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ORIGINAL CONTRIBUTION

# Inhibition of $Na_V1.8$ prevents atrial arrhythmogenesis in human and mice

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## Abstract

Pharmacologic approaches for the treatment of atrial arrhythmias are limited due to side effects and low efficacy. Thus, the identification of new antiarrhythmic targets is of clinical interest. Recent genome studies suggested an involvement of SCN10A sodium channels ($Na_V1.8$) in atrial electrophysiology. This study investigated the role and involvement of $Na_V1.8$ (SCN10A) in arrhythmia generation in the human atria and in mice lacking $Na_V1.8$. $Na_V1.8$ mRNA and protein were detected in human atrial myocardium at a significant higher level compared to ventricular myocardium. Expression of $Na_V1.8$ and $Na_V1.5$ did not differ between myocardium from patients with atrial fibrillation and sinus rhythm. To determine the electrophysiological role of $Na_V1.8$, we investigated isolated human atrial cardiomyocytes from patients with sinus rhythm stimulated with isoproterenol. Inhibition of $Na_V1.8$ by A-803467 or PF-01247324 showed no effects on the human atrial action potential. However, we found that $Na_V1.8$ significantly contributes to late $Na^+$ current and consequently to an increased proarrhythmogenic diastolic sarcoplasmic reticulum $Ca^{2+}$ leak in human atrial cardiomyocytes. Selective pharmacological inhibition of $Na_V1.8$ potently reduced late $Na^+$ current, proarrhythmic diastolic $Ca^{2+}$ release, delayed afterdepolarizations as well as spontaneous action potentials. These findings could be confirmed in murine atrial cardiomyocytes from wild-type mice and also compared to $SCN10A^{-/-}$ mice (genetic ablation of $Na_V1.8$). Pharmacological $Na_V1.8$ inhibition showed no effects in $SCN10A^{-/-}$ mice. Importantly, in vivo experiments in $SCN10A^{-/-}$ mice showed that genetic ablation of $Na_V1.8$ protects against atrial fibrillation induction. This study demonstrates that $Na_V1.8$ is expressed in the murine and human atria and contributes to late $Na^+$ current generation and cellular arrhythmogenesis. Blocking $Na_V1.8$ selectively counteracts this pathomechanism and protects against atrial arrhythmias. Thus, our translational study reveals a new selective therapeutic target for treating atrial arrhythmias.

**Keywords** Antiarrhythmic drugs · Atrial arrhythmias · $Na^+$ channel · Late sodium current

## Introduction

Atrial arrhythmias, in particular atrial fibrillation (AF), contribute to the morbidity and mortality of western societies [4]. However, pharmacological therapeutic options are still

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limited due to moderate potency and severe side effects. Therefore, identification and evaluation of new targets involved in atrial arrhythmogenesis are of clinical interest. The mechanisms for atrial arrhythmogenesis include electrical remodelling and disturbances in ion homeostasis [16]. Both can cause focal triggered activity, which might evoke atrial arrhythmias or promote re-entry mechanisms. One potent substrate in promoting electrical disturbances and focal triggered activity in the atria is an increased late $Na^+$ current ($I_{NaL}$), which is a persistent $Na^+$ influx throughout the action potential [3, 16, 22, 33]. By prolonging the duration of the action potential, $I_{NaL}$ increases the probability of early afterdepolarizations (EADs), which constitute a trigger for arrhythmias. Moreover, by increasing cytosolic [$Na^+$] an enhanced $I_{NaL}$ may lead to $Na^+/Ca^{2+}$ exchanger (NCX)-mediated $Ca^{2+}$ overload [26]. Consecutively, this induces arrhythmogenic $Ca^{2+}$ release events ($Ca^{2+}$ sparks) from the sarcoplasmic reticulum (SR) during diastole [12, 13]. Increasing diastolic $Ca^{2+}$ levels may promote a depolarizing inward current ($I_{ti}$), resulting in delayed afterdepolarizations (DADs), which serve as a trigger for irregular action potentials and focal arrhythmias [32]. However, the mechanisms involved in $I_{NaL}$ generation in the atria are not fully understood.

While SCN5A sodium channels ($Na_V1.5$) are the predominant isoform in the heart [14], recent evidence suggested the involvement of SCN10A sodium channels ($Na_V1.8$) in atrial conduction [8]. Moreover, genome-wide association studies showed that variants of $Na_V1.8$ are associated with the development of atrial fibrillation [17, 21, 25]. Therefore, the aim of our study was to fundamentally investigate the molecular and functional role of $Na_V1.8$ in the human and murine atria. Moreover, we studied the involvement of $Na_V1.8$ in atrial arrhythmogenesis and evaluated the channel as a specific target for antiarrhythmic pharmacotherapy.

## Materials and methods

### Human myocardial tissue

All procedures were performed according to the Declaration of Helsinki and were approved by the local ethics committee. Informed consent was obtained from all patients. Human atrial myocardium from patients with sinus rhythm or atrial fibrillation was acquired from atrial resections during open heart surgery (for patient characteristics, see Table 1). For molecular purposes, we utilized left ventricular myocardium from healthy donor hearts that were not transplanted due to technical reasons.

### SCN10A$^{-/-}$ and wild-type mice

SCN10A$^{-/-}$ and respective wild-type mice (WT) were studied to reveal the functional consequence of genetic $Na_V1.8$ ablation [2]. The animal investigations conform to the "Guide for the Care and Use of Laboratory Animals" published by the US National Institutes of Health (publication No. 85-23, revised 1996) and the guidelines from Directive 2010/63/EU of the European Parliament on the protection of animals used for scientific purposes. Murine atrial cardiomyocytes were isolated as previously reported [13]. Mice used for cardiomyocyte isolation were sacrificed under isoflurane 133 inhalation anesthesia (5%) by cervical dislocation. Mice

**Table 1** Clinical characteristics of patients with sinus rhythm ($n = 34$) and patients with atrial fibrillation ($n = 10$)

| Patient data | Sinus rhythm ($n = 34$) | Atrial fibrillation ($n = 10$) | Non-failing ventricle ($n = 10$)[a] |
|---|---|---|---|
| Male sex (%) | 73.5 | 50.0 | N/A |
| Age (Mean ± SEM, y) | 64.56 ± 1.55 | 75.2 ± 2.55 | N/A |
| EF (Mean ± SEM, %) | 56.89 ± 1.95 | 51.25 ± 3.03 | N/A |
| Ischemic heart disease (%) | 100.0 | 100.0 | N/A |
| Diabetes (%) | 41.4 | 20.0 | N/A |
| ACE inhibitors (%) | 83.3 | 80.0 | N/A |
| β-Blockers (%) | 81.8 | 100.0 | N/A |
| Digoxin (%) | 0.0 | 0.0 | N/A |
| Catecholamines (%) | 0.0 | 0.0 | N/A |
| Amiodaron (%) | 0.0 | 30.0 | N/A |
| PDE inhibitors (%) | 0.0 | 0.0 | N/A |
| Statin (%) | 83.9 | 90.0 | N/A |

Values are mean ± SEM or *n* (%). Clinical data could not be completely obtained from every patient

*EF* ejection fraction, *ACE* angiotensin-converting enzyme, *PDE* phosphodiesterase

[a]Blinded due to ethical reasons

used for in vivo studies were anesthetized via intraperitoneal injection of medetomidine (0.5 mg/kg), midazolam (5 mg/kg) and fentanyl (0.05 mg/kg body weight) and killed by cervical dislocation after the procedure.

## Human atrial cardiomyocytes isolation

Atrial myocardium from patients with sinus rhythm was used for cellular experiments. Before starting isolation, human atrial tissue was cleared from fat and blood, then cut into very small pieces and rinsed thoroughly. Cardiomyocytes were isolated using collagenase (Worthington type 1, 370 U/mg) and proteinase (Sigma Type XXIV, 7.0–14.0 U/mg) as described previously [15]. Enzymatic digestion was stopped by adding BCS (2%). The supernatant containing dispersed cells was centrifuged (58 g, 5 min) and cells were resuspended in storage medium. Only cell solutions containing elongated cardiomyocytes with clear cross-striations were selected for experiments, plated on laminin-coated recording chambers, and left to settle for 30 min. A representative isolated human atrial cardiomyocyte is shown in Fig. 2a. Cellular experiments were performed at room temperature.

## Murine atrial cardiomyocytes isolation

Atrial cardiomyocytes from SCN10A$^{-/-}$ and respective WT mouse hearts were isolated as previously described [13]. Cellular experiments were performed at room temperature.

## Quantitative real-time PCR (qPCR)

Human atrial tissue or ventricular non-failing tissue were snap-frozen in liquid nitrogen and stored at − 80 °C. RNA was isolated by use of the SV total RNA isolation System (Promega). Primer sequences (forward and four reverse) of SCN10A (Origene, cat No HP209444), SCN5A and GAPDH were used for quantitative RT-PCR.

