An interplay between Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII\textsubscript{δc}) and late Na\textsuperscript{+} current (I\textsubscript{NaL}) is known to induce arrhythmias in the failing heart. Here, we elucidate the role of the sodium channel isoform Na\textsubscript{v}1.8 for CaMKII\textsubscript{δc}-dependent proarrrhythmia. In a CRISPR-Cas9-generated human iPSC-cardiomyocyte homozygous knock-out of Na\textsubscript{v}1.8, we demonstrate that Na\textsubscript{v}1.8 contributes to I\textsubscript{NaL} formation. In addition, we reveal a direct interaction between Na\textsubscript{v}1.8 and CaMKII\textsubscript{δc} in cardiomyocytes isolated from patients with heart failure (HF). Using specific blockers of Na\textsubscript{v}1.8 and CaMKII\textsubscript{δc}, we show that Na\textsubscript{v}1.8-driven I\textsubscript{NaL} is CaMKII\textsubscript{δc}-dependent and that Na\textsubscript{v}1.8-inhibition reduces diastolic SR-Ca\textsuperscript{2+} leak in human failing cardiomyocytes. Moreover, increased mortality of CaMKII\textsubscript{δc}-overexpressing HF mice is reduced when a Na\textsubscript{v}1.8 knock-out is introduced. Cellular and in vivo experiments reveal reduced ventricular arrhythmias without changes in HF progression. Our work therefore identifies a proarrrhythmic CaMKII\textsubscript{δc} downstream target which may constitute a prognostic and antiarrrhythmic strategy.
Voltage-gated sodium channels (Na\(_V\)) play a critical role in physiological cardiac conduction. Na\(_V\) channels become inactive within a few milliseconds after activation under physiological conditions. However, in cardiac pathologies such as ischemia, hypoxia, oxidative stress, and heart failure (HF), some Na\(_V\) remain persistently open or reopen, generating a small but persistent Na\(^+\) current, referred to as the late Na\(^+\) current (I\(_{\text{NaL}}\)). This current slows the repolarization rate and thereby prolongs the action potential duration (APD). Augmented I\(_{\text{NaL}}\) may additionally cause Na\(^+\)-dependent Ca\(^{2+}\) overload in cardiomyocytes, thereby playing an essential role for arrhythmogenesis and diastolic dysfunction\(^{1,2,6}\). Furthermore, Na\(^+\)/Ca\(^{2+}\) overload caused by augmented I\(_{\text{NaL}}\) can give rise to early afterdepolarizations (EADs), delayed afterdepolarizations (DADs), and ventricular fibrillation and sudden cardiac death\(^{24}\). In the failing heart, increased I\(_{\text{NaL}}\) induces an influx of Na\(^+\) into the cardiomyocyte, which in turn stimulates Ca\(^{2+}\) influx via the reverse mode of Na\(^+\)/Ca\(^{2+}\) exchanger (NCX)\(^{12,13}\). Cytosolic Ca\(^{2+}\) may now bind to calmodulin (CaM), forming a Ca\(^{2+}\)/CaM complex, which activates Ca\(^{2+}\)/calmodulin-dependent protein kinase II\(\delta\) (CaMKII\(\delta\)), a multifunctional serine/threonine protein kinase\(^{8,10,14,19}\). CaMKII\(\delta\) is expressed in four isoforms \(\alpha, \beta, \gamma, \) and \(\delta\). CaMKII\(\delta\) is the predominant isoform in heart\(^{16}\) while the \(\delta\) isoform is mainly located in the cytosol. Once CaMKII\(\delta\) is activated, it may cause hyperphosphorylation of the ryanodine receptor 2 (RyR2) residing within the sarcoplasmic reticulum (SR)-sarcosome junction, leading to spontaneous arrhythmogenic SR-Ca\(^{2+}\) release events in HF\(^{13,17-20}\). Further, this augmented CaMKII\(\delta\) activity can also induce I\(_{\text{NaL}}\), significantly reduced following pharmacological inhibition and genetical knockout of Na\(_V\)\(_{1.8}\) in human and murine cardiomyocytes. In isolated murine CaMKII\(\delta^{+/T}\) cardiomyocytes, I\(_{\text{NaL}}\) was augmented to ~150% compared to wildtype (WT), whereas the specific Na\(_V\)\(_{1.8}\) blockers A-806734 or PF-01247324 reduced I\(_{\text{NaL}}\) by ~40% in the same background (CaMKII\(\delta^{+/T}\)) (Fig. 2a, b). These results indicate that CaMKII\(\delta\)-induced I\(_{\text{NaL}}\) can be clearly ameliorated by inhibiting Na\(_V\)\(_{1.8}\). A current–voltage relationship of Na\(_V\)\(_{1.8}\)-dependent I\(_{\text{NaL}}\) in isolated ventricular myocytes from CaMKII\(\delta^{+/T}\) mice is presented in the Supplementary information (Supplementary Fig. 3).

In the human failing heart, CaMKII\(\delta\) and I\(_{\text{NaL}}\) are upregulated in parallel\(^{22,33}\). Therefore, we inhibited Na\(_V\)\(_{1.8}\) by using PF-01247324 and compared its ability to reduce I\(_{\text{NaL}}\) to CaMKII\(\delta\) inhibition using autacamide-2-related inhibited peptide (AIP) in human failing ventricular cardiomyocytes. In addition, we blocked Na\(_V\)\(_{1.8}\) and CaMKII\(\delta\) in parallel by exposing human failing cells simultaneously to PF-01247324 and AIP. I\(_{\text{NaL}}\) measurements demonstrated that Na\(_V\)\(_{1.8}\) inhibition alone (PF-01247324) led to a ~40% decrease and CaMKII\(\delta\) inhibition

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**Results**

**Na\(_V\)\(_{1.8}\) and CaMKII\(\delta\) interaction in human HF.** Since interaction between CaMKII\(\delta\) and Na\(_V\)\(_{1.5}\) was shown previously, we hypothesized that CaMKII\(\delta\) interacts also with Na\(_V\)\(_{1.8}\) and therefore performed co-immunoprecipitation using human ventricular tissue homogenates. Indeed, we found that CaMKII\(\delta\) associates with Na\(_V\)\(_{1.8}\) in human non-failing as well as HF myocardium (Fig. 1a). Using immunofluorescence stainings, CaMKII\(\delta\) and Na\(_V\)\(_{1.8}\) were found to be co-localized in isolated human cardiomyocytes (Fig. 1b). Of note, SCN10A mRNA expression in tissue from non-failing and HF hearts, as well as isolated cardiomyocytes from human HF hearts was found to be much lower than SCN5A. Further, RT-qPCR experiments revealed that a relevant part of SCN10A mRNA in the heart originates from cardiomyocytes (Supplementary information, Supplementary Figs. 1, 2).

