=0.15$) or slope factor $\chi$ ($P=0.56$; null: $n=15$, $N=3$; WT: $n=12$, $N=3$, $MRE$ analysis). $Scn2b$ deletion had no effect on the voltage dependence of activation ($P=0.005$, null: $n=12$, $N=6$; WT: $n=9$, $N=6$, $MRE$ analysis) but decreased the slope factor $\chi$ ($P=0.05$, null: $n=12$, $N=6$; WT: $n=9$, $N=6$, $MRE$ analysis).}

$Scn2b$ Deletion Results in Impaired Steady-State $K^+$ Current in Ventricular Myocytes

We recorded $I_{K}$ density from RVOT myocytes to ask whether alterations in repolarizing currents may underlie prolongation of late-phase APD in the nulls (Figure 3E and 3F). We observed no differences in $I_{K,peak}$ density between genotypes (Figure 3E). The decay phase of the current was fit with a biexponential function to assess the contributions of slowly inactivating $I_{K,slow}$ and transient outward current $I_{to}$.16 There were no differences between genotypes for $I_{K,slow}$ or $I_{to}$ density (Figure 3E). There were no differences between genotypes in the time constants of inactivation for...
Role of Sodium Channel β2-Subunits in Heart

### Table 1. Ventricular $I_{Na}$ Biophysical Properties

<table>
<thead>
<tr>
<th></th>
<th>WT, N=6</th>
<th>Scn2b Null, N=6</th>
<th>P Value</th>
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<tbody>
<tr>
<td><strong>Voltage dependence of activation</strong></td>
<td></td>
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<tr>
<td>$V_{1/2}$ (mV)</td>
<td>−55.9 (-60.66 to −51.12)</td>
<td>−61.9 (-66.26 to −57.53)</td>
<td>0.065</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>6.2 (5.4–7)</td>
<td>4.5 (3.8–5.2)*</td>
<td>0.005</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>12</td>
<td></td>
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<tr>
<td><strong>Voltage dependence of inactivation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{1/2}$ (mV)</td>
<td>−91.2 (-96.25 to −86.2)</td>
<td>−92.4 (-96.7 to −88)</td>
<td>0.712</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>−6 (-643 to −5.58)</td>
<td>−6.1 (-6.5 to −5.76)</td>
<td>0.632</td>
</tr>
<tr>
<td>C</td>
<td>0.03 (0.008–0.058)</td>
<td>0.02 (0.01–0.042)</td>
<td>0.390</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>12</td>
<td></td>
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<tr>
<td><strong>Kinetics of inactivation</strong></td>
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<tr>
<td>$\tau_{inact}$ (ms)</td>
<td>5.2 (3.32–7.13)</td>
<td>4.42 (2.54–6.3)</td>
<td>0.516</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>$\tau_{fast}$ (ms)</td>
<td>1.96 (1.39–2.53)</td>
<td>1.59 (1.07–2.11)</td>
<td>0.317</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td><strong>Steady-state persistent current</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of peak current</td>
<td>3.9 (2.8–4.9)</td>
<td>2.7 (1.7–3.7)</td>
<td>0.100</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td><strong>Kinetics of recovery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\kappa$, ms</td>
<td>10.4 (7.33–13.4)</td>
<td>8.6 (5.84–11.3)</td>
<td>0.344</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>10</td>
<td></td>
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</table>

Data are expressed as mean (95% confidence interval). Kinetics of inactivation were recorded at a test potential of −45 mV. Persistent $I_{Na}$ was recorded at a test potential −45 mV, 50 to 52 ms after the voltage step. n indicates number of cells tested; N, number of animals tested; and WT, wild type.

*Significance determined by Mixed Effects Regression analysis with P<0.05.

### Scn2b Null Ventricles Are Arrhythmogenic

To assess the susceptibility of null ventricles to arrhythmia, we performed optical mapping with simultaneous, volume-conducted ECGs in isolated hearts (Figure 4). Episodes of spontaneous, nonsustained ventricular tachycardia were observed in 3 of 10 null hearts, and premature ventricular complexes (PVCs) were observed in 4 of 10 null hearts, but none in the 11 WT hearts tested (Figure 4A). Figure 4B and 4C shows anatomic views of the heart during the optical mapping and epicardial activation during a sinus beat, respectively. As expected, during the sinus beat, 2 breakthroughs appeared on the LV and RV free wall close to the apex and the excitation wavefront then propagated toward the base of the heart. All spontaneous aberrant rhythm events appeared with a PVC within an average of 25 to 26 minutes after cannulation and before pacing or administration of drugs (Figure 4D). Episodes of spontaneous nonsustained ventricular tachycardia (VT) were recorded, and phase maps were generated to analyze activation patterns during tachycardia (Figure 4D through 4F). Rotors were observed (Movie I in the Data Supplement), implicating functional reentry as the underlying mechanism of arrhythmia in null hearts. Singularity points, the organizing centers of each rotor, were concentrated in the null RVOT (Figure 4E), suggesting that wavebreaks were more likely to occur in this area. Rotors were not confined to the RVOT but observed to meander to the left and right anterior ventricular free walls over time (Figure 4F), giving rise to the polymorphic appearance of the ECG (Figure 4D). Closely coupled PVCs served as triggers for nonsustained ventricular tachycardia initiation in null hearts. Chronological snapshots taken from a phase movie of a null heart, capturing initiation of an episode of spontaneous polymorphic VT, illustrate the interplay between a PVC and the arrhythmogenic substrate leading to arrhythmia (Figure III in the Data Supplement). During the sinus beat preceding VT, the epicardium was homogeneously activated from apex to base (Figure IIIA in the Data Supplement). A PVC then emerged (Figure IIIB and IIIC in the Data Supplement) as an epicardial breakthrough on the anterior RV free wall near the apex. This premature impulse traveled to the RVOT and was blocked, likely because of prolonged APD and maintenance of refractoriness (Figure IIIC and IIID in the Data Supplement). The impulse then detoured to the LV, circumventing the RVOT. When the RVOT was finally activated, because of slowed impulse conduction within this region, the