## Western blots

Human atrial tissue samples from patients with sinus rhythm (SR) and atrial fibrillation (AF) as well as human ventricular samples from healthy donors (NF) were homogenized in Tris buffer and complete protease and phosphatase inhibitor cocktails (Roche Diagnostics). Protein concentration was determined by BCA assay (Pierce Biotechnology). Mouse monoclonal anti-$Na_V1.8$ antibodies (1:1,000, LSBio, LS-C109037), rabbit polyclonal anti-$Na_V1.5$ (1:2,000, Alomone labs, ASC-005), and mouse monoclonal anti-GAPDH (1:20,000, BIOTREND, BTMC-A473-9) were used. ImmobilonTM Western Chemiluminescent HRP Substrate (Millipore) was used for the chemiluminescent detection.

## Pharmacological interventions

For selectively blocking $Na_V1.8$, isolated cardiomyocytes were treated with either A-803467 (30 nmol/L, Sigma) or PF-01247324 (1 µmol/L, Sigma). Cells were incubated for 15 min before measurements were started. Isoproterenol (30 nmol/L, Sigma) was used for slight beta-adrenergic stimulation in all groups [10]. Moreover, we used tetrodotoxin (2 µmol/l) to inhibit $I_{NaL}$.

## Patch-clamp experiments

### $I_{NaL}$ measurements

Ruptured-patch whole-cell voltage-clamp was used to measure $I_{NaL}$ in human atrial cardiomyocytes (HEKA electronics). Cardiomyocytes were held at − 120 mV and $I_{NaL}$ was elicited using a train of pulses to − 35 mV (1 s duration, ten pulses, BCL 2 s). Recordings were initiated 3 min after rupture. The measured current was integrated (between 100 and 500 ms) and normalized to the membrane capacitance (Suppl. Fig. 3).

### Action potential recordings

For action potential recordings, the whole-cell patch-clamp technique was used (current clamp configuration, HEKA electronics). Access resistance was typically ~ 5–10 MΩ after patch rupture. Action potentials were continuously elicited by square current pulses of 0.5–1 nA amplitude and 1–5 ms duration at a frequency of 1 Hz. For assessing DADs and spontaneous action potentials, stimulation was paused for 15 s and for 30 s.

## Confocal $Ca^{2+}$ spark measurements

Isolated atrial cardiomyocytes were loaded with the $Ca^{2+}$ indicator Fluo 4-AM (10 µmol/L for 15 min, Molecular Probes) at RT. The solution was substituted and cells were incubated for 15 min with Tyrode's solution and the respective agents. Line scans for $Ca^{2+}$ spark measurements were obtained with a laser scanning confocal microscope (Zeiss). Line scans were recorded during rest after loading the sarcoplasmic reticulum with $Ca^{2+}$ by continuous field stimulation at 1 Hz. $Ca^{2+}$ sparks were analysed with the program SparkMaster for ImageJ.

## In vivo arrhythmia studies

For electrophysiological studies, SCN10A$^{-/-}$ and respective wild-type mice [2] were anesthetized and temperature controlled (37 °C). As previously described, a Millar 1.1F octapolar EP catheter (EPR-800; Millar Instruments) was

inserted via the right jugular vein [20]. Right atrial pacing was performed using 2 ms current pulses delivered by an external stimulator. Atrial capture was confirmed by atrial pacing prior to the arrhythmia protocol. Inducibility of atrial arrhythmias was tested by decremental burst pacing (5 episodes/mice). AF was defined as the occurrence of rapid and fragmented atrial electrograms with irregular AV nodal conduction and ventricular rhythm for at least 1 s.

### Statistics

All data are presented as the mean values ± SEM. For statistical analysis of two groups containing parametric data Student's *t* test was used, for non-parametric data Mann–Whitney test was used.

For analysis of parametric data comparing more than two groups, one-way ANOVA was used. *P* values were corrected for multiple comparisons by the Tukey method. For analysis of proportions, Fisher's exact test was used. Analysis was performed using GraphPad Prism 8. *P* values are two-sided and considered statistically significant if $P < 0.05$.

## Results

### Expression of $Na_V1.8$ in human atrial myocardium

To investigate whether $Na_V1.8$ is expressed in the human atrium, we used myocardium from patients with SR and patients with AF for mRNA and protein analysis. At the protein level, we could confirm the existence of $Na_V1.8$ in the human atria. Moreover, $Na_V1.8$ protein expression is significantly higher in the human atria ($n = 6$) compared to the human ventricle ($n = 5$ Fig. 1a, b). Using qPCR, we detected the expression of $Na_V1.8$ mRNA in human atrial tissue, which was 3.0 ± 0.9-fold higher in the human atrium as compared to ventricular non-failing myocardium (ventricle: $n = 10$ patients, atria: $n = 7$ patients, Fig. 1c). To evaluate whether $Na_V1.8$ or the major cardiac sodium channel isoform $Na_V1.5$ might be differentially regulated in atrial fibrillation (AF) compared to sinus rhythm (SR), we investigated atrial myocardium from patients with SR or with AF. However, neither $Na_V1.8$ (SR: $n = 14$ patients, AF: $n = 14$ patients) nor $Na_V1.5$ (SR: $n = 14$ patients, AF: $n = 13$ patients) protein expression levels were different between myocardium from patients with AF and SR (Fig. 1d–f). Moreover, while $Na_V1.8$ mRNA was lower compared to $Na_V1.5$, we found no changes between SR versus AF for $Na_V1.8$ (SR: $n = 8$ patients, AF: $n = 8$). $Na_V1.5$ mRNA levels differed between SR ($n = 8$ patients) and AF ($n = 8$, Fig. 1g), which however did not translate into protein expression differences. Therefore, $Na_V1.8$ was confirmed to be present in the human atria without being regulated in patients with AF.

### Effects of $Na_V1.8$ on the cardiac action potential

To investigate the effect of $Na_V1.8$ on the action potential properties in human atrial cardiomyocytes, we performed ruptured-patch whole-cell current clamp experiments using freshly isolated cardiomyocytes from patients with sinus rhythm (Fig. 2a). Action potential amplitude (APA), maximum upstroke velocity (*dv/dt*), action potential duration (APD) as well as resting membrane potential (RMP) were investigated from five different patients with sinus rhythm (control: $n = 14$ cardiomyocytes; A-803467: $n = 12$; PF-01247324: $n = 11$). APA (100.9 ± 4.1 mV) and *dv/dt* (54.88 ± 4.8 mV/ms) were not altered after inhibition of $Na_V1.8$ by A-803467 (APA: 104.9 ± 5.5 mV; *dv/dt*: 58.08 ± 7.0 mV/ms) or PF-01247324 (APA: 99.8 ± 6.7 mV; *dv/dt*: 56.33 ± 8.2 mV/ms), which indicates that $Na_V1.8$ has negligible effects on peak $Na^+$ current (Fig. 2b, c). Also, RMP (-77.1 ± 2.3 mV) was not changed after $Na_V1.8$ inhibition (A-803467: − 75.6 ± 2.3 mV; PF-01247324: − 73.9 ± 2.5 mV, Fig. 2d). APD at 50% repolarization ($APD_{50}$) was 28.8 ± 2.6 ms in control compared to A-803467 (25.0 ± 2.3 ms) and PF-01247324 (28.2 ± 2.8 ms, Fig. 2e). However, $APD_{90}$ was slightly abbreviated ($APD_{90}$; 121.0 ± 11.0 ms) after exposure to A-803467 (96.3 ± 6.9 ms) and PF-01247324 (97.2 ± 9.1 ms, Fig. 2f), which, however, did not reach statistical significance. We further evaluated the effects of $Na_V1.8$ on the action potential in atrial cardiomyocytes from $SCN10A^{-/-}$ ($n = 10$ mice, control: $n = 16$ cells, PF-01247324: $n = 15$ cells) and WT mice ($n = 8$ mice, control: $n = 14$ cells, PF-01247324: $n = 13$ cells, Fig. 3a). According to the human data, we could confirm that $Na_V1.8$ has no effects on APA (Fig. 3b), *dv/dt* (Fig. 3c), RMP (Fig. 3d) as well as $APD_{50}$ (Fig. 3e) or $APD_{90}$ (Fig. 3f). Accordingly, we observed no effects of pharmacological $Na_V1.8$ inhibition using PF-01247324 in $SCN10A^{-/-}$ and WT. These experiments indicate that $Na_V1.8$ has negligible effects on the human and murine atrial action potential, which is of importance for further translational studies.