**Na\(_V\)\(_{1.8}\) inhibition reduces I\(_{\text{NaL}}\) in human failing, mouse CaMKII\(\delta\) transgenic cardiomyocytes, and in SCN10A knockout iPSC-cardiomyocytes.** In functional experiments, we could show that I\(_{\text{NaL}}\), induced by increased CaMKII\(\delta\) activity, was significantly reduced following pharmacological inhibition and genetical knockout of Na\(_V\)\(_{1.8}\) in human and murine cardiomyocytes. In isolated murine CaMKII\(\delta^{+/T}\) cardiomyocytes, I\(_{\text{NaL}}\) was augmented to ~150% compared to wildtype (WT), whereas the specific Na\(_V\)\(_{1.8}\) blockers A-806734 or PF-01247324 reduced I\(_{\text{NaL}}\) by ~40% in the same background (CaMKII\(\delta^{+/T}\)) (Fig. 2a, b). These results indicate that CaMKII\(\delta\)-induced I\(_{\text{NaL}}\) can be clearly ameliorated by inhibiting Na\(_V\)\(_{1.8}\). A current–voltage relationship of Na\(_V\)\(_{1.8}\)-dependent I\(_{\text{NaL}}\) in isolated ventricular myocytes from CaMKII\(\delta^{+/T}\) mice is presented in the Supplementary information (Supplementary Fig. 3).

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**Fig. 1 CaMKII\(\delta\) interacts with Na\(_V\)\(_{1.8}\) in human myocardium and isolated cardiomyocytes.** a Co-immunoprecipitation of CaMKII\(\delta\) and Na\(_V\)\(_{1.8}\) from left ventricular homogenates of human non-failing and failing hearts (NF: \(n=7\); HF: \(n=7\)). b Co-localization of CaMKII\(\delta\) and Na\(_V\)\(_{1.8}\) in human failing cardiomyocytes with immunofluorescence staining. Scale bar: 10 \(\mu\)m (staining was performed in cardiomyocytes isolated from five heart failure patients).
by AIP to a ~53% reduction of $I_{\text{nax}}$ in human failing cardiomyocytes (Fig. 2c, d). However, preincubation with AIP and PF-01247324 together decreased $I_{\text{nax}}$ comparable to AIP alone suggesting that CaMKIIδ inhibition might already suppress NaV1.8-driven $I_{\text{nax}}$. Further, peak $I_{\text{nax}}$ measurements revealed that $I_{\text{nax}}$ reduction due to NaV1.8 inhibition is not caused by a reduction of overall Na+ current (Supplementary information, Supplementary Fig. 4).

As the existence of NaV1.8 and its role in cardiomyocytes is still a matter of debate, we generated homozygous NaV1.8 knockout (KO) cardiomyocytes isolated from CaMKIIδ transgenic cardiomyocytes, and in SCN10A knockout iPSC-cardiomyocytes (Fig. 3a, b). We incubated CaMKIIδ transgenic cardiomyocytes, and in parallel resulted in a significant proarrhythmic SR-Ca2+ release events. Accordingly, we investigated whether inhibition of the NaV1.8-mediated $I_{\text{nax}}$ could attenuate the increase of the proarrhythmogenic SR-Ca2+ spark frequency (CaSpF) caused by overexpression of CaMKIIδ (Fig. 3a, b). We incubated CaMKIIδ transgenic cardiomyocytes with NaV1.8 inhibitors and measured the CaSpF. A ~50% reduction of CaSpF was observed in both NaV1.8 inhibitor groups compared to untreated cells (Fig. 3a, b). These results display that SR-Ca2+ leak due to increased CaMKIIδ expression and activity can be reduced by inhibiting NaV1.8.

It is well known that inhibition of CaMKIIδ can attenuate SR-Ca2+ leak. However, therapeutic general inhibition of CaMKIIδ in humans may not be suitable because of its pivotal involvement in different vital pathways such as learning processes. We explored whether the NaV1.8 inhibitor PF-01247324 exerts similar effects comparable to the inhibition of CaMKIIδ. Incubation of human failing cardiomyocytes with either the CaMKIIδ inhibitor AIP or the NaV1.8 inhibitor PF-01247324 resulted in a similar reduction of CaSpF compared to untreated cells (Fig. 3c, d). Furthermore, blocking CaMKIIδ and NaV1.8 in parallel resulted in a significant reduction of CaSpF, comparable to AIP or PF-01247324 alone in human failing cardiomyocytes (Fig. 3c, d).

We further investigated whether NaV1.8 inhibition modulates the Ca2+ transient amplitude and SR-Ca2+ load in cardiomyocytes isolated from CaMKIIδ transgenic mice. NaV1.8 inhibition using PF-01247324 did not pose any effect on either the Ca2+ transient

**Fig. 2 Reduced $I_{\text{nax}}$ upon NaV1.8 inhibition in human failing and mouse CaMKIIδ transgenic cardiomyocytes, and in SCN10A knockout iPSC-cardiomyocytes.**

a. Original traces of $I_{\text{nax}}$ in WT and CaMKIIδ+/+ mouse ventricular cardiomyocytes elicited using the protocol shown in the inset. b. Mean data ± SEM along with individual values shown in the graph plotting WT (n = 10 cells/5 mice; Supplementary Fig. 5). c. Incubation of human failing cardiomyocytes with NaV1.8 inhibitors and measured the CaSpF. A ~50% reduction of CaSpF was observed in both NaV1.8 inhibitor groups compared to untreated cells (Fig. 3a, b). These results display that SR-Ca2+ leak due to increased CaMKIIδ expression and activity can be reduced by inhibiting NaV1.8.