### Table 2. Atrial $I_{Na}$ Biophysical Properties

<table>
<thead>
<tr>
<th></th>
<th>WT, N=3</th>
<th>Scn2b Null, N=3</th>
<th>P Value</th>
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</thead>
<tbody>
<tr>
<td><strong>Peak $I_{Na}$ density, pA/pF</strong></td>
<td></td>
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</tr>
<tr>
<td>−54.8 (−80.1 to −29.6)</td>
<td>−59.7 (−83.4 to −36)</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>mV of Peak $I_{Na}$</td>
<td>−46.7±2.2</td>
<td>−50.7±5.2</td>
<td>0.23</td>
</tr>
<tr>
<td><strong>Persistent $I_{Na}$ density at −45 mV, pA/pF</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>−2.14 (−3.11 to −1.2)</td>
<td>−1.88 (−2.7 to −1)</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Voltage dependence of activation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{Na}$ (mV)</td>
<td>−59.4 (−64 to −54.8)</td>
<td>−64.7 (−68.8 to −60.5)</td>
<td>0.15</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>4.19 (3.37–5)</td>
<td>3.8 (3.11–4.6)</td>
<td>0.56</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>Voltage dependence of inactivation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$V_{Na}$ (mV)</td>
<td>−91.8 (−97.3 to −86.3)</td>
<td>−93.9 (−99.2 to −88.7)</td>
<td>0.59</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>−6.9 (−7.6 to −6.2)</td>
<td>−7.4 (−8.1 to −6.7)</td>
<td>0.39</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>15</td>
<td></td>
</tr>
</tbody>
</table>

Data are expressed as mean (95% confidence interval). $P$ values were determined with Mixed Effects Regression analysis or Student t test (for mV of peak $I_{Na}$ with $P<0.05$). Kinetics of inactivation were recorded at a test potential of −45 mV. Persistent $I_{Na}$ density was recorded at a test potential −45 mV, 50 to 52 ms after the voltage step. n indicates number of cells tested; N, number of animals tested; pA/pF; pico-amps per pico-farad; and WT, wild type.
Conduction velocity is decreased in the null right ventricular outflow tract (RVOT). A, Conduction velocity is decreased in the RVOT but not RV free wall (null: N=9; wild type [WT]: N=11) paced at cycle lengths (CLs) of 100 ms \( P=0.011 \); 125 ms, \( P=0.032 \); 150 ms *\( P=0.015 \); 175 ms, \( P=0.012 \); Student \( t \) test or Mann–Whitney rank-sum test where applicable. B, Langendorff heart prep during optical mapping. Box: region of measurement. C, Representative activation maps from null and WT at 150 ms CL. White lines: isochrone lines. Numbers indicate time in ms. Isochrone lines show conduction slowing in the null RVOT. N indicates number of animals tested.
Figure 3. Action potential (AP) and potassium current ($I_K$) in single right ventricular outflow tract (RVOT) myocytes. A, Late phase action potential duration values (APD$_{70–90}$) were prolonged in nulls (wild type [WT]: n=10–14, N=8; null: n=12–16, N=5; *$P$=0.01–0.04, as indicated, Mixed Effects Regression [MRE] analysis). WT: black. Null: red. B, Representative AP traces. WT: black. Null: red. The final phase of the null AP is prolonged compared to WT. C, Null myocytes exhibited a higher incidence of early after depolarizations (EADs) than WT ($P$=0.024, Fisher exact test). D, Representative null EAD trace paced at 4Hz. Null myocytes have normal $I_{K,peak}$, $I_{K,slow}$ and $I_{to}$ densities. Current ($I$)–voltage ($V$) curves for $I_{K,peak}$ (left), $I_{K,slow}$ (center), and $I_{to}$ (right), measured in RVOT myocytes. Peak current at +40 mV for (Continued)
rest of the heart had finished repolarizing and was ready for re-excitation. As a result, the excitation wavefront turned at the RVOT and initiated a rotor (Figure III E and IIIF in the Data Supplement).

**Scn2b Null Mice Have a Normal Cardiac Conduction System**

We performed intracardiac recordings to measure the conduction time for each segment along the conduction

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**Figure 3 Continued.** $I_{\text{K,peak}}$ (WT: 33.35 [24.46–42.24] pA/pF, n=13, N=5; null: 32.43 [22.98–41.88] pA/pF, n=10, N=6; $P=0.88$), $I_{\text{K,slow}}$ at +40 mV (WT: 7.96 [4.15–11.77], n=13, N=5; null: 10.81 [6.98–14.64] pA/pF, n=10, N=6; $P=0.28$), $I_{\text{K,SS}}$ at +40 mV (WT: 12.18 [8–16.36], n=13, N=5; null: 12.35 [7.8–16.9] pA/pF, n=10, N=6; $P=0.95$). WT: black. Null: red. F. Scn2b null myocytes have normal $I_{\text{K,peak}}$ density and reduced $I_{\text{K,SS}}$ density.

Left. No differences in inward rectifying current ($I_{\text{K1}}$) between null (red) and WT (black) RVOT myocytes. Center. Bar graph of $I_{\text{K1}}$ at −120 mV (WT: −6.47 [−9.35 to −3.58], n=11, N=5; null: −8.24 [−10.89 to −5.59] pA/pF, n=12, N=6; $P=0.35$). Right. $I_{\text{K,SS}}$ at +20 mV (WT: 10.60 [7.47–13.73], n=13, N=5; null: 5.70 [2.50–8.91] pA/pF, n=10, N=6; $P=0.04$), at +30 mV (WT: 12.54 [8.91–16.17], n=13, N=5; null: 7.02 [3.41–10.64] pA/pF, n=10, N=6; $P=0.04$), and at +40 mV (WT: 14.13 [9.77–18.49], n=13, N=5; null: 8.38 [4.02–12.62] pA/pF, n=10, N=6; $P=0.07$). WT: black. Null: red. Results in E and F are reported as mean (95% confidence interval), with significance (*) determined using MRE analysis.