### Role of $Na_V1.8$ for generation of $I_{NaL}$

The contribution of $Na_V1.8$ in $I_{NaL}$ generation was studied in human atrial cardiomyocytes from patients with sinus rhythm using ruptured-patch whole-cell voltage-clamp. Patch-clamp recordings of isolated human atrial cardiomyocytes showed that $Na_V1.8$ inhibition caused a significant reduction of $I_{NaL}$ by 44.9 ± 13.5% after exposure to A-803467 ($n = 12$ cardiomyocytes/4 patients) and by 53.5 ± 12.7% after PF-01247324 ($n = 10/4$) compared to control ($n = 15/6$, Fig. 4a, b). Furthermore, isolated atrial cardiomyocytes from $SCN10A^{-/-}$ mice ($n = 11$ cells/ 5 mice) showed a significantly lower $I_{NaL}$ compared to WT ($n = 8$ cells/ 4 mice, Fig. 4c–e). While pharmacological

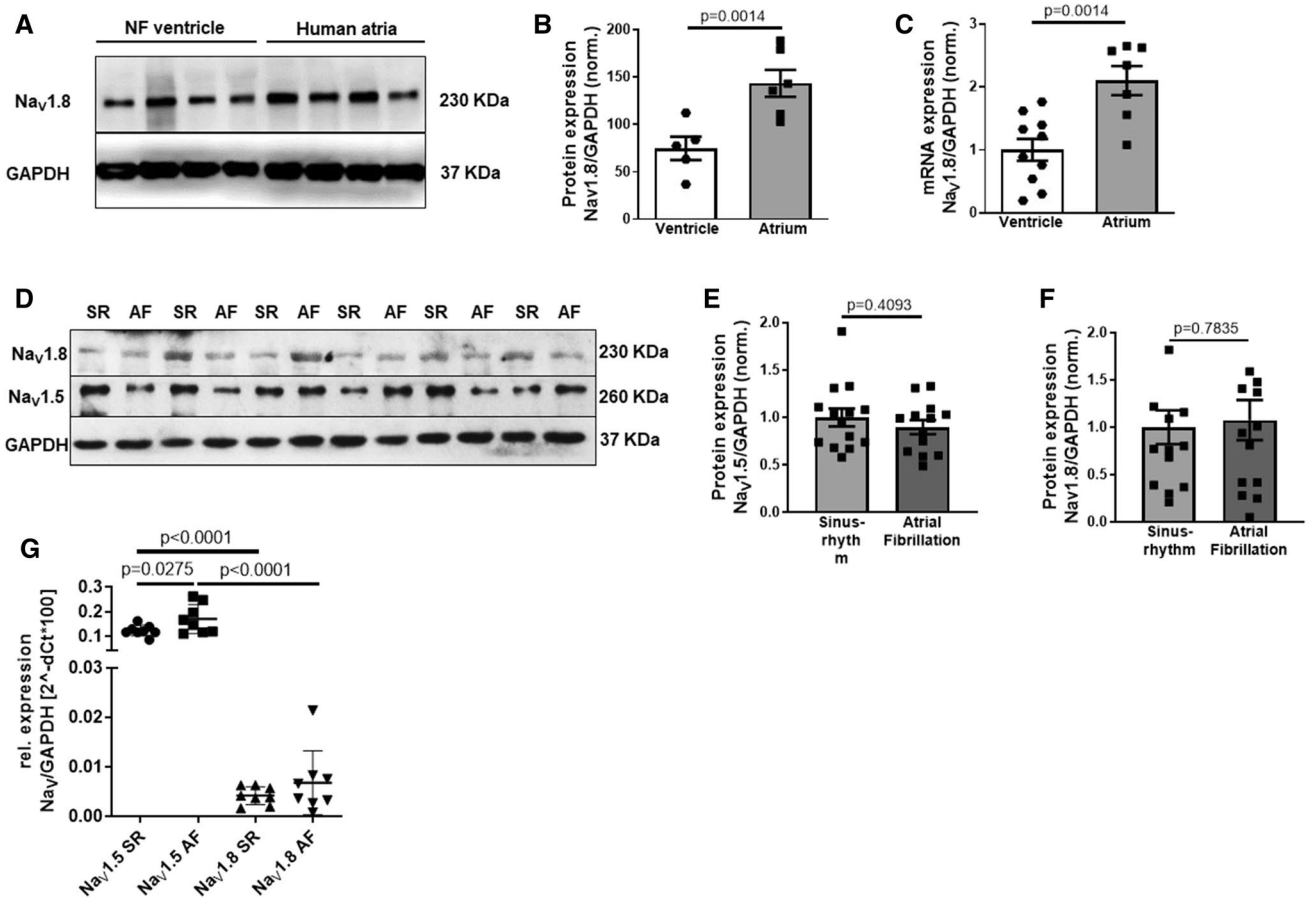


**Fig. 1** Expression of $Na_V1.8$ in the human atrium. Data are presented as scatter plot with mean ± SEM. *P* values were calculated using unpaired Student's *t* test. **a** Original Western blot for $Na_V1.8$ expression in atrial myocardium from patients with sinus rhythm compared to ventricular myocardium from non-failing donors (NF). **b** Normalized densitometry data comparing the protein expression of $Na_V1.8$ in human atria ($n=6$) and human ventricle ($n=5$). GAPDH was used as an internal loading control in all blots. **c** Normalized mRNA expression of $Na_V1.8$/GAPDH in human atrial myocardium ($n=7$ patients) compared to ventricular myocardium from healthy subjects ($n=10$ patients). **d** Original Western Blot for $Na_V1.8$ and $Na_V1.5$ protein in human atria from patients with sinus rhythm (SR) or atrial fibrillation (AF). **e** Normalized densitometry data from Western Blots using atrial myocardium from patients with SR or AF showing the protein expression of $Na_V1.5$ (SR: $n=14$ patients, AF: $n=13$) and (**f**) $Na_V1.8$ (SR: $n=14$ patients, AF: $n=14$). GAPDH was used as an internal loading control in all blots. **g** Normalized mRNA expression of $Na_V1.5$/GAPDH (SR: $n=8$ patients, AF: $n=8$) and $Na_V1.5$/GAPDH (SR: $n=8$ patients, AF: $n=8$) in human atrial myocardium from SR compared to AF

$Na_V1.8$ inhibition by PF-01247324 ($n=7$ cells/ 5 mice) exerted no effect on $I_{NaL}$ in SCN10A$^{-/-}$, $I_{NaL}$ could be significantly reduced by application of PF-01247324 in atrial WT cardiomyocytes ($n=7$ cells/ 4 mice, Fig. 4c–e). To determine the contribution of $Na_V1.8$ to $I_{NaL}$ generation, we also performed measurements with TTX (2 µmol/L) to globally inhibit $I_{NaL}$. We observed a trend towards a lower $I_{NaL}$ in TTX-treated SCN10A$^{-/-}$ cardiomyocytes and also compared to PF-01247324-treated WT cardiomyocytes (Fig. 4e) suggesting that other $Na_V$-dependent $I_{NaL}$ is still relevant under these conditions.

## Role of $Na_V1.8$ for sarcoplasmic $Ca^{2+}$-leak generation

It is well known that $I_{NaL}$ can potently induce arrhythmogenic diastolic SR $Ca^{2+}$ release events [13]. To investigate whether the reduction of $I_{NaL}$ caused by $Na_V1.8$ inhibition may lead to a diminished incidence of diastolic SR $Ca^{2+}$ release in human atrial cardiomyocytes we used confocal microscopy. The frequency of diastolic SR $Ca^{2+}$ sparks (CaSpF) in line scans of human atrial cardiomyocytes was $3.2 \pm 0.5 \times 100$/µm/s ($n=84$ cardiomyocytes/13 patients) which could be significantly attenuated to $1.2 \pm 0.2$