**Fig. 3 Reduced $I_{\text{nax}}$ upon NaV1.8 inhibition in human failing and mouse CaMKIIδ transgenic cardiomyocytes, and in SCN10A knockout iPSC-cardiomyocytes.**

a. Original traces of $I_{\text{nax}}$ in WT and CaMKIIδ+/+ mouse ventricular cardiomyocytes elicited using the protocol shown in the inset. b. Mean data ± SEM along with individual values shown in the graph plotting WT (n = 10 cells/5 mice; Supplementary Fig. 5). c. Incubation of human failing cardiomyocytes with NaV1.8 inhibitors and measured the CaSpF. A ~50% reduction of CaSpF was observed in both NaV1.8 inhibitor groups compared to untreated cells (Fig. 3a, b). These results display that SR-Ca2+ leak due to increased CaMKIIδ expression and activity can be reduced by inhibiting NaV1.8.
amplitude or Ca$^{2+}$ transient decay measured at different stimulation frequencies (Fig. 3e–h). Furthermore, diastolic Ca$^{2+}$, SR-Ca$^{2+}$ content, and SERCA2a activity were not affected by inhibition of NaV1.8 (untreated: \( n = 10 \) cells/5 mice) at different stimulation frequencies (Fig. 3e–h). Data were presented as mean ± SEM.

**Scn10a** knockout improves survival in CaMKIIc$^{+/+}$ mice in the absence of structural ventricular changes. To study whether inhibition of Na$\alpha_{1.8}$ influences the development of HF, arrhythmogenesis, or survival in CaMKIIc$^{+/+}$ mice, we crossbred these mice with Na$\alpha_{1.8}$ knockout mice. Interestingly, SCN10A$^{-/-}$/CaMKIIc$^{+/+}$ mice showed a significantly improved survival compared to CaMKIIc$^{+/+}$ (median survival 98 vs. 72 days, 64 vs. 43 animals, Hazard Ratio 0.6) as assessed in blinded investigations. Specifically, CaMKIIc$^{+/+}$ mice showed only a 37% survival at 12 weeks, whereas SCN10A$^{-/-}$/CaMKIIc$^{+/+}$ died at a slower rate, with 67% survival at this age (Fig. 4a). These data illustrate that Na$\alpha_{1.8}$ knockout is capable to counteract the lethal phenotype of CaMKIIc$^{+/+}$ overexpression to a relevant extent.

Detailed investigations of hearts from SCN10A$^{-/-}$/CaMKIIc$^{+/+}$ double-mutant and CaMKIIc$^{+/+}$ mice by the age of 12 weeks exhibited comparably enlarged heart chambers (Fig. 4b). Heart weight to tibia length ratio was similarly increased in double-mutant and CaMKIIc$^{+/+}$ mice (Fig. 4c). To investigate whether Na$\alpha_{1.8}$
knockout influences CaMKIIδ-mediated hypertrophy we prepared histological heart stainings. CaMKIIδ overexpression led to an increase of cardiomyocyte cross-sectional area (CSA) compared to WT and SCN10A+/-/CaMKIIδ-/- in mouse left ventricles. However, in double-mutant mice CSA did not differ from CaMKIIδ+/T alone (Fig. 4d, e). In addition, Scn10a knockout did not change the expression of Nav1.5 (Snr5a) and CaMKIIδ on protein and mRNA levels in hearts from WT and CaMKIIδ+/T mice (Supplementary information, Supplementary Figs. 7, 8).

Serial echocardiography recordings revealed a HF phenotype in CaMKIIδ+/- and SCN10A+/-/CaMKIIδ+/- with a significant reduction of left ventricular ejection fraction (EF) (Fig. 4f, g). Moreover, significantly enlarged left ventricular end-diastolic diameters (LVEDD) were measured in CaMKIIδ+/- and SCN10A−/−/CaMKIIδ−/−/CaMKIIδ+/-/CaMKIIδ−/− compared to WT or SCN10A−/−/CaMKIIδ−/−/CaMKIIδ+/-/CaMKIIδ−/− (Fig. 4f, h). There were no changes between WT and SCN10A−/−/CaMKIIδ−/−/CaMKIIδ+/- and SCN10A−/−/CaMKIIδ−/−/CaMKIIδ+/- mice with respect to LVEDD and EF.