**Figure 4.** Arrhythmic events captured in Scn2b null hearts by optical mapping. A. Incidence of premature ventricular complex (PVC) and ventricular tachycardia (VT) in wild type (WT; 0/11) vs null (4/10 and 3/10, respectively; $P=0.02$ and $P=0.09$, respectively, Fisher exact test). B, Anatomic view of the heart during optical mapping. C, Activation map of a sinus beat. Normal epicardial activation during the sinus beat had 2 breakthroughs on the left ventricular (LV) and right ventricular (RV) free wall close to the apex and then the excitation wavefront (Continued)
pathway and to assess the electrophysiological properties of the sinoatrial node and atrioventricular node. Atrial-His conduction time, His-ventricular conduction time, sinoatrial node recovery time, atrioventricular node refractoriness, and Wenckebach periodicity were similar between genotypes (Table 3), suggesting that the conduction system is intact in Scn2b null hearts.

Scn2b Null Mice Have Increased Susceptibility to AF In Vivo
AF can be reproducibly induced in the mouse heart by a combination of endocardial pacing and administration of the cholinergic agonist, carbachol. We applied burst pacing before and after carbachol administration to test whether null hearts are prone to atrial arrhythmias. The efficacy of carbachol was assessed by the observation of decreased heart rate after drug administration. We defined AF as episodes of 1 s or longer in duration, before or after carbachol administration, during burst pacing or the S1–S2 protocol, in which the heart is paced with a train of pulses (S1) followed by a premature stimulus (S2). Using these criteria, we found that AF susceptibility was higher in null (7/9) compared with WT (1/7; \( P=0.02 \), Figure 5A). Durations of induced AF were variable in null mice, ranging from just over 1 s to 11 minutes (Figure 5B). In contrast, the majority of AF episodes in WT mice had durations of \(<1\) s (not shown). Figure 5C shows a representative surface ECG lead-II trace and atrial electrogram acquired during AF/AT in a null mouse. This is compared with traces from a WT mouse that was resistant to induction of tachyarrhythmia.

Reentry Underlies the Mechanism of AF in Scn2b Null Atria
To investigate the mechanism of atrial arrhythmia in null hearts, we performed ex vivo optical mapping (Figure 6A). Consistent with in vivo observations, we induced AF by burst pacing in 5 of 11 null atria compared with 1 of 10 WT atria in the absence of carbachol (\( P=0.073 \)). As expected from previous work,, carbachol administration increased the level of AF induction in both genotypes (6/11 Scn2b null versus 3/10 WT, \( P=0.39 \)). Phase movies recorded during AF in null atria showed variable forms of rotors, which served as drivers to maintain the arrhythmia. Figure 6B and Movie II in the Data Supplement demonstrate AF driven by a single rotor in the RA. Figure 6C and Movies III and IV in the Data Supplement show AF driven by 2 rotors in the figure-of-eight configuration (2 counterclockwise rotating rotors that share a common pathway) in the RA in the absence of carbachol and then driven by a single rotor in the pulmonary vein region of the left atria after carbachol administration. Figure 6D and Movie V in the Data Supplement show AF driven by 3 independent rotors at different dominant frequencies (17.7 and 26.5 Hz, respectively). One rotor was located in the RA, whereas the other 2, with figure-of-eight configuration at the higher common frequency, were located in the pulmonary vein region. In this case, the AF terminated and then was spontaneously reinitiated by a sinus beat because of wavebreak in the RA. This reinitiated AF was sustained by a single rotor in the RA (Figure 6D). Spontaneous AF was also observed in 1 null atrium but not in any of the WT atria, suggesting higher atrial arrhythmogenicity in the mutant animals (Figure IV in the Data Supplement). Taken together, the dynamic, complex, and nonhierarchical organization of rotors in Scn2b null atria suggests that the substrates for AF/AT may be functionally and anatomically heterogeneous.

APD Is Heterogeneously Prolonged in Scn2b Null Atrium
In contrast to the RVOT, we observed no changes in atrial conduction velocity between genotypes (Figure V in the