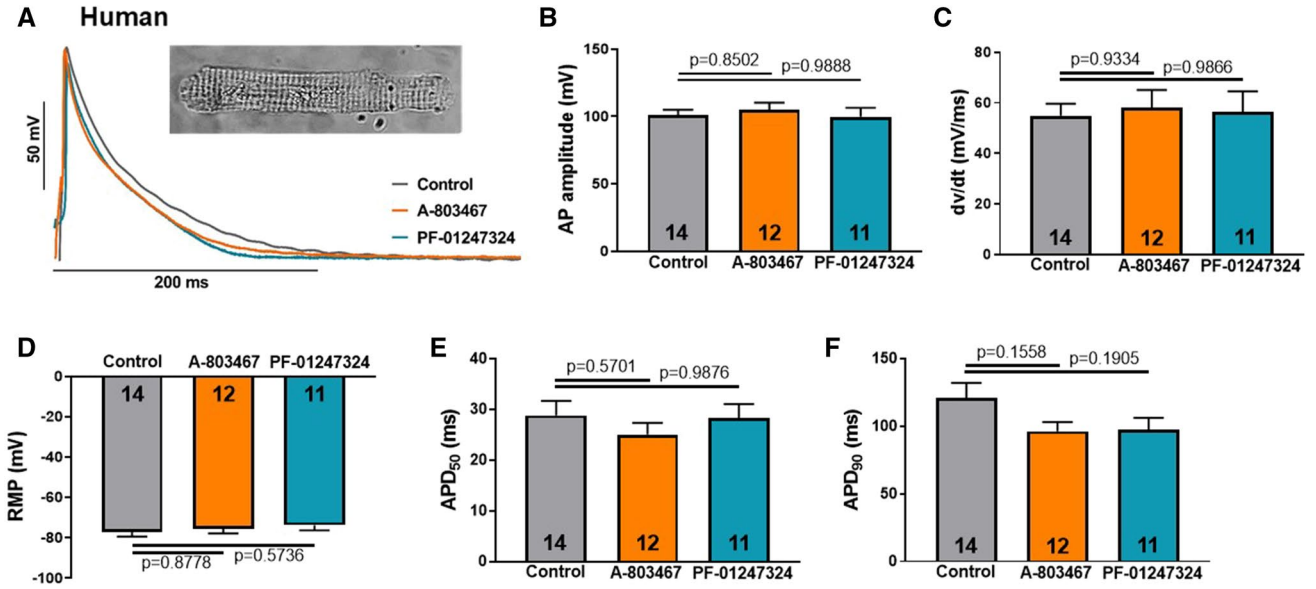


**Fig. 2** Effects of $Na_V1.8$ on the human atrial action potential. Data are presented as mean ± SEM. *P* values were computed using one-way ANOVA with Tukey's test for multiple comparisons. **a** Representative action potential recordings (1 Hz stimulation). Inset: isolated human atrial cardiomyocyte. **b** Effects of $Na_V1.8$ inhibition by A-803467 ($n = 12$ cardiomyocytes/5 patients) or PF-01247324 ($n = 11/5$) compared to control ($n = 14/5$) on action potential (AP) amplitude, (**c**) maximum upstroke velocity (*dv/dt*), (**d**) resting membrane potential (RMP) and action potential duration at (**e**) 50% ($APD_{50}$) and (**f**) 90% repolarization ($APD_{90}$)

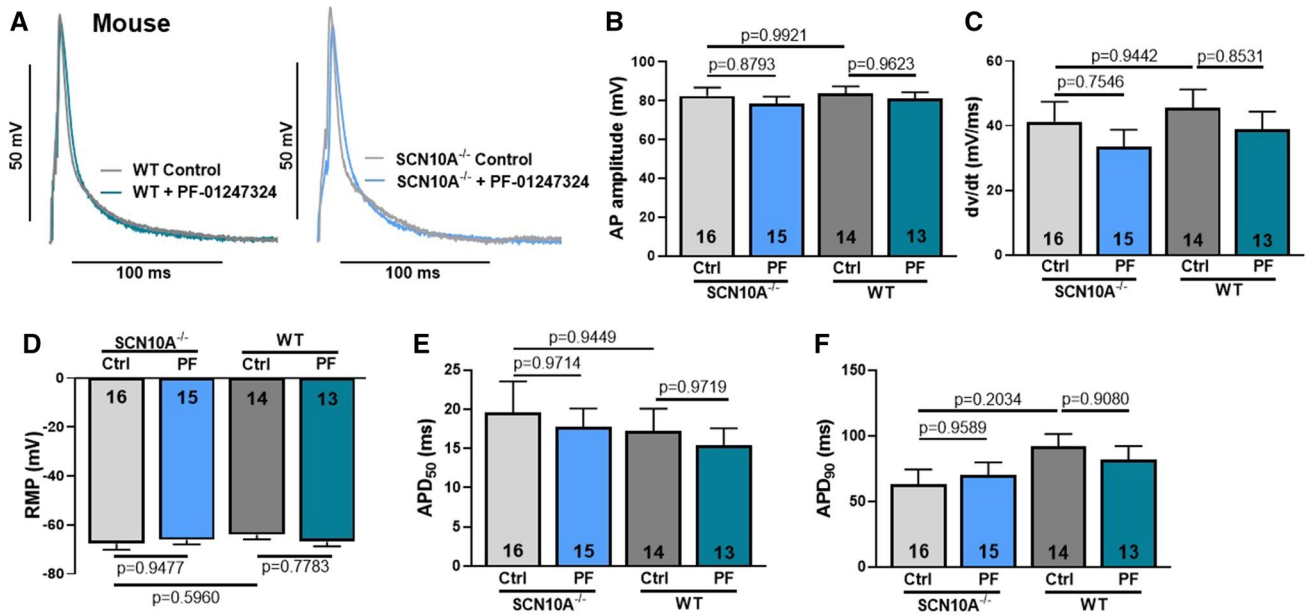


**Fig. 3** Effects of $Na_V1.8$ on the murine atrial action potential using $SCN10A^{-/-}$ and WT mice. Data are presented as mean ± SEM. *P* values were calculated using one-way ANOVA with Tukey's test for multiple comparisons. **a** Representative action potential recordings (1 Hz stimulation) of isolated murine atrial cardiomyocytes. **b** Effects of genetic ablation of $Na_V1.8$ ($SCN10A^{-/-}$: $n = 16$ cells/10 mice) compared to WT ($n = 14$ cells/8 mice) and effects of pharmacological inhibition of $Na_V1.8$ by PF-01247324 in each genotype ($SCN10A^{-/-}$: $n = 15$ cells/10 mice and WT: $n = 13/8$) on action potential (AP) amplitude, (**c**) maximum upstroke velocity (*dv/dt*), (**d**) resting membrane potential (RMP) and action potential duration at (**e**) 50% ($APD_{50}$) and (**f**) 90% repolarization ($APD_{90}$)

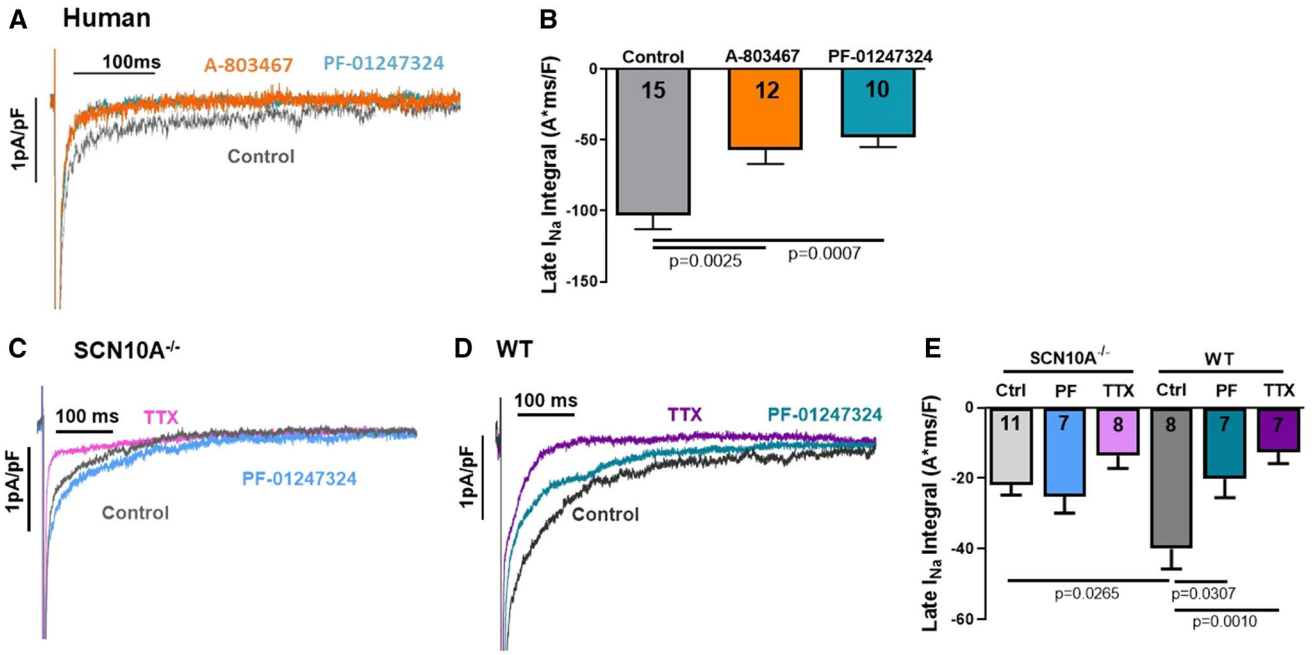


**Fig. 4** Role of $Na_V1.8$ in $I_{NaL}$ generation. Values are reported as mean ± SEM. One-way ANOVA with Tukey's test for multiple comparisons was used to calculate *P* values. **a** Original traces of $I_{NaL}$ in human atrial cardiomyocytes and (**b**) mean data of $I_{NaL}$ (integral 100–500 ms) showing the effects of $Na_V1.8$ inhibition with either A-803467 ($n = 12$ cardiomyocytes/4 patients) or PF-01247324 ($n = 10/4$) on $I_{NaL}$ compared to control ($n = 15/6$). **c** Original $I_{NaL}$ traces representing $I_{NaL}$ in SCN10A$^{-/-}$ mice as well as (**d**) WT and $I_{NaL}$ after $Na_V1.8$ inhibition by PF-01247324 and tetrodotoxin treatment respectively. **e** Mean values of $I_{NaL}$ (integral 100–500 ms) showing the effects of genetic ablation of $Na_V1.8$ (SCN10A$^{-/-}$: $n = 11$ cells/5 mice) compared to WT ($n = 8$ cells/4 mice) and effects of pharmacological inhibition of $Na_V1.8$ by PF-01247324 (SCN10A$^{-/-}$: $n = 7$ cells/5 mice and WT: $n = 7/4$) and TTX (SCN10A$^{-/-}$: $n = 8$ cells/5 mice and WT: $n = 7/4$) in each genotype