**Reduction of proarrhythmic activity in SCN10A−/−/CaMKIIδ−/−/CaMKIIδ+/- cardiomyocytes.** To test whether enhanced I_{NaL} in CaMKIIδ−/−/CaMKIIδ+/- mice can be ameliorated by genetic knockout of Nav1.8, we measured I_{NaL} in isolated mouse cardiomyocytes (Fig. 5a, b). While CaMKIIδ+/- cardiomyocytes showed significantly enhanced I_{NaL},...
Fig. 5 Knockout of Snca (NaV1.8) in CaMKIIc°/T mice (SCN10A°/CaMKIIc°/T) significantly reduces INaL and proarrhythmic triggers. a Original traces of INaL in WT, SCN10A°/−, CaMKIIc°/T, and SCN10A°/−/CaMKIIc°/T mouse ventricular cardiomyocytes elicited using the protocol shown in the inset. b Mean data ± SEM along with individual values shown in the graph plotting (WT: n = 7 cells/4 mice, SCN10A°/−: n = 10 cells/5 mice, CaMKIIc°/T: n = 9 cells/5 mice; SCN10A°/−/CaMKIIc°/T: n = 4 cells/4 mice), there was a significantly reduced INaL in SCN10A°/−/CaMKIIc°/T cardiomyocytes compared to CaMKIIc°/T. Data were analyzed by one-way ANOVA with post hoc Bonferroni’s correction. c Original traces of action potentials showing triggered action potentials originating from delayed afterdepolarizations (DADs) in CaMKIIc°/T and SCN10A°/−/CaMKIIc°/T cardiomyocytes. d Graph of mean data ± SEM along with individual values showing DADs per minute in WT (n = 10 cells/5 mice), SCN10A°/− (n = 12 cells/5 mice), CaMKIIc°/T (n = 21 cells/5 mice) and SCN10A°/−/CaMKIIc°/T (n = 15 cells/5 mice) cardiomyocytes. There were significantly less events of afterdepolarizations in SCN10A°/−/CaMKIIc°/T compared to CaMKIIc°/T cardiomyocytes. Data were analyzed by one-way ANOVA with the post hoc two-stage step-up method of Benjamini, Krieger, and Yekutieli. e Original traces of action potential showing early afterdepolarizations (EADs) in CaMKIIc°/T and SCN10A°/−/CaMKIIc°/T cardiomyocytes. f Graph of mean data ± SEM along with individual values showing EADs per minute in WT (n = 10 cells/5 mice), SCN10A°/− (n = 12 cells/5 mice), CaMKIIc°/T (n = 21 cells/5 mice) and SCN10A°/−/CaMKIIc°/T (n = 16 cells/5 mice) cardiomyocytes. There were significantly less events of afterdepolarizations in SCN10A°/−/CaMKIIc°/T compared to CaMKIIc°/T cardiomyocytes. Data were analyzed by one-way ANOVA with the post hoc two-stage step-up method of Benjamini, Krieger, and Yekutieli. g Original confocal line scans images of CaMKIIc°/T and SCN10A°/−/CaMKIIc°/T cardiomyocytes showing diastolic Ca²⁺ waves. h Percentage of cells exhibiting waves was significantly less in SCN10A°/−/CaMKIIc°/T (n = 74 cells/7 mice) compared to CaMKIIc°/T (n = 104 cells/9 mice). Data were analyzed by Chi-square test, two-tailed analysis. I Significantly decreased number of Ca²⁺ waves per minute in SCN10A°/−/CaMKIIc°/T compared to CaMKIIc°/T. Data were analyzed by one-way ANOVA with post hoc Bonferroni’s correction. Cells/mice studied, WT: n = 48 cells/5 mice, SCN10A°/−: n = 52 cells/5 mice, CaMKIIc°/T: n = 104 cells/9 mice; SCN10A°/−/CaMKIIc°/T: n = 74 cells/7 mice. Data were presented as mean values ± SEM.
knockout of Na<sub>v</sub>1.8 resulted in a ~45% decrease in I<sub>Na</sub> in SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> mice compared with CaMKII<sup>c+/T</sup>. Of note, at basal/unstimulated conditions, I<sub>Na</sub> did not differ between WT and SCN10A<sup>−/−</sup>.

To evaluate whether chronic ablation of Na<sub>v</sub>1.8 in CaMKII<sup>c+/T</sup> mice may reduce proarrhythmic cellular activity, we performed electrophysiological measurements (Fig. 5c–f). Patch-clamp experiments revealed that CaMKII<sup>c+/T</sup> cardiomyocytes exhibited approximately fivefold more delayed afterdepolarizations/min (DADs) compared to WT and SCN10A<sup>−/−</sup>. Scn10a knockout reduced the fraction of CaMKII<sup>c</sup>-induced DADs/min by ~60% (Fig. 5c, d). Comparable observations were made regarding the occurrence of early afterdepolarizations (EADs, EADs, Fig. 5e, f). While WT and SCN10A<sup>A−/−</sup> cardiomyocytes developed almost no EADs, 13.6 ± 3.2 EADs/min were recorded in CaMKII<sup>c+/T</sup>. Na<sub>v</sub>1.8 knockout caused an 80% reduction of EADs/min in these cells. A detailed description of the action potential characteristics of these measurements is provided in the Supplementary information (Supplementary Table 1).

Furthermore, to evaluate whether inhibition of Na<sub>v</sub>1.8 may decrease the number of cardiomyocytes exhibiting Ca<sup>2+</sup>-derived proarrhythmic events, we quantified the occurrence of Ca<sup>2+</sup>-waves in mouse ventricular cardiomyocytes. The fraction of cardiomyocytes developing Ca<sup>2+</sup>-waves was significantly less in SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> versus CaMKII<sup>c+/T</sup> (14.8 vs 27.8%, Fig. 5g, h). However, some cardiomyocytes showed more than one Ca<sup>2+</sup>-wave. Therefore, we calculated the occurring events per minute. In CaMKII<sup>c+/T</sup> the frequency of Ca<sup>2+</sup>-waves was ~2.5-fold higher compared to WT and SCN10A<sup>−/−</sup> and was reduced by ~55% in cardiomyocytes from SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> compared to CaMKII<sup>c+/T</sup> (Fig. 5i). Of note, Ca<sup>2+</sup>-transient amplitude and Ca<sup>2+</sup>-extrusion from cytosol was unchanged between SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> and CaMKII<sup>c+/T</sup> (Supplementary information, Supplementary Fig. 9). In summary, we describe a cellular rescue of the proarrhythmic CaMKII<sup>c+/T</sup> phenotype due to genetic ablation of Na<sub>v</sub>1.8, which is associated with improved animal survival.

**Scn10a knockout reduces ventricular arrhythmias in CaM-KII<sup>c+/T</sup> mice.** To further investigate whether the proarrhythmic potential of Scn10a knockout on the cellular level is translatable into the in vivo situation, we implanted telemetric monitors into 8 week old SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> and CaMKII<sup>c+/T</sup> mice. After 10 days of recovery from surgery, telemetric measurements were performed twice a week for 24 h over a period of 2 weeks. Baseline ECG characteristics are present in the Supplementary information (Supplementary Table 2). There was no relevant difference in overall physical animal activity between the groups (Fig. 6b). Binned analysis revealed that SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> showed a strong trend towards a reduction of premature ventricular contractions (PVC) by ~93% compared to CaMKII<sup>c+/T</sup> (Fig. 6a, c, p = 0.08). Most importantly, the incidence of ventricular tachycardia (VT) was significantly lower (91%) in SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> (Fig. 6a, d). Therefore, the observed improved survival of SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup>-animals is associated with reduced ventricular arrhythmias.