---

**Table 3. Intracardiac Electrophysiological Values**

<table>
<thead>
<tr>
<th>Value</th>
<th>WT</th>
<th>Scn2b Null</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH interval, ms</td>
<td>27.2±2</td>
<td>27.8±2.5</td>
<td>0.87</td>
</tr>
<tr>
<td>HV interval, ms</td>
<td>9.5±0.5</td>
<td>10.4±0.6</td>
<td>0.31</td>
</tr>
<tr>
<td>SNRT90, ms</td>
<td>N=5</td>
<td>N=7</td>
<td></td>
</tr>
<tr>
<td>SNRT100, ms</td>
<td>74.9±11.9</td>
<td>62.4±11</td>
<td>0.23</td>
</tr>
<tr>
<td>AVERP80, ms</td>
<td>106±9.9</td>
<td>83.1±14.3</td>
<td>0.22</td>
</tr>
<tr>
<td>AVERP90, ms</td>
<td>47.6±2.6</td>
<td>48.5±2.3</td>
<td>0.79</td>
</tr>
<tr>
<td>AVERP100, ms</td>
<td>N=7</td>
<td>N=8</td>
<td></td>
</tr>
<tr>
<td>AERP90, ms</td>
<td>58.1±2.2</td>
<td>58.6±2</td>
<td>0.89</td>
</tr>
<tr>
<td>AERP100, ms</td>
<td>26.3±2.4</td>
<td>22.3±1.8</td>
<td>0.2</td>
</tr>
<tr>
<td>AERP115, ms</td>
<td>19.8±1.1</td>
<td>19.4±1</td>
<td>0.78</td>
</tr>
<tr>
<td>N=7</td>
<td>N=7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WP, ms</td>
<td>72.6±2.1</td>
<td>73.3±1.1</td>
<td>0.77</td>
</tr>
<tr>
<td>WP11, ms</td>
<td>53.7±2.1</td>
<td>52.2±1.6</td>
<td>0.59</td>
</tr>
<tr>
<td>N=7</td>
<td>N=8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VERP80, ms</td>
<td>45.2±2.5</td>
<td>38.7±3</td>
<td>0.13</td>
</tr>
<tr>
<td>N=6</td>
<td>N=6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VERP100, ms</td>
<td>42.5±3.2</td>
<td>39.2±3.2</td>
<td>0.48</td>
</tr>
<tr>
<td>N=4</td>
<td>N=5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means±SE. \( P \) values were determined using Student t test and Mann–Whitney rank-sum test. AERP indicates right atrial effective refractory period; AH, interval from atrial to His-signal; AVERP, atrial-ventricular effective refractory period; HV, interval from His to first QRS movement in surface ECG; N, number of mice; SNRT, sinus node recovery time; VERP, right ventricular effective refractory period; WP, Wenckebach periodicity; and WP111, Wenckebach periodicity at 2:1 conduction. Subscripts indicate S1S1 drive cycle lengths.
Data Supplement). AP recordings from single RA myocytes showed no differences in amplitude, maximum upstroke velocity, or resting membrane potential between groups (Figure 7A). We observed a significant increase in the later phases of the APD (APD_{50-90}) in null myocytes compared with WT at the highest pacing frequency tested (Figure 7B). Figure 7C shows representative, superimposed AP waveforms from each genotype to illustrate the AP prolongation in null atrial myocytes compared with WT. In Figure 7D, we plot the range of APD values for atrial myocytes of each genotype to illustrate the degree of heterogeneity in the null atrial data set. For comparison, Figure VI in the Data Supplement shows the range of APD values for ventricular myocytes of each genotype. We propose that this dispersion of repolarization in atrial myocytes generates functionally heterogeneous substrates in the null atrium that predispose the tissue to wavebreak during fast pacing.

Increased Fibrosis in *Scn2b* Null Right Atrium
Evidence connects cardiac VGSC dysfunction to fibrosis.\textsuperscript{21,23} To assess the presence of fibrosis in the null model, the posterior walls of the RA and left atria were sectioned in the coronary plane parallel to the posterior wall and stained using Masson trichrome protocol. We observed increased levels of fibrosis in the null RA, but not the left atria, compared with WT (Figure 8A and 8B). We propose that fibrotic tissue deposited in the null RA interstitial space creates anatomic substrates for wavebreaks and anchor points for rotors and may explain transitions from AF to atrial flutter in the null animals (eg, Figure 8C).

Discussion
This study presents the first investigation of the effects of *Scn2b* deletion on cardiac electrophysiology in mice. Loss of VGSC β2 results in reduction of $I_{\text{Na}}$ density because of...
impaired channel cell surface expression over a defined voltage range and reduction of $I_{K,SS}$ density in ventricular myocytes, with subsequent slowing of conduction in the RVOT. Slowed conduction and prolonged repolarization in the RVOT interact with PVCs to initiate functional reentry, increasing the susceptibility of null hearts to ventricular arrhythmia. In contrast to the ventricle, atrial $I_{Na}$ density and biophysical properties were not different between genotypes,
Figure 7. Action potential (AP) recordings from right atrial myocytes. A, No differences between genotypes in AP amplitude, AP maximum upstroke velocity, or resting membrane potential (RMP). Wild type (WT): black. Null: red. B, APD_{50-90} was prolonged in the nulls at a pacing frequency of 5 Hz (*P<0.05, Mixed Effects Regression analysis). C, Representative superimposed AP traces from null and WT myocytes. D, Distribution of the APD_{90} data paced at 5 Hz from atrial myocytes. APD lengthening in atrial myocytes is heterogeneous, resulting in a dispersed distribution of the data set (null: n=13, N=4; WT: n=12, N=5, Student t test). WT: black. Null: red. Blue: mean. APD indicates action potential duration; n, number of cells tested; and N, number of animals tested.
Figure 8. Increased fibrosis in Scn2b null right atrium. A, Masson trichrome staining shows increased fibrosis (blue) in null right atria (RA) but not left atria (LA) compared with wild type (WT). B, Quantification of fibrosis ($P=0.002$, Mann-Whitney rank-sum test). N=6 per genotype. C, Transition of atrial fibrillation to atrial flutter in null atrium. Increased levels of fibrosis are proposed to provide anchoring points for rotors underlying the transition.
demonstrating region-specific functionality of β2 in the heart. Finally, null mice are more susceptible to AF/AT and have increased atrial fibrosis compared with WT. We propose that increased functional and anatomic heterogeneity in the atrium because of repolarization dispersion and fibrosis contribute to the mechanism of atrial arrhythmia in Scn2b null hearts.

**Scn2b Null Mice May Mimic Some Aspects of BrS**
BrS is a rare cardiac disease that results in increased risk of ventricular fibrillation and sudden cardiac death. Approximately 35% of BrS cases are attributable to known pathogenic gene variants, with ≈30% of these resulting from loss of $I_{Na}$. The RVOT is the most affected region in human heart in BrS. Similarly, Scn2b null mice have reduced $I_{Na}$ density throughout the ventricle with decreased impulse propagation in the RVOT. Although the mechanism for selective propagation slowing in the RVOT here and in human BrS is not completely understood, previous work has shown that Scn5a mRNA and Na 1.5 protein levels are reduced in the WT mouse RVOT compared with the rest of the RV. This normally occurring lowered conduction reserve is proposed to result in greater conduction slowing in the RVOT under conditions of reduced cardiac $I_{Na}$. For example, administration of VGSC blocking agents or the presence of loss-of-function VGSC gene mutations, increasing the susceptibility for arrhythmias. We propose that reductions in functional VGSC expression in Scn2b null ventricle result in larger decrements in conduction reserve in the RVOT compared with the remainder of the RV. In addition to aberrant depolarization, prolongation of repolarization is observed in the RVOT of BrS patients. We observed reduced $I_{Kss}$ density and prolongation of the APD in Scn2b null RVOT myocytes, results that are functionally consistent with clinical findings.