× 100/µm/s after addition of A-803467 ($n = 73/9$) or PF-01247324 to 1.1 ± 0.2 × 100/µm/s ($n = 88/11$, Fig. 5a, b). Moreover, the calculated diastolic SR $Ca^{2+}$ leak was reduced by 64.3 ± 22.2% after addition of A-803467 and by 80.6 ± 20.6% after PF-01247324 (control: $n = 84$ cardiomyocytes/13 patients, A-803467: $n = 73/9$; PF-01247324: $n = 88/11$, Fig. 5c). In line with that, atrial cardiomyocytes from SCN10A$^{-/-}$ mice ($n = 57$ cells/ 7 mice, Fig. 5d) showed a lower frequency of diastolic $Ca^{2+}$ sparks as well as a lower diastolic $Ca^{2+}$ leak compared to WT cells ($n = 57$ cells/ 7 mice, Fig. 5e–g). While PF-01247324- ($n = 62$ cells/7 mice) and TTX- ($n = 61$ cells/ 7 mice) treated cardiomyocytes had a significantly reduced $Ca^{2+}$ spark frequency and $Ca^{2+}$ leak in WT, both drugs had no further effects in SCN10A$^{-/-}$ cardiomyocytes ($n = 57$ cells/7 mice and 64/7 respectively). Interestingly, we observed no further antiarrhythmic effect of TTX compared to $Na_V1.8$ inhibition alone. Thus, the reduction of both $I_{NaL}$ and diastolic SR $Ca^{2+}$ release reveal the significant role of $Na_V1.8$ for cellular arrhythmogenesis in the human atria. Of note, we observed no effects of pharmacological $Na_V1.8$ inhibition on systolic $Ca^{2+}$ transient amplitude and SR $Ca^{2+}$ load (Suppl. Figs. 1, 2).

## Blocking $Na_V1.8$ suppresses diastolic $Ca^{2+}$ waves

To elucidate potential antiarrhythmic effects of $Na_V1.8$ inhibition, we have studied the effects of pharmacological inhibition and genetic ablation of $Na_V1.8$ on the incidence of diastolic $Ca^{2+}$ waves, which are major diastolic $Ca^{2+}$ release events and constitute proarrhythmic triggers. Indeed, human atrial cardiomyocytes from patients with sinus rhythm treated with A-803467 or PF-01247324 showed a significantly reduced frequency of diastolic $Ca^{2+}$ waves (Fig. 6a, b). Also, the percentage of cells developing diastolic $Ca^{2+}$ waves, 26.1% under control conditions ($n = 116$ cardiomyocytes/13 patients), decreased after exposure to either A-803467 (to 8.4%, $n = 90/9$) or PF-01247324 (to 8.3%, $n = 104/11$, Fig. 6c). Also, SCN10A$^{-/-}$ mice ($n = 67$ cells/7 mice) had a reduced $Ca^{2+}$ wave frequency as well as a reduced fraction of cardiomyocytes with arrhythmic events compared to WT ($n = 80$ cells/7 mice, Fig. 6d–g). Pharmacological $Na_V1.8$ inhibition with PF-01247324 exerted no effects in SCN10A$^{-/-}$ mice ($n = 67$ cells/7 mice), but significantly decreased $Ca^{2+}$ wave frequency and the percentage of cells with arrhythmic events in WT ($n = 70$ cells/7 mice, Fig. 6f–g). $I_{NaL}$ inhibition by TTX caused no further effects

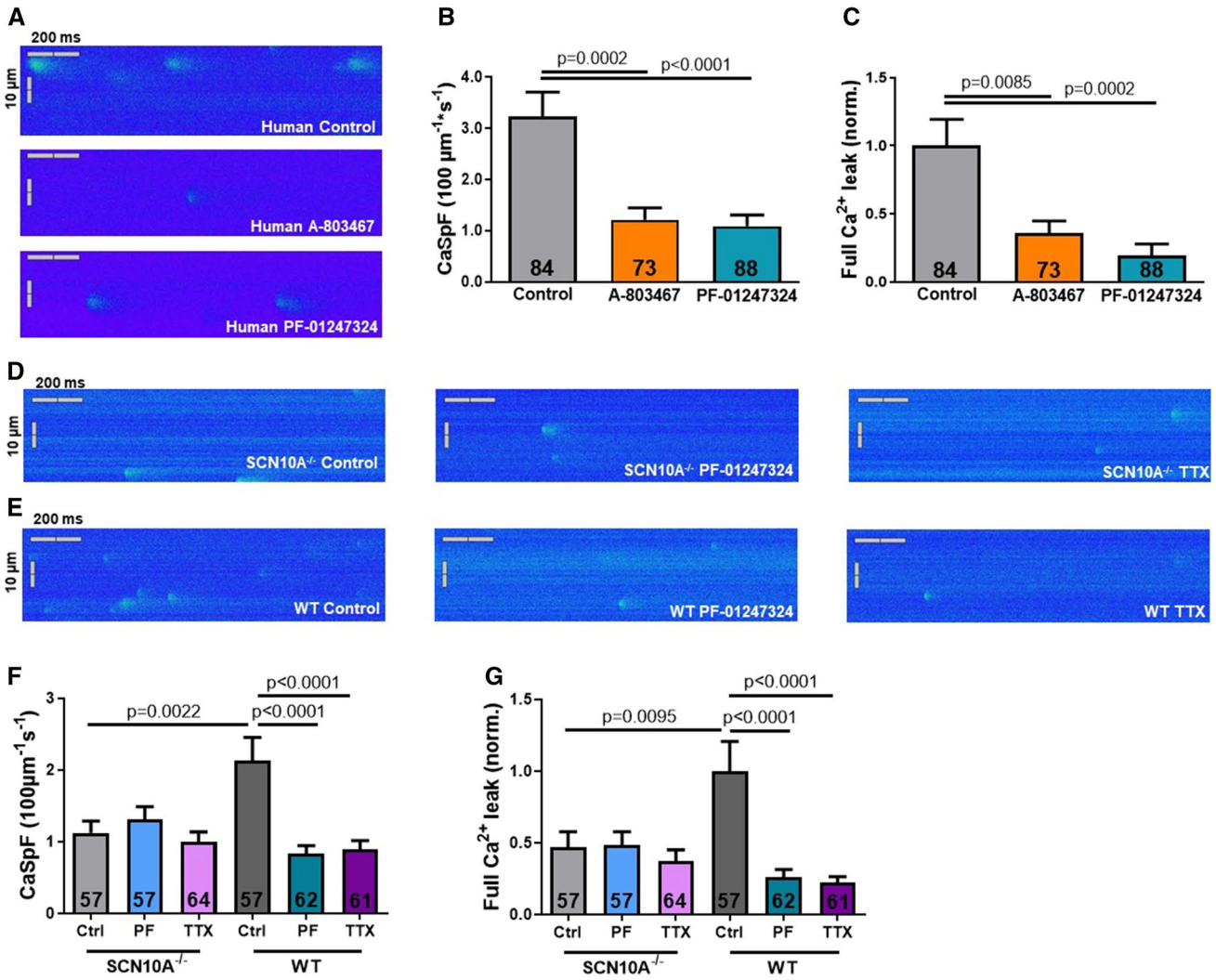


**Fig. 5** Relevance of $Na_V1.8$ for diastolic sarcoplasmic $Ca^{2+}$ leak. Values are given as mean ± SEM. One-way ANOVA with Tukey's test for multiple comparisons was used to calculate *P* values. **a** Representative confocal line scan images of human atrial cardiomyocytes loaded with the $Ca^{2+}$ indicator Fluo-4 showing $Ca^{2+}$ sparks during diastole. **b** Mean values of the frequency of $Ca^{2+}$ sparks (CaSpF) and (**c**) the total calculated diastolic $Ca^{2+}$ leak in atrial cardiomyocytes ($n = 84$ cardiomyocytes/13 patients) and after blocking $Na_V1.8$ with A-803467 ($n = 73/9$) or PF-01247324 ($n = 88/11$). **d** Original line scan images of murine atrial cardiomyocytes from SCN10A$^{-/-}$ and (**e**) WT mice. **f** Mean values of CaSpF and (**g**) the total calculated diastolic $Ca^{2+}$ leak in SCN10A$^{-/-}$ ($n = 57$ cells/7 mice) and WT ($n = 57$ cells/7 mice) mice and, respectively, effects of $Na_V1.8$ inhibition by PF-01247324 (SCN10A$^{-/-}$: $n = 57$ cells/7 mice and WT: $n = 62/7$) and TTX (SCN10A$^{-/-}$: $n = 64$ cells/7 mice and WT: $n = 61/7$)

compared to $Na_V1.8$ ablation ($n = 68$ cells/7 mice) or inhibition ($n = 67$ cells/7 mice Fig. 6f–g).