**Discussion**

In our present study, we demonstrate that CaMKII<sup>c</sup> interacts with the neuronal sodium channel Na<sub>v</sub>1.8 in human ventricular cardiomyocytes. Using different approaches in human and mouse cardiomyocytes, we demonstrated the relevance of Na<sub>v</sub>1.8 for I<sub>Na</sub> generation in HF and that an enhanced CaMKII<sup>c</sup>, indeed, regulates this Na<sub>v</sub>1.8-driven I<sub>Na</sub>. Isolated cardiomyocytes from SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> compared to CaMKII<sup>c+/T</sup> mice exhibit reduced cellular arrhythmic events. While there was no change with respect to HF progression, i.e., similar left ventricular ejection fraction and chamber diameters, we found reduced ventricular arrhythmias and an improved animal survival of SCN10A<sup>−/−</sup>/CaMKII<sup>c+/T</sup> animals. Thus, we identified a modifiable proarrhythmic CaMKII<sup>c</sup> downstream target in the failing heart.

We recently found that Na<sub>v</sub>1.8 is upregulated and thereby contributes to I<sub>Na</sub> under conditions of HF and cardiac hypertrophy where CaMKII<sup>c</sup> activity is known to be enhanced. Therefore, the aim of the current study was to investigate a potential crosstalk between Na<sub>v</sub>1.8 and CaMKII<sup>c</sup> and its consequences on I<sub>Na</sub> generation and cellular arrhythmogeneity. Accordingly, we revealed an interaction of Na<sub>v</sub>1.8 and CaMKII<sup>c</sup> in human ventricular myocardium of both non-failing and HF samples. CaMKII<sup>c</sup> is known to also interact with Na<sub>v</sub>1.5 channels at the intercalated disc where Na<sub>v</sub>1.5 and CaMKII<sup>c</sup> are part of a macro complex with Ankyrin-G and BIV-spectrin. The interaction with CaMKII<sup>c</sup> in this complex influences Na<sub>v</sub>1.5 channel function in several cardiac diseases such as HF. As Na<sub>v</sub>1.8 was previously shown to interact with Ankyrin-G in neurons in a similar fashion as it is known for Na<sub>v</sub>1.5 in cardiomyocytes, similar mechanisms of Na<sub>v</sub>1.8 interaction with CaMKII<sup>c</sup> like known for Na<sub>v</sub>1.5 are conceivable. Several Serin residues as well as CaMKII-binding consensus motifs were found to be conserved between Na<sub>v</sub>1.5 and Na<sub>v</sub>1.8 (for details see Supplementary Fig. 10).

In a variety of cardiac pathologies, enhanced CaMKII<sup>c</sup> activity and expression is a key contributor to maladaptive electrical remodeling and thereby promotes arrhythmia. CaMKII<sup>c</sup> can influence I<sub>Na</sub> magnitude by phosphorylating Na<sub>v</sub>1.8, which has been exclusively investigated for cardiac Na<sub>v</sub>1.5 before, whereas a possible CaMKII<sup>c</sup>-dependent regulation of Na<sub>v</sub>1.8 has not been investigated yet. In our study, we investigated the contribution of Na<sub>v</sub>1.8 to I<sub>Na</sub> in an HF model that was exclusively induced by chronic CaMKII<sup>c</sup> overexpression. As previously shown, I<sub>Na</sub> was augmented in cardiomyocytes from CaMKII<sup>c+/T</sup> compared to WT, while these effects were ameliorated by the application of specific Na<sub>v</sub>1.8 blockers. Therefore, at least a relevant part of CaMKII<sup>c</sup>-induced I<sub>Na</sub> appears to be driven by Na<sub>v</sub>1.8. Additional support for this conclusion comes from our I<sub>Na</sub> measurements from human failing cardiomyocytes where CaMKII<sup>c</sup> activity and I<sub>Na</sub> are both known to be increased in parallel. Inhibition of either CaMKII<sup>c</sup> or Na<sub>v</sub>1.8 reduced I<sub>Na</sub> as demonstrated before. However, simultaneous inhibition of Na<sub>v</sub>1.8 and CaMKII<sup>c</sup> had no additive effect compared to CaMKII<sup>c</sup> inhibition alone. Therefore, it can be assumed that the majority of Na<sub>v</sub>1.8-driven I<sub>Na</sub> was already abolished by CaMKII<sup>c</sup> inhibition and hence, seems to be CaMKII<sup>c</sup>-dependent. In addition, Scn10a knockout in CaMKII<sup>c+/T</sup> mice resulted in a reduction in I<sub>Na</sub> comparable to Na<sub>v</sub>1.8 inhibition upon specific blockers.

In previous studies, the functional relevance of Na<sub>v</sub>1.8 expression in cardiomyocytes was questioned as an application of specific blockers had no effects on peak I<sub>Na</sub> and I<sub>Na</sub> in healthy and unstimulated cardiomyocytes. These data are not in conflict with our findings, as we also did not observe differences in I<sub>Na</sub> magnitude in cardiomyocytes from healthy mice, while clear effects were evident under conditions of enhanced I<sub>Na</sub>, either by chronic CaMKII<sup>c</sup> overexpression or isoproterenol treatment of iPSC-cardiomyocytes. Therefore, the interaction of Na<sub>v</sub>1.8 with enhanced CaMKII<sup>c</sup> activity might be necessary to generate meaningful effects on cardiomyocyte electrophysiology while Na<sub>v</sub>1.8 appears to play a negligible role in healthy cardiomyocytes. This establishes Na<sub>v</sub>1.8 to be a disease-specific target.
An augmentation of $I_{\text{Na}}$ was demonstrated to cause a Na$^+$-dependent Ca$^{2+}$ overdrive and spontaneous SR-Ca$^{2+}$ release providing a substrate for cellular proarrhythmia$^8$,$^{15,30,44}$. We previously reported that inhibition of $I_{\text{Na}}$ by specifically targeting Nav1.8 is potent enough to reduce NCX reverse mode and thereby diastolic SR-Ca$^{2+}$ leak$^1$,$^{11,31,32}$. In the present work, we inhibited either CaMKIIc or Nav1.8 in failing humans and CaMKIIc$^{-/-}$ mouse ventricular cardiomyocytes and correspondingly observed a decrease in $I_{\text{Na}}$ and diastolic SR-Ca$^{2+}$ leak. A significant reduction of diastolic SR-Ca$^{2+}$ release events was already prominent after Nav1.8 inhibition alone in failing human cardiomyocytes which was almost comparable to the effect caused by CaMKIIc inhibition. Moreover, we did not observe a further reduction in SR-Ca$^{2+}$ leak when AIP was co-administrated with the Nav1.8 blocker suggesting that effects of Nav1.8 inhibition on SR-Ca$^{2+}$ leak act via indirect inhibition of CaMKIIc as it was previously shown for $I_{\text{Na}}$ inhibition with TTX or Ranolazine$^8$,$^{15}$. The present findings, therefore confirm that Nav1.8 inhibition abrogates the vicious proarrhythmic cycle between enhanced $I_{\text{Na}}$ and CaMKIIc.