**PVCs and Arrhythmogenesis in Scn2b Null Hearts**
PVCs are proposed to serve as triggers for arrhythmogenesis. Trigger elimination, by focal ablation of PVCs originating from the RVOT, is effective in preventing arrhythmic events in patients. The timing of emergence and spatial orientation of the excitation wavefronts of ectopic beats are key factors in arrhythmia initiation. In Scn5a−/− mice, PVCs play important roles in initiating reentrant, spontaneous polymorphic VT. In Scn2b null hearts, every recorded VT episode occurred after a PVC, suggesting a requirement for PVCs to serve as triggers. In addition, epicardial breakthrough of the initiating PVC was observed at the RV free wall near the apex rather than at the RVOT. We found no differences in the coupling interval between VT-initiating PVCs and PVCs that did not trigger VT (Figure VII in the Data Supplement). Because the morphologies of these PVC types were similar, we propose that the origins of the PVCs were focal. Thus, the subsequent occurrence of VT was dependent on the repolarizing state of the preceding sinus beat, in which case the repolarization or refractoriness was dynamic.

**SCN2B and AF**
AF is the most prevalent clinical arrhythmia, with even higher incidence in BrS compared with the general population. Both loss- and gain-of-function mutations in SCN5A have been linked to familial AF. The 2 reported AF patients with SCN2B mutations exhibited saddleback-type ST-segment elevation in the right precordial leads, suggesting that these individuals may have also experienced BrS-like symptoms. Here, Scn2b deletion resulted in effects on atrial AP repolarization and tissue remodeling, but not atrial $I_{Na}$. The APD was increased in a heterogeneous fashion in Scn2b null atrium, resulting in larger repolarization dispersion. In addition, higher levels of fibrosis were found preferentially in Scn2b null RA. These changes are proposed to provide electric and anatomic substrates that favor AF. The notion of increased heterogeneity of Scn2b null atrium is supported by our finding that the number, frequency, location, and organization of rotors are highly dynamic and complex.

Increased fibrosis in Scn2b null atrium is consistent with reported links between VGSC dysfunction and fibrosis in the heart. For example, transforming growth factor-β1-mediated fibrosis is triggered by Scn5a disruption. In other studies, relaxin suppressed AF in aged rats and in spontaneously hypertensive rats through decreased atrial fibrosis, increased $I_{Na}$, and increased atrial conduction velocity. As an alternative hypothesis, fibrosis in null mice may be triggered directly by the absence of β2 polypeptides during atrial development. β2-subunits are substrates for sequential cleavage by BACE-1 and γ-secretase. The cleaved intracellular domain of β2, at least in neurons, translocates to the nucleus to participate in transcriptional regulation of VGSC α-subunits and possibly other genes. Thus, the cleaved β2 intracellular domain may normally regulate genes that inhibit fibrosis in the atrium, resulting in uncontrolled fibrosis in Scn2b null hearts.

In summary, Scn2b null mice may be useful for modeling aspects of human ventricular arrhythmias that exhibit increased susceptibility to atrial arrhythmia. This study provides insight into the role of VGSC β2-subunits in maintaining normal cardiac electric activity and provides a novel perspective on the connection between ventricular and atrial arrhythmias in patients who carry VGSC gene mutations.

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**Disclosures**
None.

**References**
Role of Sodium Channel β-Subunits in Heart


Scn2b Deletion in Mice Results in Ventricular and Atrial Arrhythmias


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SUPPLEMENTAL MATERIAL

Detailed Methods

Animals

Male and female Scn2b null and wildtype (WT) mice, congenic on the C57BL/6J background for over 20 generations, were used at the ages indicated in the methods sub-sections. Mouse genotyping was described previously.

Cell isolation

Ventricular myocyte isolation for $I_{Na}$ recording

WT and Scn2b null cardiac myocytes were acutely isolated from 2-3 month old mice of both genders using a protocol described in Auerbach et al. In brief, hearts were isolated and placed in ice-cold perfusion buffer. The hearts were cannulated and cleared with perfusion buffer (37°C, 3 ml/min). Type-II collagenase (0.87 mg/ml, Worthington Biochemical), trypsin (0.14 mg/ml), and 12 μM CaCl2 were added to the perfusion buffer for enzymatic digestion. Hearts were then continuously perfused at 37°C in digestion buffer for 3 min. The lower two-thirds of the ventricle or the RVOT region (see below) were isolated and minced into small pieces. Stopping buffer, which included perfusion buffer plus 10% fetal bovine serum and 12.5 μM CaCl2, were then added to stop the reaction. A final concentration of 1mM CaCl2 was achieved by incrementally adding CaCl2 every 4 minutes at 37 °C. Rod shaped, striated, and quiescent myocytes were chosen for electrophysiological recordings.
Right ventricular outflow track (RVOT) myocyte isolation

RVOT myocyte isolation was similar to that described above. However, instead of isolating the lower two-thirds of the heart, the RVOT anterior free wall corresponding to the slowed conduction area in optical mapping (see below), were cut from 2-4 month old male mice. Tissues were minced in digestion buffer followed by steps similar to those described above.

Atrial myocyte isolation

This procedure was similar to the ventricular myocyte isolation described above but with the following changes: 1.25g/25ml Liberase TM (Roche) was added to the digestion buffer during perfusion. Digestion time was modified to 12-18 min. 2-3 month old mice of both genders were used. To minimize the known AP heterogeneity between left and right atria\(^3\), only the right atrial region to the right of the crista terminalis was isolated and minced.