## Inhibition of $Na_V1.8$ reduces proarrhythmic triggers in atrial cardiomyocytes

Given that $I_{NaL}$-dependent diastolic SR $Ca^{2+}$ release can induce an NCX mediated depolarizing current leading to cellular arrhythmias, we tested the effects of $Na_V1.8$ on afterdepolarizations and spontaneous action potentials. The incidence of EADs in human atrial cardiomyocytes ($3.1 \pm 0.9$/min, $n = 14$ cardiomyocytes/4 patients) could be significantly reduced by inhibiting $Na_V1.8$ with either A-803467 ($0.6 \pm 0.4$/min, $n = 11/4$) or PF-01247324 ($0.4 \pm 0.1$/min, $n = 11/4$, Fig. 7a, b). Moreover, DADs and spontaneous action potentials during rest ($13.6 \pm 2.7$/min in control, $n = 11/4$) were significantly less common when $Na_V1.8$ was inhibited (A-803467: $4.5 \pm 1.5$/min, $n = 9/4$, PF-01247324: $5.5 \pm 1.6$/min, $n = 10/4$, Fig. 7c, d). Likewise, $Na_V1.8$ inhibition by PF-01247324 ($n = 13$ cells/8 mice) strongly suppressed DAD occurrence and spontaneous action potentials during rest compared to control in WT ($n = 14$ cells/8 mice). Application of PF-01247324 ($n = 13$ cells/10 mice) had no effect in SCN10A$^{-/-}$ mice, which

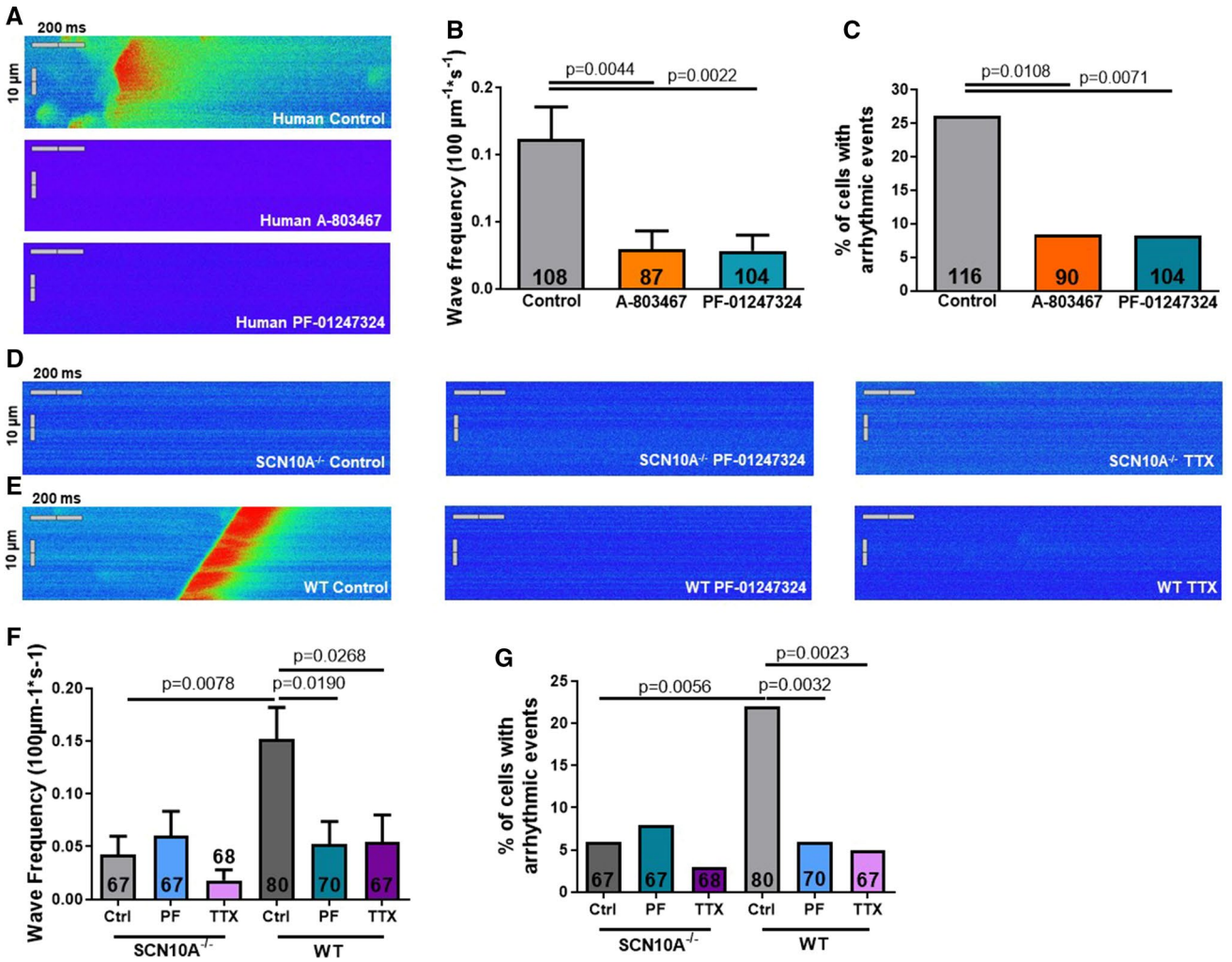


**Fig. 6** Effects of $Na_V1.8$ on diastolic $Ca^{2+}$ waves. Data are presented as mean ± SEM. *P* values were calculated using one-way ANOVA with Tukey's test for multiple comparisons or Fisher's exact test (for **c**, **g**). **a** Confocal line scan images of human atrial cardiomyocytes loaded with the $Ca^{2+}$ indicator Fluo-4 representing the occurrence of major diastolic arrhythmogenic $Ca^{2+}$ release events. **b** Mean frequency of $Ca^{2+}$ waves in control ($n = 108$ cardiomyocytes/13 patients) and after treatment with A-803467 ($n = 87/9$) or PF-01247324 ($n = 104/11$). **c** Percentage of cells developing arrhythmic events ($Ca^{2+}$ waves or spontaneous transients; $n = 24$ of 116 cardiomyocytes/13 patients) and effects of $Na_V1.8$ blockade with A-803467 ($n = 7$ of 90/9 patients) or PF-01247324 ($n = 8$ of 104/11 patients). **d** Representative original line scans of murine atrial cardiomyocytes from SCN10A$^{-/-}$ and (**e**) WT mice. **f** Mean values of $Ca^{2+}$ wave frequency and (**g**) proportion of cells showing arrhythmic events in SCN10A$^{-/-}$ ($n = 67$ cells/7 mice) and WT ($n = 80$ cells/7 mice) mice and effects of $Na_V1.8$ inhibition by PF-01247324 (SCN10A$^{-/-}$: $n = 67$ cells/7 mice and WT: $n = 70/7$) and TTX (SCN10A$^{-/-}$: $n = 68$ cells/7 mice and WT: $n = 67/7$) in both genotypes

show an already reduced incidence of arrhythmic events ($n = 13$ cells/10 mice, Fig. 7e, f). Thus, $Na_V1.8$ inhibition markedly prevented cellular arrhythmias in human and murine atrial cardiomyocytes.