Increased expression and enhanced activity of CaMKIIc is not only known to increase proarrhythmic triggers in HF but is also associated with a strong HF phenotype and increased mortality in mice$^{6,8,13,16,45}$. Our current findings illustrate that Nav1.8 plays a relevant role for arrhythmogenesis under conditions of enhanced CaMKIIc activity. For deeper mechanistic analysis, we crossbred our CaMKIIc$^{-/-}$ with Scn10a$^{+/+}$ knockout mice. In fact, we observed an improved survival in these SCN10A$^{-/-}$/CaMKIIc$^{-/-}$ mice but still with an unchanged typical phenotype of dilated cardiomyopathy in these blinded investigations. There may be three potential explanations for the improved survival, which were either reduced lethal arrhythmias, pump failure, or a combination of both. As we could demonstrate that induction and progression of HF is not influenced by the additional Scn10a knockout we propose a reduction of augmented $I_{\text{Na}}$ with subsequent lower proarrhythmic activity to constitute the underlying mechanism of this improved survival. The link between enhanced $I_{\text{Na}}$ and increased arrhythmia risk is rather complex. On the one hand, enhanced $I_{\text{Na}}$ prolongs APD and may therefore give rise to the formation of EADs. On the other hand, enhanced $I_{\text{Na}}$ causes Na$^+$ overload of the cardiomyocyte subsequently leading to Ca$^{2+}$ overload by activating NCX reverse mode$^6$,$^8$,$^{11,15}$. This may trigger the occurrence of diastolic SR-Ca$^{2+}$ release and DADs due to CaMKIIc-dependent RyR2 phosphorylation$^{8,10,15}$. In our experiments, a reduction of $I_{\text{Na}}$ in SCN10A$^{-/-}$/CaMKIIc$^{-/-}$ cardiomyocytes was observed. This was clearly associated with a reduction in the occurrence of EADs, DADs and diastolic SR-Ca$^{2+}$-release events, thereby illustrating that the CaMKIIc-induced proarrhythmic phenotype can be alleviated on the cellular level by Nav1.8 knockout.

In a recent study by our groups, we could demonstrate that selective inhibition of diastolic SR-Ca$^{2+}$ leak by the compound Rycal 536 improves survival in an HF mouse model, where $I_{\text{Na}}$ and CaMKIIc activity were also described to be enhanced$^{10,46}$. As in our study, improved survival was caused by a significant reduction of malignant arrhythmias, while the severity of HF was unchanged. Likewise, we observed less ventricular arrhythmias in SCN10A$^{-/-}$/CaMKIIc$^{-/-}$ mice in vivo. It is well known that

![Fig. 6 SCN10A$^{-/-}$/CaMKIIc$^{-/-}$ exhibit less in vivo arrhythmias compared to CaMKIIc$^{-/-}$ mice.](image-url)
detrimental proarrhythmic effects in the human HF heart and in genetic ablation of NaV1.8 can specifically influence cellular electrophysiology by increasing Ryr2-leakiness and can give rise to cellular proarrhythmic events in heart cardiomyocytes. Importantly, the results of the present study demonstrate that pharmacological inhibition and genetic ablation of NaV1.8 can specifically reverse these detrimental proarrhythmic effects in the human HF heart and in our SCN10A−/−/CaMKIIδ+/T mouse model. This is associated with improved animal survival. Therefore, targeting NaV1.8 as a specific substrate of increased CaMKIIδ activity may constitute a promising antiarrhythmic approach in HF which merits further translational investigation.

Methods

Human tissue samples. The study conforms to the declaration of Helsinki and was approved by the local ethics committee. All participants were informed about the study prior to inclusion. All patients signed informed consent. We obtained left ventricular tissues from explanted hearts of patients with end-stage HF (NYHA HF classification IV) who were undergoing heart transplantation. After explantation, the whole heart or myocardial tissues were immediately placed in a container having precooled cardioplegic solution containing (in mmol/L): NaCl 110, KCl 16, MgCl₂ 16, NaHCO₃ 16, CaCl₂ 1.2, and glucose 11. Myocardial samples for Western blot and co-immunoprecipitation were immediately frozen in liquid nitrogen and stored at −80°C. The heart tissue for cell isolation was stored in cooled cardio-protection solution containing (in mmol/L): 156 Na⁺, 1.3 K⁺, 135 Cl⁻, 25 HCO₃⁻, 0.6 Mg²⁺, 1.3 H₂PO₄⁻, 0.6 SO₄²⁻, 2.5 Ca²⁺, 11.2 glucose, and 10 2.3-butanediolmonoxime (BDM) aerated with 95% O₂ and 5% CO₂. We used healthy myocardium from healthy donor hearts that could not be transplanted for technical reasons as controls co-immunoprecipitation. Patient characteristics are presented in Supplementary Table 3. The study was approved by the local ethics committee of the University Medicine of Goettingen.