Single Cell Electrophysiology

Standard voltage and current clamp techniques were used to assess the effects of Scn2b deletion on cardiac \(I_{\text{Na}}\) and AP properties, respectively. Single cell cardiac electrophysiological properties were acquired from healthy cardiac myocytes. Borosilicate glass pipettes with resistance of <3MΩ for \(I_{\text{Na}}\) and 3-5 MΩ for AP recordings were used. Data were acquired using an Axopatch 200B amplifier and Axopatch 700B (Molecular Devices, USA). The data were acquired and analyzed using pCLAMP9-10.
(Molecular Devices, USA) and custom AP analysis software (National Instruments LabView, USA)

**Voltage Clamp Recordings**

Voltage clamp $I_{\text{Na}}$ recordings were performed at room temperature (21–22°C). The extracellular solution contained (in mM): 5 NaCl, 1 MgCl$_2$, 1 CaCl$_2$, 0.1 CdCl$_2$, 11 Glucose, 132.5 CsCl, and 20 HEPES. The filling solution contained (in mM): 5 NaCl, 135 CsF, 10 EGTA, 5 MgATP, and 5 HEPES. Upon gaining access to the cell, appropriate whole cell and series resistance compensation (<75%) and leak subtraction were applied. Assessment of transient and persistent $I_{\text{Na}}$ density, $I_{\text{Na}}$ inactivation time, and the voltage dependence of $I_{\text{Na}}$ conductance were obtained by holding the cell at -120 mV, followed by stepping to voltages between -100 and +30 mV, in 5 mV steps, for 200 ms, with 2800 ms interpulse intervals. The voltage dependence of $I_{\text{Na}}$ availability was determined by holding at various voltages (-160 mV to 0 mV, 5 mV increments, 200 ms duration) and stepping to -40 mV (30 ms), with 2770 ms interpulse intervals at -120 mV. The normalized voltage dependence of $I_{\text{Na}}$ availability and conductance (based upon each cells’ reversal potential) were fit to a Boltzmann function, and differences in the $V_{1/2}$ and slope factor were compared between groups. The time dependence of $I_{\text{Na}}$ recovery was assessed by holding at -120 mV and stepping to -30 mV for 20 ms (P1), followed by a 1–40 ms (1 ms increments) interpulse interval at -120 mV, and a second step to -30 mV for 20 ms (P2). The time dependence of $I_{\text{Na}}$ recovery was calculated by $P2/P1$ at each time point, and these results were fit to a single exponential function. The
rate of $I_{\text{Na}}$ inactivation was fit to a double exponential function. Persistent $I_{\text{Na}}$, measured 50-52 ms after the voltage step, when the current amplitude was stable.

Voltage clamp $I_{\text{K}}$ recordings were performed room temperature (21–22°C). Myocytes isolated from the right ventricular outflow tract (RVOT) region were used within 8 h of dissociation. Myocytes were bathed in a solution containing (in mM): 136 NaCl, 4 KCl, 2 MgCl$_2$, 1 CaCl$_2$, 10 HEPES, 10 Glucose, 0.02 TTX, 0.5 CdCl$_2$ (pH 7.4; 296 mOsm). Pipettes with resistance of 2-4 mOhms were filled with a solution containing (in mM): 135 KCl, 5 K$_2$ATP, 10 EGTA, 10 HEPES, 5 glucose (pH 7.2; 306 mOsm). $I_{\text{K}}$ were measured in 4.5 s depolarizing 10 mV steps from -60 to +40 mV from a holding potential of -80 mV. The decay phase of the current was fit to a two-phase exponential equation to measure $I_{\text{to,f}}$, $I_{\text{K,slow}}$, and $I_{\text{K,ss}}$, respectively. $I_{\text{K1}}$ was measured as the current at 350 ms post-pulse between -40 and -120 mV. Currents were normalized to cell capacitance.

**Current Clamp Recordings**

Current clamp recordings of APs were acquired at 37°C in standard Tyrodes solution (in mM): 148 NaCl, 0.4 NaH$_2$PO$_4$, 1 MgCl$_2$, 5.4 KCl, 1 CaCl$_2$, 5.5 glucose, 15 HEPES. The internal solution included (in mM): 140 KCl, 5 EGTA-KOH, 10 HEPES. Incremental amounts of current (100 pA steps, 1 ms) were used to map the threshold of current to evoke an AP. Then, 1.5-fold of that current was used for AP recordings at different pacing cycle lengths (200-1000 ms). Only cells with a diastolic membrane potential more negative than -65 mV were used for analysis.
Quantitative (q)RT-PCR

Total RNA was extracted from 6 Scn2b null and 6 WT left and right ventricles at 3 months of age using Trizol reagent (Life Technologies). Each sample was assayed in triplicate. 1 µg of RNA from each sample was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Transcripts of Scn5a were measured with Taqman assay Mm00451971 (Applied Biosystems) using a 7900HT fast real time PCR machine with sequence Detection system 2.2 software (Applied Biosystems). Transcripts of GAPDH were measured with Taqman assay Mm99999915 (Applied Biosystems) and served as an internal control. Negative control samples produced no detectable signals. DCt was calculated as cycles to reach threshold for the GAPDH transcript minus cycles to reach threshold for the Scn5a transcript.