## SCN10A$^{-/-}$ mice are protected against AF induction

The role of $Na_V1.8$ for in vivo arrhythmias was investigated using SCN10A$^{-/-}$ mice and respective WT. After transjugular vein catheterization, five episodes of atrial burst stimulation were performed in anaesthetized mice (Fig. 8a). Electrocardiograms of SCN10A$^{-/-}$ mice showed no changes in cardiac conduction and repolarization compared to WT (Suppl. Table 1). In WT mice undergoing atrial burst stimulation AF was inducible in all animals ($n = 5$ mice). However, only in two out of eight SCN10A$^{-/-}$ mice AF could be induced indicating a significantly lower susceptibility to AF ($n = 8$ mice, Fig. 8b). Moreover, the AF duration after respective burst episodes was markedly shorter in SCN10A$^{-/-}$ mice (12.0 ± 3.8 s) compared to WT (33.2 ± 5.5 s, Fig. 8c). These data demonstrate that $Na_V1.8$ ablation is protective against AF

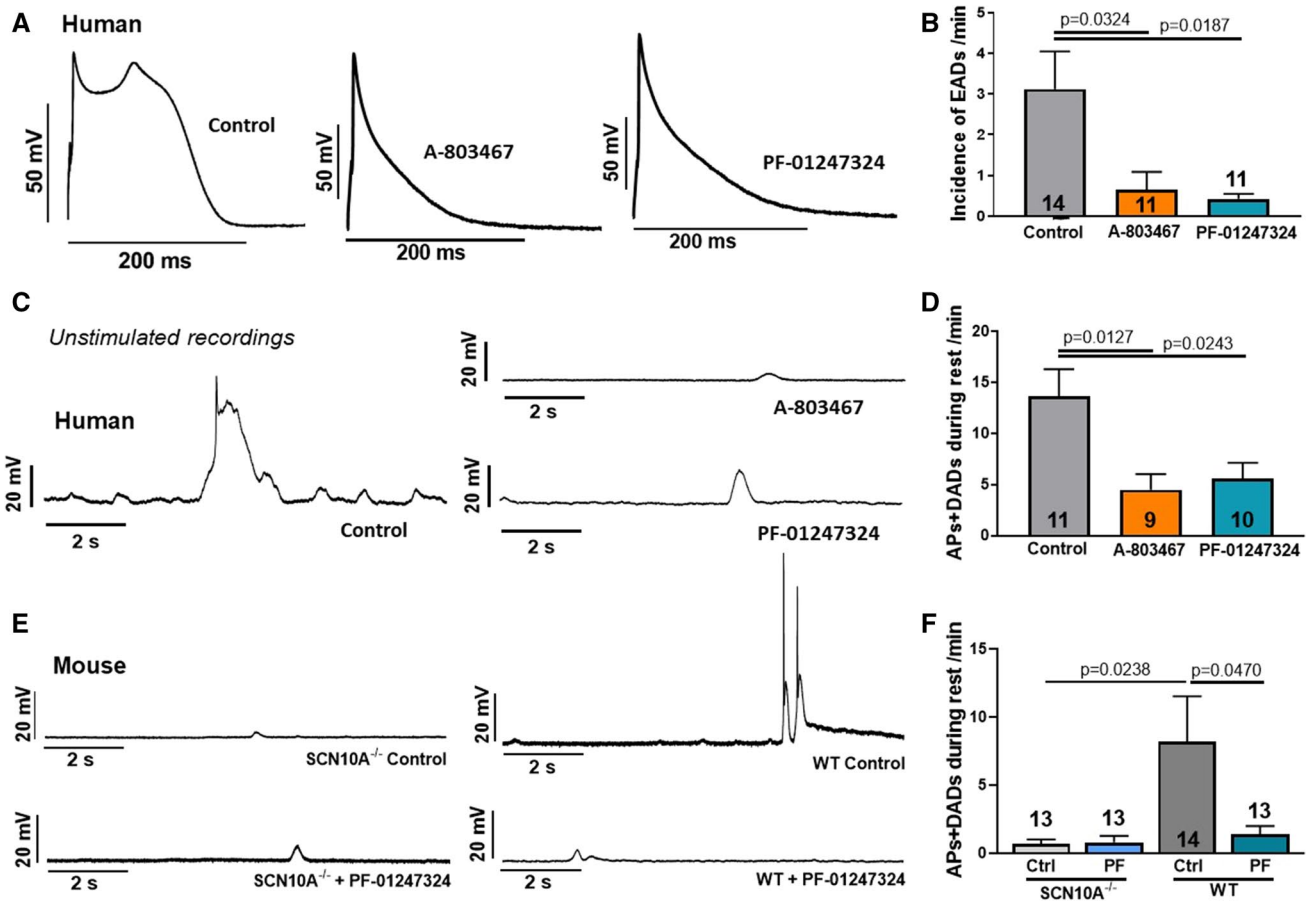


**Fig. 7** Effects of $Na_V1.8$ inhibition on cellular arrhythmogenic trigger. Data are reported as mean ± SEM. *P* values were calculated using one-way ANOVA with Tukey's test for multiple comparisons. **a** Action potential recordings (1 Hz) representing the occurrence of an early afterdepolarization (EAD). **b** Incidence of EADs/min ($n = 14$ cardiomyocytes/4 patients) and effects of inhibiting $Na_V1.8$ with A-803467 ($n = 11/4$) or PF-01247324 ($n = 11/4$). **c** Original unstimulated recordings of human atrial cardiomyocytes during 10 s rest after a series of 30 stimulated action potentials (1 Hz). **d** Mean incidence of spontaneous action potentials (APs) and delayed afterdepolarizations (DADs) during rest ($n = 11/4$) and after treatment with A-803467 ($n = 9/4$) or PF-01247324 ($n = 10/4$). **e** Original unstimulated recordings of murine atrial cardiomyocytes during 10 s rest after a series of 30 stimulated action potentials (1 Hz) from $SCN10A^{-/-}$ and WT mice. **f** Mean values of spontaneous APs and DADs during rest in $SCN10A^{-/-}$ ($n = 13$ cells/ 10 mice) and WT ($n = 14$ cells/ 8 mice) and effects of PF-01247324 in $SCN10A^{-/-}$ ($n = 13$ cells/ 10 mice) and WT ($n = 13$ cells/ 8 mice)

induction and thereby confirm its arrhythmic potency in an in vivo system.

## Discussion

This study comprehensively investigated $Na_V1.8$ in human atrial myocardium and its role in cellular electrophysiology and arrhythmogenesis. We could detect relevant $Na_V1.8$ mRNA and protein levels in the human atrium. While pharmacological $Na_V1.8$ modulation showed no significant effects on action potentials, it depicted a contribution to $I_{NaL}$ generation and thereby to diastolic SR $Ca^{2+}$ leak in human atrial cardiomyocytes. Importantly, selective inhibition of $Na_V1.8$ with two agents potently reduced cellular arrhythmogenic triggers. These findings could be confirmed in mice lacking $Na_V1.8$ ($SCN10A^{-/-}$). Finally, in vivo studies revealed that $SCN10A^{-/-}$ mice are protected against AF induction.

We not only found that $Na_V1.8$ is expressed in the human atrium but could show that mRNA and protein expression is higher in atrial compared to ventricular myocardium. The presence of $Na_V1.8$ in the human atria was indirectly suggested by genome-wide association studies (GWAS) reporting that the SCN10A gene (encoding $Na_V1.8$) impacts atrial conduction, in particular PR interval and *P* wave duration [8, 18]. Data from mice further support our findings by showing a higher $Na_V1.8$ expression in the atria compared to the ventricle [34]. Of note, one previous study reported a generally lower $Na_V1.8$ mRNA expression in the atria compared to other $Na_V$ isoforms [19] and other studies described difficulties in the detection of $Na_V1.8$, which may be due to a high

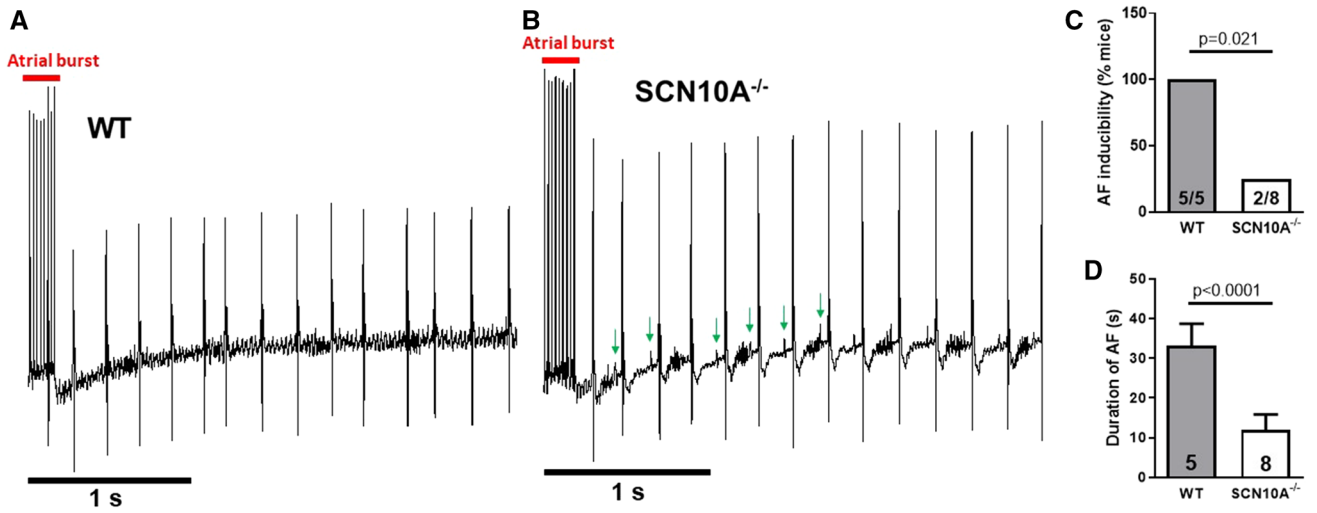


**Fig. 8** SCN10A$^{-/-}$ mice are protected against AF induction. Data are presented as mean ± SEM. **a**, **b** Representative electrocardiogram recordings of wild type (WT) and SCN10A$^{-/-}$ mice undergoing burst stimulation protocol. Arrows indicate regular *P* waves. **c** Percentage of inducible WT ($n=5$) or SCN10A$^{-/-}$ mice ($n=8$). *P* value was calculated using Fisher's exact test. **d** AF duration after burst stimulation (5 episodes/mice) in WT ($n=5$) and SCN10A$^{-/-}$ mice ($n=8$). *P* value was calculated using Mann–Whitney test