Generation of homozygous knockout iPSCs using CRISPR/Cas9 and directed differentiation into iPSC-cardiomyocytes. All procedures were performed according to the Declaration of Helsinki and were approved by the local ethics committee. Informed consent was obtained by all tissue donors before start of the study. Passage 2–4 iPSCs were electroporated with 2.5 µl (100 µM) of each gRNA, 5 µl tracrRNA (2 µM Cas9 protein) (1:1 OD) and 1 µl IDT tracrRNA (100 µM, IDT) using the Amaxa Nucleofection II Device (Lonza, program B-016) and the corresponding Human Stem Cell Nucleofection Kit (Amaxa, VPH-5022). Electroporated iPSC cells were expanded and analyzed by Sanger sequencing (Microsynth). After additional singarization two identical homozygous SCN10A knockout clones (K62.1 and K62.4) were chosen for pluripotency analysis and cardiac differentiation. Two NaV1.8 knockout iPSC lines (K62.1 and K62.4) were cultured feeder-free and adherent by cultivation on Geltrex-coated cell culture dishes in the presence of 85–95% Wnt signaling while some had signs of terminal illness therefore, heart weight toibia length ratio was documented in a database of the local facility of animal experiments. A survival curve was prepared from this database and a comparison of survival was made between the groups CaMKIIδ+/T and SCN10A−/−/CaMKIIδ+/T. Animals used for experiments were excluded from survival analysis. Before sacrificing animals, body weight was recorded while after exsanguination heart weight and tibia length were measured to tabulate animal size. Some of the animals showed signs of heart failure with fluid retention while some had signs of terminal illness therefore, heart weight to tibia length ratio was analyzed instead of heart weight to body weight ratio. The mouse study was approved by the local ethics committee of the University Medicine of Goettingen and the public authority on animal welfare.
experiments into ventricular iPSC-CMs of two NaV1.8 knockout lines and the corresponding healthy isogenic control line were used.

Cardiomyocyte isolation

Human. Human myocardium was rinsed, cut into small pieces, and incubated at 37 °C in a spinner flask filled with Joklik-MEM solution (IME; AppliChem, Darmstadt, Germany) containing 1.0 mg/mL collagenase (type 1, 185 U/mg; Worthington Biochem, New Jersey, USA) and trypsin (2.5 mL/L Life Technologies, Carlsbad, California, USA). After 45 min of digestion, the supernatant was discarded, and the tissue was incubated with fresh IMEM medium containing only collagenase. The solution was incubated for 10 to 20 min until cardiomyocytes were disaggregated using a Pasteur pipette. The supernatant containing disaggregated cells was removed and centrifuged (700 r.p.m., 5 min). Fresh IMEM with collagenase was added to the remaining tissue. This procedure was repeated 4 to 7 times. After each digestion step, the centrifuged cells were resuspended in IMEM medium containing (in mmol/L): 10 taurine, 70 glutamic acid, 2.5 KCl, 10 KH2PO4, 22 dextrose, 0.5 EGTA, and 10% bovine calf serum (pH 7.4 adjusted with KOH at room temperature). Only cell solutions containing elongated, non-granulated cardiomyocytes with cross-striations were selected for experiments, plated on laminin-coated recording chambers, and allowed to settle for 30 min.

Mouse. Explanted mouse hearts from male and female wild-type (WT), CaMKII−/−, and SCN10A−/− (rat polyclonal, Thermo Scientific, 1:5000 dilution) were homogenized in lysis buffer containing (1% CHAPS in RIPA buffer (Roche Diagnostics, Mannheim, Germany), 0.014% trypsin, and 0.1 mmol/L CaCl2 (Roche). Human myocardium was rinsed, cut into small pieces, and incubated at 37 °C for 45 min in digestion solution (113 NaCl, 4.7 KCl, 0.66 Na2HPO4, 2H2O, 1.2 MgSO4·7H2O, 12 NaHCO3, 10 KHCO3, 10 HEPES, 5.5 glucose, and 0.032 phenol red (pH 7.4, with NaOH at 37 °C) using a Langendorff perfusion apparatus. Then, 0.05 mL/mg labeable TM (Roche Diagnostics, Mannheim, Germany), 0.014% trypsin, and 0.1 mmol/L CaCl2 were added to the perfusion solution. Once the heart became flaccid, ventricular tissue was removed, cut into pieces, and dispersed using a Pasteur pipette. Cells were performed carefully by increasing Ca2+ stepwise from 0.1 to 0.4 mmol/L for the patch-clamp and to 1.6 mmol/L for Ca2+ spark experiments. Cells were plated on laminin-coated chambers, allowed to settle for 15 min, and then used for measurements.

Co-immunoprecipitation

Tissue homogenates from human ventricular myocardium were suspended in lysis buffer containing (1% CHAPPS in RIPA buffer containing (in mmol/L): 50 Tris-HCl, 120 NaCl, 200 NaF, 1 Na4VO4, 1 DTT, (pH 7.4), and complete protease and phosphatase inhibitor cocktails (Roche Diagnostics, Mannheim, Germany). After 45 min of digestion, the supernatant was used in immunoprecipitation experiments to detect NaV1.8 (mouse monoclonal, LSBio, LS-C109037). The tissue was homogenated in lysis buffer containing (in mmol/L): 80 CsCl, 40 Cs-glutamate (pH 7.2 with CsOH at room temperature). In some experiments, cardiomyocytes were incubated with tezepen (1 mmol/L, Sigma) or PF-01247324 (1 mmol/L, Sigma) for 15 min before initiating these measurements. Human cardiomyocytes were incubated with autocompand-2-related inhibitor peptide (1 mmol/L, Sigma) or PF-01247324 (1 mmol/L, Sigma) for 15 min before initiating these measurements. Controls were performed to establish the current-voltage relationship of INaL but were not used for INaL measurements. In all experiments, NaV1.8 was measured using a cell-attached configuration to measure membrane potential in single isolated cardiomyocytes. INaL measurements were performed in a cell-attached configuration with more than 1 Giga-Ohm seal was achieved and the access resistance remained < 7 Ω. When whole-cell patch configuration was achieved, cardiomyocytes were identified by the presence of Ca2+ spark events, and the seal was monitored. The current-voltage relationship of INaL was measured using a cell-attached configuration with more than 1 Giga-Ohm seal was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω. Afterward, after establishing the seal, the membrane potential was measured using a cell-attached configuration with a seal of more than 1 Giga-Ohm was achieved and the access resistance remained < 7 Ω.
after cell stabilization, which was ~5 min after rupture. Data were collected using Patchmaster 2.0 (HEKA Elektronik) and was analyzed using LabChart 7.37 (ADInstruments). Prior to experiments, cells were maintained under continuous stimulation with 0.5 Hz. An EAD was counted as an EAD when a renewed depolarization of at least 1 mV occurred before complete membrane repolarization. Criteria for counting DADs were similar, but after achieving complete membrane repolarization.