Western Blotting

Left and right ventricle membrane proteins were prepared from 4 Scn2b null and 4 WT mice at 3 months of age as previously described. 50 µg of protein were separated on 7.5% SDS-PAGE gels and transferred to PVDF membrane (Immobilon-P, Millipore). The blot was probed with anti-Na\textsubscript{v}1.5 antibody (Cell Signaling Technology #14421) followed by detection with ECL Select Western Blotting Detection Reagent (GE Healthcare). Anti-α-actin antibody (Sigma) was used as a loading control. Na\textsubscript{v}1.5 immunoreactive bands were digitized and normalized to the corresponding loading control using ImageJ software.
**Super-resolution scanning patch clamp**

This method combined scanning ion conductance microscopy (SICM) with cell-attached patch clamp technology for recording of ion channels at a particular subcellular location. A detailed description of this technique can be found in the previous work of Bhargava *et al* [4]. Briefly, after generating the topographical image of the cardiomyocyte surface with SICM, the pipette was moved to an area clear of cells or debris. At those coordinates, a custom-built program was used to clip the tip of the pipette against the bottom of the dish. The pipette resistance was continuously monitored and the clipping maneuver stopped once the current through the pipette reached the desired level. At that point, the pipette was repositioned to spatial coordinates that were selected based on the topography image recorded with the sharp pipette. The repositioned, clipped pipette was lowered to the predetermined subcellular location to record sodium channels in the cell-attached configuration.

**Optical Mapping**

*Ventricular mapping*

3-6 month old male mice were heparinized (0.5 U/g i.p.) and then euthanized. The heart was immediately excised and placed in ice-cold cardioplegic solution, then retrogradely perfused through the cannulated aorta with warm (36.8 ± 0.3 °C), oxygenated (100% O₂) Tyrodes solution containing (in mM) 130 NaCl, 1.2 NaH₂PO₄, 1.0 MgCl₂, 4.0 KCl, 1.8 CaCl₂, 5.6 glucose, and 25.0 HEPES, pH 7.4. The preparation was allowed to equilibrate for 20-30 min. Volume-conducted ECGs (pseudo lead 2) were acquired and digitized at 1 KHz (MiniDigi1A digitizer and AxoScope software; Axon Instruments).
preparation was then perfused with 7 µM Blebbistatin (Cayman Chemical), a mechanical uncoupler, to immobilize the heart. The heart was then stained with 100 µM Di-4-ANEPPS (Life Technologies), a voltage-sensitive fluorescent dye. High-resolution optical mapping studies were performed using an 80 × 80 pixel CCD camera (SciMeasure) coupled to a SMZ-1000 dissection microscope (Nikon), imaging a 6.4 × 6.4-mm area at 800 frames per second, providing a spatial resolution of 80 µm per pixel. The visualized area included the anterior ventricular epicardium (left ventricle, septum, right ventricle, and apex). Optical movies (4 s) were acquired under 532-nm fluorescence excitation (argon laser, 1 W) and 640 ± 20 nm emission. Optical movies were recorded at baseline and at progressively shorter basic cycle lengths (175 ms to 100 ms) during RV apex pacing. Analysis was performed using custom-made software. Briefly, movies were spatiotemporally filtered, background fluorescence was subtracted, and averaged activation maps were constructed under steady-state baseline and paced conditions. Phase maps were generated for arrhythmia visualization as described 5.

**Atrial Mapping**

This procedure was similar to ventricular mapping. 8-9 month old mice of both genders were used. Instead of cannulation, we excised the whole atrium along the AV rings (mitral and tricuspid), pinned the preparation to a sylgard gel coated dish and superfused the tissue with warm (36.8 ± 0.3 °C), oxygenated (100% O2) Tyrodes solution. The CCD camera was positioned to face the posterior wall of both atria. Optical movies were recorded at baseline and at progressively shorter basic cycle lengths (125 ms to 100 ms) during right atrium appendage pacing. Arrhythmia
inducibility was assessed by applying 2 atrial electric stimulations (bursting pacing at 66.67 HZ for 5 or 10 seconds) before and after the carbachol (Cch, 300 nM) administration.

**ECG and intracardiac recording**

Following the achievement of surgical anesthesia, mice were placed onto a temperature-regulated operating table. Platinum electrodes (Natus Neurology) were inserted subcutaneously in the limbs and connected to a DSI digital communication module and then to the DSI ACQ-7700 - acquisition interface (Data Sciences International) for a standard lead-II ECG. Standard ECG parameters (HR, P wave duration and PR interval, QRS duration, QT interval) were analyzed under stable baseline conditions with 1.5% vol. isofluorane. A 1.1 French octapolar stimulation-recording catheter (FTS-1113A-0518, Science) was inserted through the jugular vein and advanced into the right atrium and ventricle. Programmed electrical stimulation was assessed to determine the basal sinus node recovery time (SNRT) by applying S1S1 protocol at 120 ms and 90 ms of cycle length (CL), while the atrial refractory period (AERP), atrio-ventricular nodal effective refractory period (AVERP), and ventricular refractory period (VERP) were estimated by delivery of a S1-S1-S2 protocol with a fixed S1 (100ms and 80ms CL) and reducing S2 every 2 ms. Arrhythmia inducibility was assessed by apply 12-18 atrial bursts of pacing (50 HZ for 2 or 5 sec) before and after the i.p. carbachol (Cch, 50 ng/g) application. AF was defined as the occurrence of rapid and fragmented atrial electrograms (lack of regular P waves) with irregular AV-nodal
conduction and ventricular rhythm lasting at least 1 sec. The entire procedure was completed in less than 2 h.

**Assessment of Fibrosis**

Atria from 8-9 month old mice of each gender were collected for histology. Only posterior walls of each side were collected. For the right atrium, tissue that was to the right of the crista terminalis was excised to eliminate collection of SA nodal tissue. Tissues were fixed in 10% formalin and submitted to the University of Michigan Unit for Laboratory Animal Medicine In-vivo Animal Core facility for sectioning and further analysis using Masson’s Trichrome staining. Tissue sections were analyzed for the degree of fibrosis using Aperio® software. The color deconvolution algorithm was used to quantify the connective tissue component of each section. The slides were viewed on ImageScope®. The intensity of the blue staining was measured in areas outlined by a veterinary pathologist. The area and intensity of the staining were scored as weak, medium, or dark according to windows set by calibration of the slides using the algorithm. To account for differences in staining intensity and distribution between slides, a total score was derived from (3 x strong) + (2 x medium) + (1 x week) intensity, as recommended by the software manufacturer.