rate of alternate splicing [6, 9]. Recent genetic studies demonstrated an involvement of SCN10A in atrial cellular electrophysiology and could associate SCN10A variants with AF [17, 18, 25]. We therefore investigated whether $Na_V1.8$ compared to $Na_V1.5$ expression might be differentially regulated in patients with SR or with AF. However, we observed no differences in $Na_V1.8$ protein or mRNA expression levels between SR and AF myocardium.

We therefore investigated human atrial cardiomyocytes from patients with sinus rhythm to elucidate the cellular role of $Na_V1.8$ in the human atria. In patch clamp experiments, pharmacological inhibition of $Na_V1.8$ did not change APA, RMP or *dv/dt* in human atrial cardiomyocytes, which could be confirmed in SCN10A$^{-/-}$ mice. Since *dv/dt* is a surrogate for the fast $Na^+$ influx and hence peak $Na^+$ current [5], these observations suggest that the involvement of $Na_V1.8$ in the peak $Na^+$ current is negligible and therefore atrial conduction may not be affected. We observed a trend towards a reduced APD after $Na_V1.8$ inhibition, which however did not reach statistical significance. Thus, while we could previously show a distinct APD abbreviation upon $Na_V1.8$ inhibition in ventricular cardiomyocytes [10], the impact on atrial APD appears minor. However, APD is abbreviated in AF and APD shortening may not be a suitable strategy for the treatment of AF [16]. A critical issue is that many previous experimental reports on AF treatment strategies investigated permanent AF atria with a very short action potential. However, patients with permanent or long-standing AF are probably not suitable patients for a pharmacological rhythm strategy due to advanced remodeling. Since atrial APD is differentially regulated in different cardiac diseases, i.e., atrial APD is prolonged in patients with left-ventricular dysfunction [24], further patient-specific studies are needed.

We here demonstrate that pharmacological and genetic $Na_V1.8$ inhibition markedly reduced $I_{NaL}$ in human and murine atrial cardiomyocytes. Previous studies in animal ventricular cardiomyocytes by Yang et al. and in human ventricular cardiomyocytes by our group described a reduction of $I_{NaL}$ as well as an abbreviation of APD due to $Na_V1.8$ inhibition [10, 34]. SCN10A variants associated with AF were also found to modulate $I_{NaL}$ after transfection in ND7/23 cells, which further strengthens findings about the role of $Na_V1.8$ for $I_{NaL}$ [23]. Of note, we observed a clear trend towards a further $I_{NaL}$ reduction in SCN10A$^{-/-}$ cardiomyocytes after exposure to TTX suggesting that other $Na_V$ isoforms still contribute to $I_{NaL}$ generation. Since $I_{NaL}$ directly impacts atrial arrhythmogenesis [3, 13, 28], we consecutively evaluated whether specific $Na_V1.8$ inhibition could prevent cellular arrhythmias. We have previously shown that in the human atrium $I_{NaL}$-mediated $Na^+$ influx can induce $Ca^{2+}$ influx via reverse-mode NCX leading to an increased cytosolic $[Ca^{2+}]$ and an enhanced incidence of $Ca^{2+}$ sparks [13]. In the present study, selective inhibition or ablation of $Na_V1.8$ markedly suppressed SR $Ca^{2+}$ spark frequency and the total calculated diastolic $Ca^{2+}$ leak in atrial cardiomyocytes. Most importantly, the incidence of major diastolic $Ca^{2+}$ release events like $Ca^{2+}$ waves, which are considered as a proarrhythmic trigger, was significantly blunted after $Na_V1.8$ inhibition/ablation. Interestingly, $I_{NaL}$ inhibition by TTX showed similar antiarrhythmic effects compared to $Na_V1.8$ inhibition/ablation. Thus, $Na_V1.8$-dependent $I_{NaL}$ inhibition alone might be sufficient enough for disrupting

the vicious circle of $I_{NaL}$-dependent SR $Ca^{2+}$ leak. The electrogenic exchange of $Ca^{2+}$ against $Na^+$ via NCX can induce a transient inward current ($I_{ti}$) leading to depolarization of the cell, which serves as a trigger for spontaneous action potentials [32]. In human atrial cardiomyocytes, both $Na_V1.8$ blockers significantly diminished the incidence of EADs and prevented the generation of DADs and spontaneous action potentials during rest. Accordingly, $SCN10A^{-/-}$ mice and PF-01247324-treated WT cells also showed a lower incidence of triggered activity. Cellular afterdepolarizations as well as irregular action potentials are considered as a potent underlying mechanism for triggered ectopic activity/ectopic firing, which may promote and/or maintain atrial arrhythmias [16].

To translate our cellular experimental findings in an in vivo model, we here demonstrate that $SCN10A^{-/-}$ mice were protected against AF induction by rapid pacing and the duration of induced AF was significantly shorter in these mice. $Ca^{2+}$ sparks and DAD-related ectopic activity have been shown to trigger ectopic beats, re-entry mechanisms [7] and may also lead to dispersion of repolarization, which further increases the susceptibility to arrhythmias/AF [31]. Accordingly, $Ca^{2+}$ sparks and DAD-related ectopic activity could previously be linked to pacing induced AF in mice [20, 27]. Thus, our in vivo data in $SCN10A^{-/-}$ mice may serve as a translation of our mechanistic findings into an in vivo system.

Using genetic ablation, the proarrhythmic role of $Na_V1.8$ in the absence of pharmacological approaches and also our findings based on the $Na_V1.8$ inhibitor PF-01247324 could be confirmed. Interestingly, few association studies in patients with early onset AF also report that SCN10A variants are associated with AF susceptibility [17, 23]. Of note, as $Na_V1.8$ was discussed to modulate cardiac conduction [6, 29] the influence of SCN10A expressed in cardiac neurons/ganglia [30] may theoretically contribute to our in vivo findings. However, we demonstrate a distinct functional proarrhythmogenic role of $Na_V1.8$ on human and murine cardiomyocyte level. Notably, $Na_V1.8$ did not change *dv/dt* and amplitude of action potentials in atrial cardiomyocytes in our study as well as in ventricular cardiomyocytes [1, 10]. In addition, the QRS complex in the ECG was also unchanged in $SCN10A^{-/-}$ mice. In sharp contrast, $Na_V1.5$ inhibition (e.g., by flecainide) and reduction of peak $Na^+$ influx causing changes in cardiac conduction can adversely affect mortality by promoting arrhythmogenic mechanisms [11].

We propose $Na_V1.8$-dependent selective $I_{NaL}$ reduction and prevention of atrial arrhythmogenesis to constitute a novel antiarrhythmic approach in the human, in particular for atrial arrhythmias involving focal and/or ectopic activity. Importantly, the current study investigated atrial cardiomyocytes from patients with sinus rhythm (or murine atrial cardiomyocytes) stressed with isoproterenol. From a clinical point of view, patients with permanent or long-standing AF, which are characterized by advanced structural atrial remodeling, are likely not the optimal patients for a pharmacological rhythm strategy. Therefore, we believe that atrial samples from patients at high risk for triggered/ectopic activity or paroxysmal AF may be more appropriate to investigate from a translational point of view. Nevertheless, $Na_V1.8$ dysregulation might also have functional implications in long-standing AF.

Taken together, the herein presented functional evidence of $Na_V1.8$ in human atrial cardiomyocytes and, most importantly, the potent antiarrhythmic effects of Nav1.8 inhibition and deletion in vitro and in vivo, could lay the foundation development towards a novel therapeutic option for atrial rhythm disorders.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.



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