Ca2+ spark and Ca2+ wave measurement. Human and mouse ventricular cardiomyocytes were loaded with a Fluo-4AM (10 µmol/L for 15 min, Molecular Probes) at room temperature. For some experiments, either A-803467 (30 nmol/L, Tocris, Sigma) or 20 V. Ca2+ sparks were normalized to cell width and scan rate (100 µm/s). Cardiomyocytes exhibited Ca2+ sparks measurement section, at room temperature) for 15 min before measurements were started to enable complete de-esterification of intracellular Fura-2-AM and to allow cellular rebalance of Ca2+ sparks. Criteria for counting DADs were similar, but after achieving complete membrane repolarization. Cardiomyocytes were washed with Tyrode's solution containing (in mmol/L): 20 Tris-HCl, 200 NaCl, 20 NaF, 1 Na3VO4, 1 DTT, 1% Triton X-100 (pH 7.4), and complete protease and phosphatase inhibitor cocktails (Roche Diagnostics) such as concentration was determined by BCA assay (Pierce Biotechnology). Denatured tissue homogenates (10 min, 70 °C in 2% beta-mercaptoethanol) were separated on 8% SDS-polyacrylamide gels, then transferred to a nitrocellulose membrane, and incubated with the following primary antibodies: rabbit polyclonal anti-CaMKII (1:5000, Thermo Scientific, FAS-21268), rabbit polyclonal anti-NaV1.5 (1:2000, Alomone labs, ASC-005), and mouse monoclonal anti-GAPDH (1:20000, BIOTREND, BTMCA-AT73-9) at 4 °C overnight. Secondary antibodies included HRP-conjugated goat anti-rabbit and goat anti-mouse (1:20000, Jackson Immunoresearch, 111-035-144 and 115-035-062, respectively). The membrane was incubated with secondary antibodies for 1 h at RT. ImmobilonTM Western Chemiluminescent HRP Substrate (Millipore) was used for chemiluminescent detection. Analysis was performed using Image Studio Lite Ver. 5.2. The full scan blots for NaV1.5 and CaMKII are shown in the Data Availability file.

Histology. Freshly explanted hearts were fixed in 4% buffered formaldehyde overnight, paraffin-embedded, sectioned (5 µm), and stained with fluorescein-conjugated wheat germ agglutinin (WGA-Alexa Fluor 594; Invitrogen, USA) for cross sectional area (CSA) assessment. At least 300 randomly selected cardiomyocytes per animal from different sections and different regions (basal, mid-ventricular, apical) of each heart were measured using the ImageJ software (Bethesda, USA).

Mouse echocardiography. For echocardiography, mice were anesthetized using 1.5% isoﬂurane, and echocardiography was performed using a VS-VEVO 660/230 (VisualSonics, Canada). During this procedure, the core temperature was maintained at 37 °C, and heart rates were kept consistent between experimental groups at 400–500 b.p.m. Electrocardiogram monitoring was obtained using hind limb electromagnetic vector geometry and systolic function was assessed by using standard 2D parasternal long and short-axis views. The examiner was blinded towards group assignment.

Telemetric ECG recordings in mice. Mice were implanted with an intraperitoneal telemetric ECG transmitter (TA11ETA-F10, Data Sciences International) with its Cable in lead II configuration. After a postoperative recovery period of 10 days, we recorded ECGs during periods of normal activity (24 h continuous recording; twice a week for 2 weeks). ECG parameters were analyzed, and the QT interval was corrected (QTc) using Bazett’s formula, QTc = QT/RR(100/RR)1/2 for established for mice with QT and RR expressed in ms. Premature ventricular contractions and ventricular tachycardia (>3 beats) were counted per 24 h and a medium value was calculated per animal was calculated from all measurements. Further, physical activity was recorded by the telemetric monitors.

Statistics. All data were expressed as the mean ± standard error of the mean (SEM). Where appropriate, one-way ANOVA with multiple comparison tests (post hoc Bonferroni’s and two-stage step-up methods of Benjamini, Krieger, and Yekutieli correction) was used. Mixed-effects analysis with Holm–Steinhaus’s post hoc test was used to analyze I-V curves. Otherwise, Student’s unpaired t-tests were used. The Chi-Square test was used to compare the occurrence of diastolic Ca2+ waves. Log-rank (Mantel–Cox) test was used to compare survival between CaMKII+/− and SCN10A−/−/CaMKII+/− mice. Two-sided p ≤ 0.05 was considered significant.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. All data generated or analyzed during this study are available within the Article and its Supplementary Information. All raw data supporting the findings from this study are available from the corresponding author upon reasonable request. Source data are provided with this paper. Any remaining raw data will be available from the corresponding author upon reasonable request.

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Author contributions
P.B., S.A., and S.S. conceived the study. K.S.-B., P.B., and S.S. designed the experiments and wrote the manuscript. P.B., N.D., P.T., S.A., B.M., N.H., M.C.K., W.M., S.P., and M.T. carried out experimental work and analyzed the data. K.S.-B. designed the CRISPR experiments. J.G., H.M., and S.L.-H. acquired and provided human tissue. K.T. applied for the authorization to carry out animal experiments and helped to analyze in vivo data. J.M., S.W., L.S.M., and G.H. provided expertise and feedback. All authors discussed the results and had the opportunity to comment on the manuscript.

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