**Statistics**

Statistical significance was evaluated using Mixed Effects Regression analysis, Student’s T-test, Mann-Whitney rank sum test, or Fisher’s exact test, as indicated. P < 0.05 was considered significant. Calculations were performed using SigmaPlot 11.2
(Systat Software Inc, San Jose, California USA), GraphPad Prism 7 (GraphPad Software, Inc, San Diego, California), or R version 3.1.1 (Copyright (C) 2014 The R Foundation for Statistical Computing). Two packages were used in R (NLME: http://cran.r-project.org/pub/R/web/packages/nlme/citation.html; IME4: https://cran.r-project.org/web/packages/lme4/citation.html).
References:


Supplemental figures

**Fig. S1.** Scn2b deletion does not alter the distribution of $I_{Na}$ in the t-tubules or crest. Ordinates show percent of recordings (relative to total number of attempts). Abscissae show number of open channels detected. Number of open channels in each recorded patch was estimated from the average peak current amplitude during a voltage step to $-30$ mV from a holding potential of $-120$ mV, and an estimated unitary conductance of 10 pS. The number of separate recordings reported in each histogram is indicated in the top left of each panel. The pipette was placed on the opening of the T-tubules (top) or on the crest (bottom). Significance determined using Fisher's exact test.
**Fig. S2.** AP recordings from single RVOT myocytes. A. No differences between genotypes in AP amplitude when paced at 1-5 Hz (WT: N=8, n=10-14; Null: N=5, n=12-16). B. No differences between genotypes in AP maximum upstroke velocity (P=0.19-0.64 from 1-5Hz; WT: N=8, n=10-14; Null: N=5, n=11-16). C. No differences between genotypes in resting membrane potential (p=0.59; WT: N=8, n=14; Null: N=5, n=18). D - F. Similar time courses of early phase repolarization (APD_{25-50}) between WT and null myocytes. Significance was determined using Mixed Effect Regression analysis.
**Fig. S3. Proposed mechanism of VT initiation and rotor formation.** Chronological snapshots of a phase movie that captured initiation of an episode of spontaneous polymorphic VT illustrate that the interplay between a PVC and the arrhythmogenic substrate of *Scn2b* null hearts leads to arrhythmia. A. During the sinus beat preceding VT, the epicardium was homogeneously activated from apex to base. B and C. A PVC then emerged and resulted in breakthrough to the epicardium in the anterior RV free wall close to apex. C and D. The early impulse traveled to the RVOT region where it was blocked due to prolonged APD and increased refractoriness. E. Thus the impulse detoured to the LV and circumvented the RVOT. When the RVOT was finally activated, due to slowed conduction within this region, the rest of the heart had finished repolarization and was ready for re-excitation. E and F. As a result, the excitation wave front turned around at the RVOT and initiated a rotor. Inset: Corresponding ECG.
**Fig. S4. Spontaneous atrial tachyarrhythmia in ex vivo atrium preparation.** Sudden acceleration of the atrial bipolar electrogram was observed in one of the *Scn2b* null atria but not in WT. No electrical pacing or drug was applied when the atrium developed tachyarrhythmia. Multiple episodes of atrial arrhythmia were observed, the longest episode lasted for 24.5 sec at a frequency of ~20 Hz. The morphology of tachyarrhythmic beats is different from sinus beats, suggesting that this was not simply an acceleration of sinus automaticity.
**Fig. S5.** Conduction velocity was not altered in *Scn2b* null atria. Right atria, *Scn2b* null: N=9; WT: N=9, Left atria, *Scn2b* null: N=7; WT: N=7. Significance determined using Student’s T-test.
Fig. S6. Distribution of the APD$_{90}$ data paced at 5 Hz from ventricular myocytes.

The increase in ventricular APD observed in nulls is homogenous, shifting the entire data set upward. (Null: N=4 n=13; WT: N=5 n=12).
**Fig. S7. No differences in PVC coupling interval.** A. An independent PVC and a PVC that triggered VT. B. No differences were found in the coupling interval between these two categories in Scn2b null heart (P=0.459, Mann-Whitney Rank Sum Test). Numbers below the box plot are the total number of PVCs that were included for analysis in each group.
**Movie legends**

**Movie S1.** Phase map movie corresponding to the ECG traces shown in Figure 5B. Phase map movie in a Scn2b null heart showing three episodes of NSVT initiated at the RVOT region by a triggering PVC and eventually meandering out of the RVOT, giving rise to polymorphic appearance of the ECG.

**Movie S2.** Atrial arrhythmia was driven by a single rotor. Atrial tachyarrhythmia was induced by burst pacing in Scn2b null right atrium under carbachol challenge. A single, stably spinning rotor in the right atrium was maintained throughout the episode.

**Movies S3-S4.** Atrial arrhythmia can be driven by one or two rotors in an atrium from one animal. Movie 3: arrhythmia driven by figure-of-eight (two count-rotating rotors sharing a common pathway) in one Scn2b null right atrium before carbachol administration. Movie 4: after carbachol application, atrial arrhythmia was driven by a single rotor located in the left atrium pulmonary vein (PV) region with 2:1 conduction to the right atrium, indicating dynamic locations and forms of rotors can be formed in one animal.

**Movie S5.** Scn2b null atrial arrhythmia can be driven by up to three rotors spinning at independent frequencies. Arrhythmia can be driven by three independent rotors at different dominant frequency (17.7 Hz and 26.5 Hz, respectively). One was located in the right atrium and the other two (figure-of-eight) were in the PV region. The arrhythmia self-terminated and spontaneously re-initiated in the right atrium. Then, the re-initiated arrhythmia was sustained by a single rotor in the right atrium.